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Assessment of Anterior Loop of Inferior Alveolar Nerve – A Cone Beam Computed Tomography Study

Abstract

Introduction: The anterior loop (AL) of the inferior alveolar nerve (IAN) and the mental foramen (MF) are important anatomical structures that should be taken into consideration during preoperative planning in the anterior mandibular region. This study aims to assess the prevalence, length, and type of the AL of the IAN in males and females in a South Indian population. Additionally, the distance from the MF to the lower border of the mandible is assessed. **Materials and Methods:** A total of 115 cone-beam computed tomography (CBCT) scans of 230 sides of mandibles were taken using a Carestream Select CS9300 CBCT machine (Carestream Dental LLC, Atlanta, USA). The scans were selected from archives and divided into three different age groups (<40, 41–60, >61 years). The scans were analyzed to detect the canal of the AL of the inferior alveolar nerve canal (IANC). The IANC, along with the AL canal and part of the incisive nerve canal, was traced using the CS 9300 software. The length of the mandibular nerve was measured for each patient in cross-sectional and panoramic views. **Results:** The AL was present in 92.6% of the 115 participants. Overall anterior loop length ranged between 0.9 and 15.2 mm. The mean length of AL in panoramic view on the right side was 3.284 ± 2.314 mm and it was 3.015 ± 2.151 mm on the left side. Y-shaped AL was more predominant when compared to T-shaped AL in the sample. **Conclusion:** The prevalence of AL was relatively higher in subjects ranging from 41 to 60 years with a slight increase in female predominance.

Keywords: Anterior loop, cone-beam computed tomography, mental foramen, inferior border of the mandible

Introduction

The mandibular nerve is the third division of the trigeminal nerve. After passing through the mandibular foramen, it is called the inferior alveolar nerve (IAN). The section of the nerve anterior to the mental foramen (MF) and just before its ramification to the incisive nerve is called the anterior loop (AL) of IAN. Surgeries in that region may result in neurosensory disturbances such as paresthesia of the lip and chin.^[1]

The AL of IAN cannot be detected clinically, but the inferior alveolar nerve canal (IANC) can be seen in diagnostic imaging, which includes dental panoramic radiography, multi detector panoramic computed tomography (MDCT scans), and cone-beam computed tomography (CBCT). CBCT superiorly visualizes the anatomical variations of IANC, which infers the path of the IAN. Visualization of the path of the nerve can

prevent nerve damage and resultant complications during surgery.^[1] While similar studies have been carried out on other populations, this study aims to determine whether the differences found in a South Indian population are significant enough to warrant separate surgical recommendations.

Materials and Methods

The present study was carried out using CBCT images of patients taken for various clinical indications during the period from December 2018 to May 2020. All images were obtained using a single Carestream select 9300 CBCT machine (Carestream Dental LLC, Atlanta, USA). Analysis of the AL of the IANC was carried out for both sides of each scan by tracing the inferior alveolar canal and its anterior

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branches. Ethical approval for this study (Pr.149/IEC/SIBAR/2018) was provided by the ethical committee of Sibar Institute of Dental Sciences, Guntur, on 29/10/2018.

In this study, the assessment of AL and its measurements were done in all three projections (axial, coronal cross-sectional, and panoramic views) using the CBCT scans viewed with the Carestream software. The IANC was traced with the software along with the AL and part of the incisive nerve canal. The entry and exit of the MF were then located from the cross-sectional view. Vertical lines were drawn on the panoramic view from the slice that corresponded to the anterior and posterior walls of the MF and the most anterior point of the anterior nerve loop length (ANLL) as seen from the cross-sectional view. The vertical length of the nerve was estimated from the canal to the opening of the MF from the cross-sectional view and translated to the panoramic view and marked as point 1. Point 2 was marked on the most anterior confinement of the AL, and a measurement was made from point 1 to point 2 [Figure 1]. This provided an actual length for the ANLL compared with the anteroposterior spread of the loop as it followed the diagonal trajectory of the nerve in panoramic view.

The visualization of sections in the three spatial dimensions (axial, sagittal, and coronal sections) is known as multiplanar

reconstruction. The type of AL in the sagittal view and length of the AL in the axial view was measured by marking three lines. The posterior part of the MF was drawn as the third line, the anterior part of the MF was drawn as the second line, and the anterior most confinement of the AL was drawn as the first line. Measurement was made from the first line to the second line. This provided an actual length for the AL [Figure 2] in axial view.

Cross sections of 1 mm thickness were obtained in cross-sectional slices. The length of the loop has been measured by counting the number of consecutive contiguous vertical cross sections performed between the anterior border of the MF and the anterior border of the loop. This number is multiplied by the resolution of the slice [Figure 3].

We used the classification described by Solar *et al.*^[2] for evaluating the various types of AL of IAN [Figure 4].

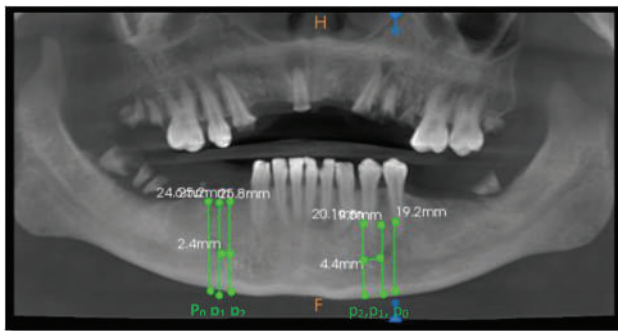


Figure 1: CBCT images with anterior loop length in panoramic view. CBCT, cone-beam computed tomography

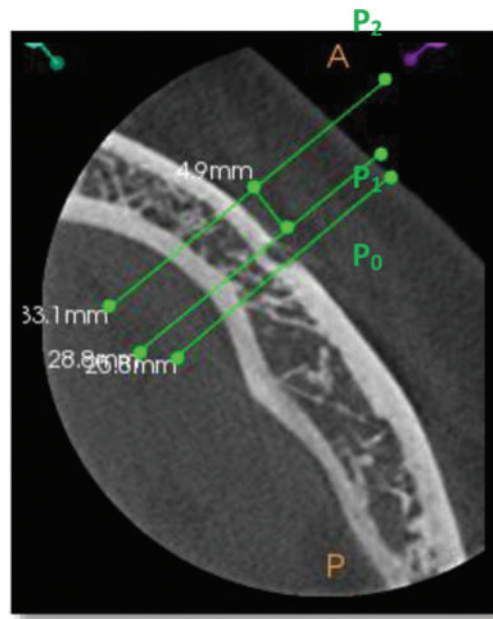


Figure 2: CBCT images with anterior loop length in oblique view. CBCT, cone-beam computed tomography

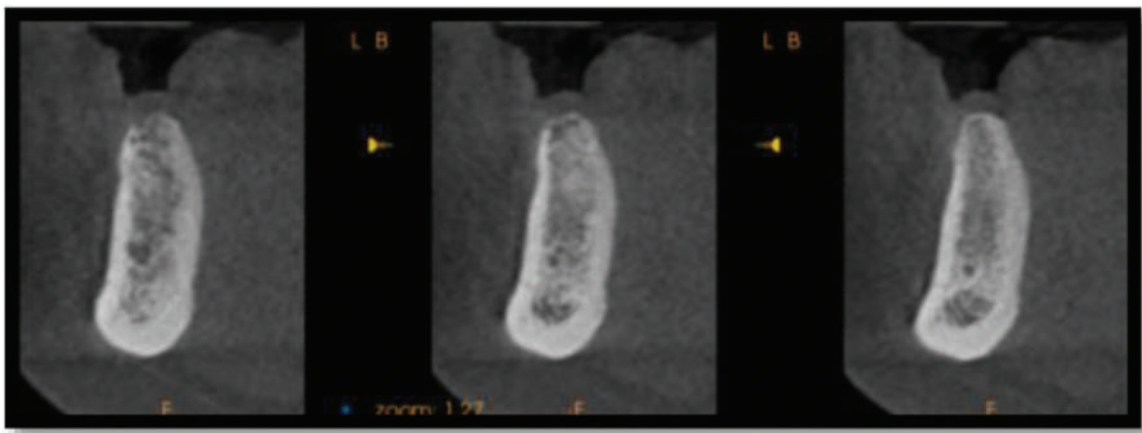


Figure 3: CBCT images with anterior loop length in cross-sectional slice view. CBCT, cone-beam computed tomography

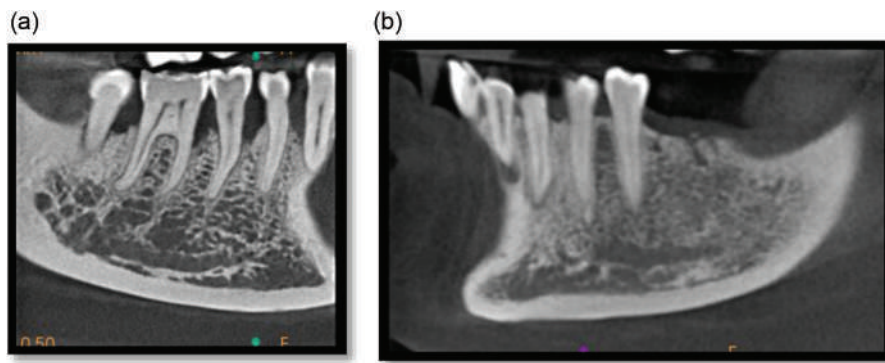


Figure 4: (a) CBCT images depicting T shape of the anterior loop. (b) CBCT images depicting Y shape of anterior loop. CBCT, cone-beam computed tomography

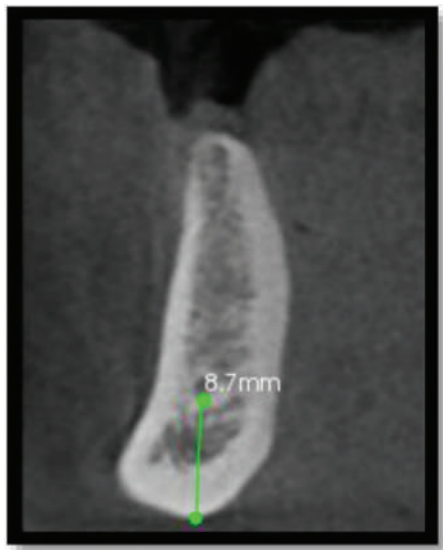


Figure 5: CBCT images of distance from mental foramen to the lower border of the mandible. CBCT, cone-beam computed tomography

- Type 1 – the AL is absent.
- Type 2 – the AL is T shaped.
- Type 3 – the AL is Y shaped.

The distance from the lower point of the MF to the lower border of the mandible was drawn and measured [Figure 5].

Results

The total number of subjects included in the study group was 115 and the total number of sides observed was 230. The AL was present in 92.6% of the 230-subject sample [Table 1]. The subjects in the study group comprised 33.9% (39) males and 66.1% (76) females, suggesting a female predominance in this sample. The length of the AL was found to be greater in the females on the left side, whereas it was found to be greater in males on the right side in all the three age groups. The AL prevalence and its length were greater in the 41 to 60 years age group than in other age groups. Overall anterior loop length (ALL) ranged from 0.9 to 15.2 mm, with a mean length

Table 1: Prevalence of anterior loop

	Present		No data/ Absent	
	N	%	N	%
Anterior loop right side	110	95.7%	5	4.3%
Anterior loop left side	103	89.6%	12	10.4%

Table 2: Length of the anterior loop in panoramic, oblique, and cross-sectional slicing views

Length of anterior loop	N	Mean	Standard deviation	t value	Significant
Panoramic side	Right	115 3.284	2.3149	15.215	0.0001*
	Left	115 3.015	2.1512	15.029	0.0001*
Oblique side	Right	115 3.211	1.8317	18.801	0.0001*
	Left	115 3.313	2.2083	16.088	0.0001*
Cross-sectional slicing side	Right	115 2.61	1.197	23.367	0.0001*
	Left	115 2.43	1.352	19.318	0.0001*

One sample, t test, *statistically significant

of AL in panoramic view on the right side and left side being 3.284 ± 2.314 and 3.015 ± 2.151 mm, respectively [Table 2]. Measurements were also done in oblique and cross-sectional views, in which the oblique view demonstrated similar measurements to the panoramic view, whereas in cross-sectional view, it showed a variation in the length of AL. The Y shape type of AL was more predominant when compared to the T shape in the sample [Chart 1]. Analyzing the relationship between the length of the AL and distance from the MF to the lower of the mandible on the right side depicts that with a one unit increase in distance from the right MF to the lower border of the mandible, the length of AL increased by 0.136 times and on the left side, with a one unit increase in distance from right MF to the lower

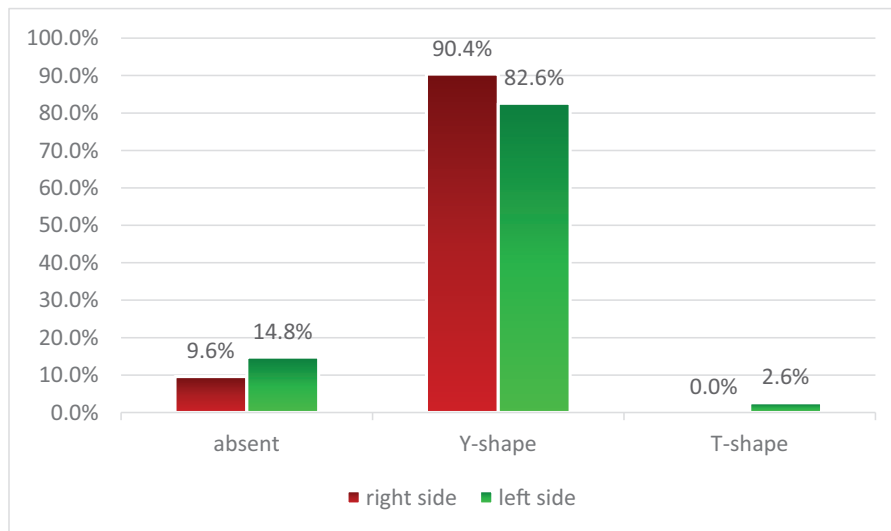


Chart 1: Type of anterior loop

border of the mandible, the length of AL increased by 0.163 times. This finding is statistically significant with a $P < 0.05$.

Discussion

The AL can be described as the extension of the mandibular canal anterior to the MF. It is formed just before the ramification of the mandibular canal into the incisive canal.^[3] It includes the mental and incisive nerves simultaneously; therefore, caution should be taken during surgical procedures in the interforaminal region to avoid nerve damage.^[3] Surgery in the area of the anterior mandible may violate the AL, resulting in neurosensory disturbances in the area of the lower lip and chin.^[1]

Many researchers have made attempts to detect the prevalence and location of the AL, as well as the anterior loop length (ALL).^[4] To avoid injury to the IAN, a 5 mm “safe” distance to the most distal fixture from the AL and a 5 mm distance from the MF for harvesting chin bone for grafts have been proposed.^[1]

CBCT is a highly efficient imaging modality for the assessment of vital anatomical structures. Moreover, CBCT provides acceptable three-dimensional (3D) views and accessibility to the craniofacial structures in comparison with the conventional radiographic techniques. Because of this, CBCT is preferred to MDCT and most often used in dental practice due to its advantages, including a lower cost and a lower radiation dose, in combination with the fact that CBCT image quality is comparable or even superior to that of MDCT for evaluating dento-maxillofacial structures.^[5]

In the present study, 230 CBCT scans of the right and left sides were evaluated, in which the AL of the IAN canal was identified in 213 scans (92.6%). Similar studies done in the literature using 3D CBCT scans had proven to exhibit high precision and reliability in the diagnosis of the AL and other

bony anatomical landmarks of the mandible with great reliability to identify the presence and measurement of length. In a study done by Wong and Patil,^[3] it was noted that the AL was present in 94% of the 100 CBCT scans in three different ethnic groups (33 Malaya, 33 India, 34 Chinese). Lu *et al.*^[6] had done a study on 366 CBCT images in the USA population in which the prevalence of AL was seen in 85.2% of scans. Parnia *et al.*^[7] had done a study on 96 CBCT scans in the Irani population, of which the AL was visualized in 84% of the CBCT scans.

In the present study, 115 CBCT scans were evaluated, out of which 33.9% were of males and 66.1% were of females. On comparing the prevalence of AL in both genders, AL was found to be more in females than in males, but this was not statistically significant. In a previous study done by Lu *et al.*^[6] on 68 CT scans of the mandible for the presence of AL on each side, 113 sides, that is, 68 patients out of 136 mandible sides, exhibited no difference in the prevalence of AL between males and females. Here some scans are single sided Siddiqui *et al.*^[1] conducted a similar study on 193 CBCT scans. The prevalence of AL was 40.4% among males and 33.7% among females. Nakib *et al.*^[8] had done a study on 400 CBCT images in which AL was noted in 56% males and 44% females, but this variable was statistically significant.

In the present study, the prevalence of AL was categorized based on age into three groups: less than 40 years, 41 to 60 years, and greater than 60 years. The prevalence of AL was found to be slightly greater in the age group of 41 to 60 years. Kajan and Salari^[9] had done a study on 84 mandibular CBCT scans, and they found a significant difference in determining the visibility of AL in different age groups. Their results showed that the prevalence of the AL: was lower in patients over 40 years of age. do Nascimento *et al.*^[5] conducted a study on CBCT scans of 250 patients among different age groups, and they reported the highest prevalence of AL in subjects in the second to fifth decades of life. Apostolakis and

Brown^[10] had done a study on 93 CBCT images in the Greek population in which the prevalence of AL was 48%. No significant correlation with the age ranges of individuals was found.

In the present study, the mean length of AL in the panoramic view was 3.284 ± 2.314 mm on the right side and 3.015 ± 2.151 mm on the left side ($P = 0.001$), whereas in the oblique view, it was 3.211 ± 1.8317 mm on the right side and 3.313 ± 2.2083 mm on the left side ($P = 0.001$). In cross-sectional slicing, it was 2.61 ± 1.19 mm on the right side and 2.43 ± 1.35 mm on the left side ($P = 0.001$). The greatest length of AL was 15.1 mm and the lowest length was about 0.9 mm. Panoramic and oblique view measurements of the length of AL did not demonstrate any statistical variation, but in cross-sectional slicing, there was a marked difference. This finding might be attributed to the thickness of the slicing in this view. Rosa *et al.*^[11] conducted a study on 352 CBCT scans of the mandible and reported that in 21 of the images, the length of the AL exceeded 4.5 mm, with the greatest length being 7 mm. Apostolakis and Brown^[10] had done a study on 93 CBCT scans with a prevalence of AL in 62% of patients, and they found the mean and range of ALL as 0.89 and 0.0 to 5.7 mm, respectively. The longest loop measured was 5.7 mm. In a study done by Kaya *et al.*^[12] on 73 panoramic radiographs and spiral CT, they reported that the mean length of AL was 0.71 ± 0.21 mm greater in panoramic radiographs than in MDCT.

Parnia *et al.*^[7] conducted a study on 96 CBCT scans in the Irani population and noticed the mean length of AL to be 3.54 mm in the study group. do Nascimento *et al.*^[5] performed a study on 250 CBCT scans and noticed that the mean length of the AL was 1.1 mm, and the largest ALL was 4 mm. The AL length had shown a mean of 0.2 to 7.61 mm presentation in various studies, but most of these studies had shown a mean length concentrating around 2 to 3 mm. Therefore, the proposed 5 mm safe distance appears to be ideal for surgical intervention in that region.

In the present study, comparing the length of AL in both genders, the mean length of AL was greater on the right side in males and on the left side in females, with a statistically significant variation. According to the study conducted by Rosa *et al.*,^[13] the AL was significantly longer in men than in women. do Nascimento *et al.*^[5] performed a study on 250 CBCT scans, and their results showed the mean length of the AL was 1.1 mm, and AL was more prevalent in male patients compared to the females.

In the present study, the mean length of the AL was found to be greater in the 41 to 60 age group. do Nascimento *et al.*^[5] had done a study on 250 CBCT scans where they found that AL was more prevalent in young patients compared to the older individuals.

In the present study, Y-shaped AL was noticed in 90.4% of cases on the right side and 82.6% of cases on the left side. On

comparing the type of AL, Y shape was more predominant when compared to the T shape in the sample, with no significant difference between the right and left sides of the mandible. Shaban *et al.*^[4] conducted a study on 71 CBCT images, of which 36 were males and 35 were females, and they found that type-III AL was a more prevalent variant and type-I (Y type) was the least prevalent type. Demir *et al.*^[14] performed a study on 279 CBCT scans in the Turkish population. It was found that type-III AL (not appreciated) was a more prevalent variant and type-I AL (Y shape) was the least prevalent type.

In the present study, the mean distance from the MF to the lower border of the mandible was greater on the right side (8.454 mm) than on the left side (7.939) and greater in males than in females. It was also greater in the age groups between 41 and 60 years on both sides. Similar studies in the literature also showed a varying presentation. Shalash *et al.*^[15] conducted a study on 120 CBCT scans and found that the MF was present, on average, 10.27 mm above the inferior border of the mandible, with no significant gender difference. In a study done by Kalender *et al.*^[16] examining 386 CBCT scans, MF was found to be 12.4 mm above the lower border, which is more compared to our study.

In the present study, linear regression was also evaluated. With one unit increase in distance from the right side of the MF to the lower border of the mandible, the length of AL increased by 0.136 times, whereas with one unit increase in distance from the left side of the MF to the lower border of the mandible, the length of AL increased by 0.163 times.

Conclusion

The prevalence of AL in this study was relatively higher and the AL prevalence was greater in subjects ranging from 41 to 60 years, with a slight increase in female predominance. The mean length of AL in panoramic and oblique views did not show any statistical variation, but measurement in cross-sectional slices showed a marked difference, which could be attributed to the thickness of the slicing in this method. Length of AL was greater on the right side in males, and on the left side, it was greater in females, with a significant variation in Y-shaped AL noticed more predominantly than in T shape. The distance from the MF to the inferior border of the mandible was greater on the right side than on the left side. In comparison, it was greater in females than in males.

After analyzing the data, it has been concluded that CBCT has emerged as a superior diagnostic modality for the evaluation of AL and thereby is useful in preoperative surgical planning. There are similar studies reported in the literature, but they have been carried out in different geographical areas. The present study depicts the ANL presentation in a South Indian population. The findings of this study support the present recommended surgical considerations. Further studies with a greater sample size would be required in order to make a

strong recommendation with regard to safety margins required in the anterior region of the mandible.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Evaluation of influence of bone quality on onset of action and the volume of anesthetic solution required: An original research

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Abstract---Aim: To evaluate the drug volume and onset of action of local anesthetic agent in different bone densities. Methodology: Present study was conducted on 40 subjects (20 male and 20 female) between 25 to 50 years attending to Sibar Institute of Dental Sciences, A 1.8ml 2% lignocaine with adrenalin was injected in the buccal mucosa(1ml) and palatal mucosa (0.8ml) in desired site of evaluation in maxilla. The duration of onset of action in minutes and drug volume used in milliliters was evaluated, where mainly comparison was done between two different densities of bone (D2,D3) according to

Misch classification. Results: Areas of maxillary which had a denser cortical bone (D2) needed more volume (1.8-3.6 ml) of local anaesthetic solution (mean \pm sd= 2.98 \pm 1.02) for achieving its mode of action and subsequently lead to numbness when compared to D3 bone which was more of a porous variety which needed less volume of anaesthetic solution (mean \pm sd= 1.63 \pm 0.78) to achieve results. The comparative result was statistically significant (P=0.037). Conclusion: The denser the bone, more time it would take to achieve adequate level of anesthesia.

Keywords---local anesthesia, cortical bone, infiltration anesthesia.

Introduction

Over the past century, there is perhaps no greater contribution to the practice of clinical dentistry than the development and application of local anaesthesia. What were once considered painful procedures have now been made routine by the deposition and action of local anaesthetics. The majority of commonly used dental local anaesthetics fall into the amide category (lidocaine, mepivacaine, bupivacaine, prilocaine), though there are some amide-type local anaesthetics that also contain an additional ester linkage (articaine). While both types of local anaesthetics have the same mechanism of action, they differ slightly in their metabolism. Intraosseous anaesthesia (IO) allows the anaesthetic solution to be injected directly into the cancellous bone. The anaesthetic solution immediately reaches the periapical region, and thus the axonal area of the nerve, where it can temporarily disable the sodium pump. The anaesthetic effect can be achieved with almost no time lag, and only a small amount of anaesthesia is needed. The transcortical injection technique avoids nerve injuries that are commonly related to the use of intraoral nerve z blocks in cases of symptomatic pulpitis. Accidental vessel injection or lingual nerve injuries can be prevented.¹ The duration of action of a local anaesthetic is contingent on two factors: the protein binding and redistribution of the local anaesthetic. Protein binding of the local anaesthetic is an inherent drug characteristic – the more protein-bound a drug is, the longer the duration of action. Duration of action on dental pulp and soft tissues is contingent almost completely on diffusion away from the site of action of the local anaesthetic. If an area is more vascular, the faster the drug will be absorbed into systemic circulation and away from the target tissue.² Use of local anaesthesia is necessary to achieve an uninterrupted field of operation and necessary for the comfort of the patient. The knowledge of pharmacology is a paramount for safe and optimum use of this group of drugs. There are many factors that influence the mechanism of action of local anaesthetic solution like the type of drug used, volume of solution, bone quality in the area of usage, local condition of tissues, technique of injection etc. Computed tomography (CT) scan has been commonly used for pre-operative quantitative and qualitative assessment of implant sites and is routinely used to determine the bone density (quality) of the bone. It allows precise 3D evaluation of anatomic structures and direct measurement of bone density, expressed in Hounsfield units (HUs).³ HUs are standard numbers originating from CT imaging. HUs represent the relative density of body tissues according to a calibrated gray-level scale, based on values for air (-1000 HU),



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water (0 HU), and bone density (+1000 HU).^{4,5} Today, CBCT is increasingly substituting multislice CT in dentistry for evaluating mineralized tissues because it provides adequate image quality, associated with a lower exposure dose.⁶⁻⁸ CBCT also offers advantages such as low cost, fast scanning time, and lower number of image artifacts.^{9,10} Over CT, it provides shorter acquisition times, submillimeter resolution, and also delivers good spatial resolution, gray density range, and contrast, as well as a good pixel/noise ratio.^{11,12} The current study focuses on the evaluation of influence of bone quality on onset of action and the volume of anaesthetic solution required.

Aim of the present study

To evaluate the drug volume and onset of action of local anesthetic agent in different bone densities. Correlation with onset of action and drug volume in different bone densities was evaluated.

Methodology

The present study was conducted on 40 subjects (20 male and 20 female) between 25 to 50 years attending to Sibar Institute of Dental Sciences, A 1.8ml 2% lignocaine with adrenalin was injected in the buccal mucosa(1ml) and palatal mucosa (0.8ml) in desired site of evaluation in maxilla. The duration of onset of action in minutes and drug volume used in milliliters was evaluated. Cone beam computed tomography (CBCT) was done to evaluate the quality of bone and the samples was categorized into different qualities. Clinicians were blinded and their opinion on the bone quality. Bone quality was determined according to Misch classification ¹³ (Table 1) Statistical analysis was conducted with the help of SPSS 25.0 from the data obtained from the CBCT values using Kruskal–Wallis test.

Results

It was observed that in maxilla, the areas which had a denser cortical bone (D2) needed more volume (1.8-3.6 ml) of local anaesthetic solution (mean \pm sd= 2.98 \pm 1.02) for achieving its mode of action and subsequently lead to numbness when compared to D3 bone which was more of a porous variety which needed less volume of anaesthetic solution (mean \pm sd= 1.63 \pm 0.78) to achieve results. The comparative result was statistically significant (P=0.037). However, when the time of the onset of anaesthesia was calculated, it was seen that bone which had D3 density achieved faster onset (average =2.8 minutes) as compared to D2 bone, due to more porous and vascular bone in the former which helps the anaesthetic solution to travel faster to the affected area. When the type of tooth which were anesthetized were compared, more volume of solution was needed to achieve the result in anterior teeth (mean \pm sd= 2.157 \pm 1.813) as compared to posterior teeth. The result was not statistically significant (p=1.36). (Table 2)

Discussion

Pain control with irreversible pulpal pain or oral hard and soft tissue pathologies is one of the greatest challenges. Emphasis should be placed on the very precise anaesthetic effect into the jaw, and therefore, a small amount of anaesthetic

solution is needed. This is a benefit, especially for treating patients with risks. A j Certosimo et al., conducted a study on reliability of electric pulp tester on predicting the level of anaesthesia. The study was performed in vivo on patients requiring operative therapy. All teeth were pulp tested preoperatively for vitality using the electric pulp tester. After injection of local anaesthesia, traditional parameters of dental anaesthesia were verified (lip numbness, mucosal sticks). Teeth were then retested with the electric pulp tester and the results recorded and concluded that electric pulp tester is a valuable clinical aid for practicing dentists, which could enable them to objectively monitor the course of anaesthesia prior to the restorative procedure.¹⁴ In a study stated that Hounsfield units (HU) was derived from Grey Scale Values obtained from CBCT, using a mathematical formula ($HU = -61.098 + 1.178 \times GSV$) derived by Razi et al. This can provide the exact bone quality in region required. The estimation of exact bone quality at surgical site can help clinicians, interpret, and plan before commencement of treatment.¹⁵ Farhad et al.¹⁶ included 60 patients and investigated the success rate, effect on blood pressure, and pain relief. After injecting 3% mepivacain, the success rate of pulpal anaesthesia was compared using pulp tests in both groups. The systolic and diastolic blood pressures of the patients were documented before and after injection. The severity of pain during injection was rated using the VAS. The success rate of pain relief of IO during treatment (56.7%) was significantly higher than that of Inferior alveolar nerve block (IANB) (23.3%) ($P = 0.008$). Both techniques showed no significant difference in pain during injection ($P = 0.304$), and systolic ($P = 0.080$) or diastolic ($P = 0.28$) blood pressure after injection. In a commentary of the study by Collier et al., he highlighted that the success rate of IANB as the primary technique for lower molars with irreversible pulpitis was assumed to be 18.9% only¹⁷ and that this may be caused by methodological flaws. This further emphasizes the suitability of the IO as the primary technique for anaesthesia for these teeth. Martinez et al. tested IO in comparison with IANB for hot teeth in the posterior mandibular region.¹⁸ In our study, it was evident that D2 bone which was more denser than the D3 bone, needed more of anaesthetic solution as well as there was increased duration related to onset of action of anaesthesia.

Conclusion

The results of this study will help in assessing the influence of bone quality on onset of action and the volume of anesthetic solution required. The denser the bone, more time it would take to achieve adequate level of anaesthesia.

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Tables

Table 1- Misch classification of assessing bone density.

Density	Hounsfield units	Description
D1	>1250 HU	Dense cortical bone
D2	850-1250HU	Thick dense to porous cortical bone on crest and coarse trabecular bone within
D3	350-850HU	Thin porous cortical bone on crest and fine trabecular bone within
D4	150-350HU	Fine trabecular bone
D5	<150HU	Immature, non-mineralized bone

Table 2- Efficacy of local anesthetic solution based on bone density

	Volume of Local anaesthetic solution (mean±SD)	Time of onset of anaesthesia (mean ±SD)	P value
Based on density of bone			
D2	2.98 ± 1.02	2.03±0.56	0.037
D3	1.63± 0.78	1.49±0.21	
Based on which tooth affected			
Anterior teeth	2.157±1.813	2.39± 2.01	1.36
Posterior teeth	1.889±1.002	1.97 ±1.16	

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Evaluation of Immediately Loaded Parallel Conical Connection Implants with Platform Switch in the Maxillary Esthetic Zone: A Prospective Clinical Study

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ABSTRACT

Aim: To assess immediately loaded parallel conical connection (Nobel Biocare) implants with platform switch design in the maxillary esthetic zone for soft and hard tissue changes.

Materials and methods: A total of 20 patients ($n = 20$) underwent prosthetic replacement of the missing maxillary anterior tooth, with an immediately loaded parallel conical connection implant (Noble Biocare, Sweden) having a platform switch design. The size of the implant was 3.75 mm in width and 13 mm in length for all patients and placement followed a standardized surgical protocol. Postoperatively, acrylic provisionalization was done within 48 hours followed by a definitive zirconia prosthesis in the 3rd month. Clinically and radiographically, the implants were evaluated for hard tissue (bone density, implant stability, crestal bone loss) and soft tissue changes (mucosal thickness—MT, sulcus probing depth—PD, bleeding on probing—BOP, width of keratinized gingiva—KG) at baseline till 36 months with follow-up intervals after loading.

Results: All patients showed uneventful healing. The difference in implant stability and density scores was significant ($p < 0.05^*$) from baseline to 36 months indicating bone formation and osseointegration of the implant. Bleeding on probing was not observed, and probing depth remained within the acceptable range (≤ 5 mm) at all time intervals after loading. The marginal bone loss was minimal (≤ 0.2 mm annually) with the absence of implant mobility and without any peri-implant radiolucency. The thickness of the gingiva (3.47 ± 0.34 mm) and width of keratinized gingiva (2.46 ± 0.39 mm) remained within reasonable limits at the 36th month with acceptable esthetic appearance.

Conclusion: In the present study, immediate loading of Nobel parallel conical connection implant in the maxillary anterior region provided adequate primary stability, minimal marginal bone loss, and increased bone density indicating earlier osseointegration. Decreased probing depth, absence of bleeding on probing, and adequate tissue collar at the neck showed better soft tissue emergence in the esthetic zone. The platform switch design demonstrated promising results and therefore can be used as an alternative to the conventional method.

Clinical significance: The present study results suggest that parallel conical connection implants (Nobel Biocare) with TiUnite surface, built-in platform switch combined with conical connection interface, parallel walled body, tapered apex, and double threads from tip to platform are all designed to provide high primary stability and support immediate function protocol, hence can be used flexibly in different bone densities.

Keywords: Esthetics, Immediate loading protocol, Nobel parallel conical connection implant, Platform switch, Prosthesis replacement.

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INTRODUCTION

The dentist must use considerable clinical skill to help patients cope with the effects of partial or complete edentulism. Dental implants are assisting in solving more difficult problems of restoration associated with missing teeth. Because of advances in implantology, patients are now enjoying fixed implant rehabilitation with good available bone. Even the patients with a single missing tooth in an esthetic zone can receive a restoration analogous to the missing natural tooth. Per Ingvar Branemark, an orthopedic surgeon in the University of Lund, Sweden, in 1952 experiment showed the fusion of titanium cylinders into the thigh bone of a rabbit, and this phenomenon is known as “osseointegration” that produced a major leap for dental implants. Osseointegration is defined as “a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant.” Further Branemark followed the orthopedic literature recommendations to place dental implants with primary stability in a submerged fashion to avoid movement during healing. The long-term follow-up study of these implants was first published in 1977 and confirmed that primary stability

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and a stress-free healing period were the two main requirements for osseointegration.¹ Several clinicians and scientists published their experience and agreed that the osseointegration requires primary stability and a period of submerged stress-free healing for 3–6 months before loading with the prosthesis. The success rate of 95–99% was observed in edentulous patients that were restored with implant-supported fixed prosthesis after following 6–15 years using this protocol. But, in this two-stage protocol, the waiting period from placement of an implant to loading was long usually 6 months in the maxilla and 3 months in the mandible. So, the functional concerns of patients during this healing period for osseointegration were raised.^{1,2} The paradigm shift in loading of the implant-supported prosthesis from delayed and early loading to immediate loading was initiated in recent years. This shift in the loading protocol is patient-driven. In the recent past, many clinical protocols were limited to provisional restorations that have shortened treatment time for the return to function but the final restoration time remained the same as conventional.³ So, various implant designs have been introduced to meet the immediate loading need. According to the fourth International Team for Implantology (ITI) consensus conference, immediate loading is defined as “dental implants that are connected to the prosthesis within 1 week after the implant placement.” The literature suggests that primary stability is an important parameter to yield proper survival of implants for all placement protocols. Insertion torques ≥ 30 N cm, implant stability quotient (ISQ) ≥ 60 , and minimal implant length ≥ 10 mm with a minimum diameter of 3 mm are recommended if accelerated loading protocols are utilized.⁴ The implant design, abutment interface, and fixation of prosthesis to implant body have changed in recent years to meet the changing clinical scenario to achieve the goal of immediate restoration that can deliver predictable treatment results in the maxillary esthetic zone.

Based on the above-mentioned parameters and guidelines, the present study assessed the immediate loading of parallel connection implants (Nobel Biocare) with platform switch design in the anterior maxilla. The objectives were evaluation of marginal bone loss, implant stability, bone density, sulcus probing depth, mucosal thickness, keratinized gingival width, and bleeding on probing.

PATIENTS AND METHODS

Study Design and Material

The present study was performed in the Department of Prosthodontics, Crown and Bridge, including Implantology during 2017–2021. A total of 20 patients with an age range of 20–45 years of either gender with missing anterior maxillary tooth and willing to take part in the study protocol were enrolled. The protocol was approved by institutional ethics committee.

Inclusion and Exclusion Criteria

- Systemically healthy patients with a history of a missing maxillary anterior tooth not less than 6 months with adequate bone quality and quantity (≥ 6 mm width \times 14 mm height) reluctant for removable or tooth-supported fixed partial dentures were included.
- Patients with a history of head and neck radiation, psychological problems, and parafunctional habits, like clenching, bruxism, deep bite, smoking, tobacco chewing, and alcohol abuse, were not included.

Source of support: Nil

Conflict of interest: None

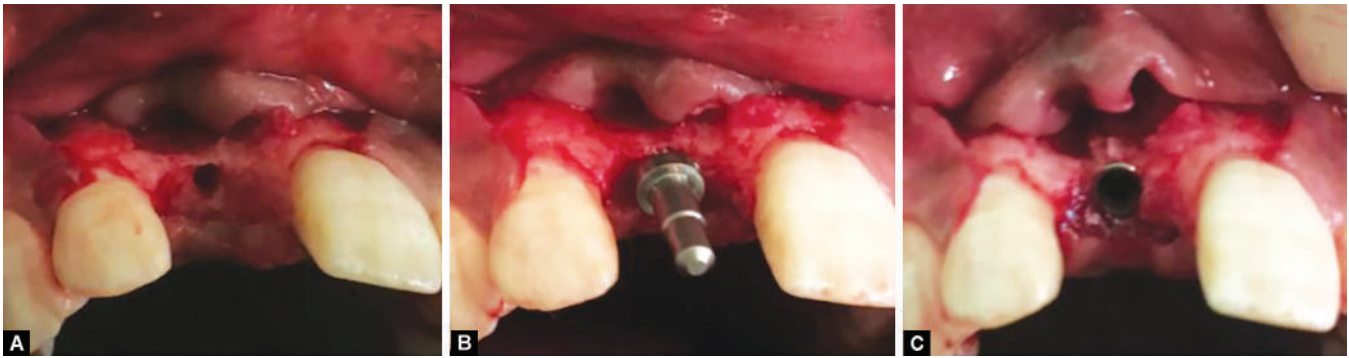
Method

Treatment planning for implant-supported restoration was done after obtaining thorough medical and dental history followed by clinical and radiographic evaluation of hard and soft tissues for each patient according to a predetermined clinical protocol. Routine blood investigations were performed to rule out any systemic or metabolic diseases. Standard intraoral periapical radiographs (IOPAR) with paralleling technique were obtained using an X-ray grid to evaluate available bone height. A bone caliper was used to assess bone width under local anesthesia. The interarch relationship was assessed using mounted diagnostic casts. Preoperative 3-dimensional cone-beam computed tomography (3D-CBCT) was obtained for further evaluation and surgical planning. A conventional surgical guide using self-cure acrylic was fabricated on study models for each patient (Fig. 1).

Recently introduced parallel conical connection (Nobel Biocare) implants with platform switch measuring 3.75 mm diameter and 13 mm length (Nobel Biocare, Sweden) were placed in all the patients (Figs 2A to C). The implants were loaded immediately on the same day and provisional restoration was given within 48 hours. Each patient was recalled after 3 months for definitive restoration. Clinically and radiographically, the implants were evaluated for hard (bone density, implant stability, crestal bone loss) and soft tissues changes (mucosal thickness—MT, sulcus probing depth—PD, bleeding on probing—BOP, width of keratinized gingiva—KG) at baseline up to 36 months with follow-up intervals after loading. The stability of the implant was assessed using Osstell-resonance frequency analyzer measured in ISQ:1–100. Bone density values were measured using IOPA digital radiographs with Digora software (Fig. 3). Crestal bone loss was measured using standard IOPAR with the help of grids. The distance between the implant shoulder and the alveolar crest was measured at the mesial and distal areas of the implants. Mucosal thickness (MT) was assessed using endodontic file number 20 with a rubber stopper. The file was inserted at the midpoint of the attached gingiva between the mucogingival junction and an imaginary line drawn from adjacent tooth cemento-enamel junction (CEJ) (Fig. 4A). Distance between the



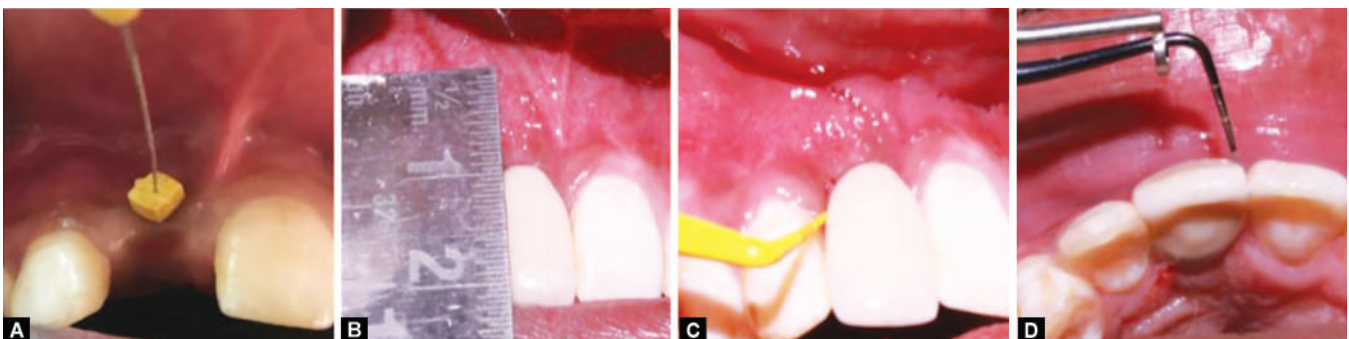
Fig. 1: Fabrication of conventional surgical stent on the diagnostic cast for implant placement in relation to maxillary right central incisor



Figs 2A to C: (A) Pilot drill after mucoperiosteal flap elevation in relation to maxillary right central incisor; (B) Verifying parallelism with adjacent tooth using guide pin; (C) Placement of parallel CC (Nobel Biocare) implant



Fig. 3: Measurement of bone density scores using DIGORA software in relation to maxillary right central incisor



Figs 4A to D: Evaluation of (A) Mucosal thickness using endodontic file number 20; (B) Width of keratinized gingiva using metal scale measured from mucogingival junction to the base of the sulcus; (C) Sulcus probing depth using plastic probe; (D) Bleeding on probing using a pressure-sensitive probe

tip of the file and rubber stopper was recorded as mucosal thickness using a digital caliper (to the nearest 0.1 mm). The width of the keratinized gingiva was measured using William's probe mid-facially

from the gingival margin to the mucogingival junction (1 week after suture removal) (Fig. 4B). Sulcus probing depth (PD) was measured using William's probe (Hu-Friedy Colorvue® probe) at mesial, distal

line angle, and middle of labial/palatal areas (Fig. 4C). Bleeding on probing (BOP) was measured using a pressure-sensitive probe that was passed along the gingival sulcus with a force of 0.25 N and waited for 30 seconds to score the bleeding index (Fig. 4D). The scoring criterion was adopted from the modified sulcular bleeding index by Muhlemann and son: score 0—no bleeding, score 1—pinpoint bleeding, score 2—the thin linear rim of bleeding, and score 3—profuse bleeding. All the radiological parameters were assessed by two observers.

Surgical and Prosthetic Procedure

Under local anesthesia, the thickness of soft tissue was measured at a predetermined point using endodontic file no. 20. One crestal and two vertical relieving incisions were made, and a full-thickness mucoperiosteal flap was reflected to expose the underlying bone.

Point of entry was gained through a surgical guide using a precision drill. Later, sequential drilling was done as per manufacturer instructions (2.0, 2.4/2.8, 2.8/3.2, cortical drill of 3.75 mm, screw tap of 3.75 mm in case of dense bone) with a drilling speed of 800–2000 rpm (Figs 2A to C). A narrow platform implant (NP) was placed slightly below the crestal level (0.5 mm) with a torque of 35 N cm. Primary stability values were checked using a resonance frequency analyzer (Osstell). Implant abutment was fixed, and soft tissue edges were sutured by interrupted sutures using 3-0 Mersilk. Antibiotics, analgesics, and mouthwash were prescribed for all patients.

Primary impressions were made using alginate for the fabrication of provisional crowns (indirect method). Immediately after implant placement, IOPAR with an X-ray grid was taken to assess the bone density and marginal bone level at baseline.

Provisional crown (self-cure acrylic) was cemented with temporary luting cement (Eugenol free Zinc Oxide) after 48 hours, which was replaced by zirconia prosthesis after 3 months. Each patient was given postoperative instructions and recalled after 7 days for suture removal. Later all patients were assessed at follow-up intervals of 3rd, 6th, 9th, 12th, 24th, and 36th months.

Statistical Analysis

The observations were tabulated using Microsoft Excel, and statistical analysis was carried out using the statistical package for social sciences (SPSS Version 26.0) software. Descriptive statistics along with Friedman and Wilcoxon matched-pairs tests were used. The quantitative variables were represented in means and standard deviations. The *p*-value ≤ 0.05 was considered to be statistically significant. The degree of interobserver agreement was assessed using the kappa correlation for radiological assessment.

RESULTS

Clinical Observations

All the patients with a mean age of 32.5 years showed a well-healed wound postimplant placement and loading. The present study included the replacement of missing right central (60%), left central (30%), and left lateral (10%) incisors. Implant stability was increased gradually from baseline (56.96 ± 2.96 ISQ) to 3rd (58.22 ± 2.18 ISQ), 6th (64.66 ± 4.15 ISQ), 9th (64.66 ± 4.15 ISQ), 12th (64.54 ± 4.01 ISQ), 18th (66.54 ± 3.60 ISQ), 24th month (66.92 ± 3.87 ISQ), and 36th month (66.92 ± 3.87 ISQ) (Fig. 5). The mucosal thickness scores at baseline and 3rd month were 3.44 ± 0.35 mm and 3.47 ± 0.34 mm, respectively, and the remaining scores till 36th month were presented in Figure 6. The

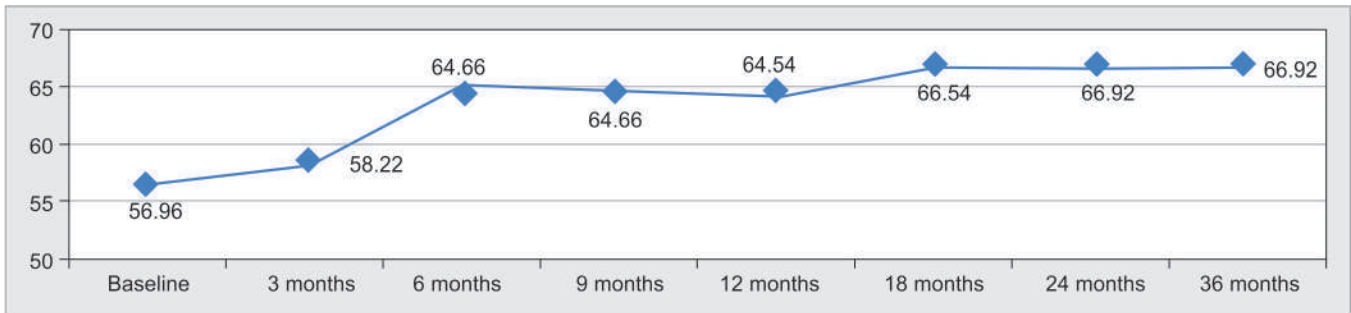


Fig. 5: Comparison of mean stability scores from baseline to 36 months with the follow-up intervals

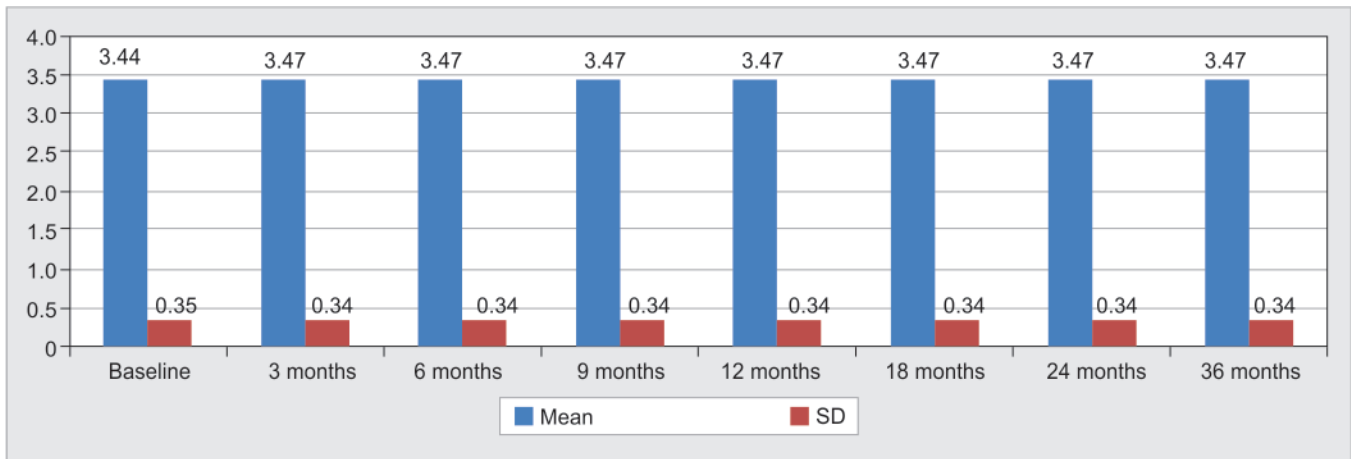


Fig. 6: Comparison of mean mucosal thickness scores from baseline to 36 months with the follow-up intervals

mean width of the gingiva was 4.105 ± 0.476 mm at baseline and was decreased to 2.46 ± 0.39 mm by the 36th month (Table 1). The mean sulcus probing depth was 1.45 ± 0.19 mm at the 3rd month and was decreased to 1.35 ± 0.33 mm at 36th month during the

follow-up period (Fig. 7). The mean rank of bleeding on probing was 3.4 at 3rd month reduced to 2.45 at 36th month during the follow-up period (Table 2). At the end of 36-month follow-up, none of the implants showed clinical signs of failure.

Table 1: Comparison of the mean width of gingiva from baseline to 36 months with the follow-up intervals (Friedman test)

Interface (Mean ± SD)	Timepoints	Mean	Standard deviation	Test statistic	p value
Baseline (4.10 ± 0.47)	3 months	3.00	0.53	1.3	0.1
	6 months	2.96	0.50	1.85	0.05*
	9 months	2.88	0.49	3.05	0.002*
	12 months	2.81	0.51	3.95	0.000*
	18 months	2.73	0.45	4.85	0.000*
	24 months	2.46	0.39	6.0	0.000*
	36 months	2.46	0.39	6.0	0.000*
3 months (3.00 ± 0.53)	6 months	2.96	0.50	0.55	0.5
	9 months	2.88	0.49	1.75	0.07
	12 months	2.81	0.51	2.65	0.006*
	18 months	2.73	0.45	3.55	0.000*
	24 months	2.46	0.39	4.7	0.000*
	36 months	2.46	0.39	4.7	0.000*
6 months (2.96 ± 0.50)	9 months	2.88	0.49	1.2	0.2
	12 months	2.81	0.51	2.1	0.03*
	18 months	2.73	0.45	3.0	0.002*
	24 months	2.46	0.39	4.15	0.000*
	36 months	2.46	0.39	4.15	0.000*
9 months (2.88 ± 0.49)	12 months	2.81	0.51	0.9	0.3
	18 months	2.73	0.45	1.8	0.06
	24 months	2.46	0.39	2.95	0.002*
	36 months	2.46	0.39	2.95	0.002*
12 months (2.81 ± 0.51)	18 months	2.73	0.45	0.9	0.3
	24 months	2.46	0.39	2.05	0.03*
	36 months	2.46	0.39	2.05	0.03*
18 months (2.73 ± 0.45)	24 months	2.46	0.39	1.15	0.2
	36 months	2.46	0.39	1.15	0.2
24 months (2.46 ± 0.39)	36 months	2.46	0.39	—	—

$p \leq 0.05^*$ considered as statistically significant

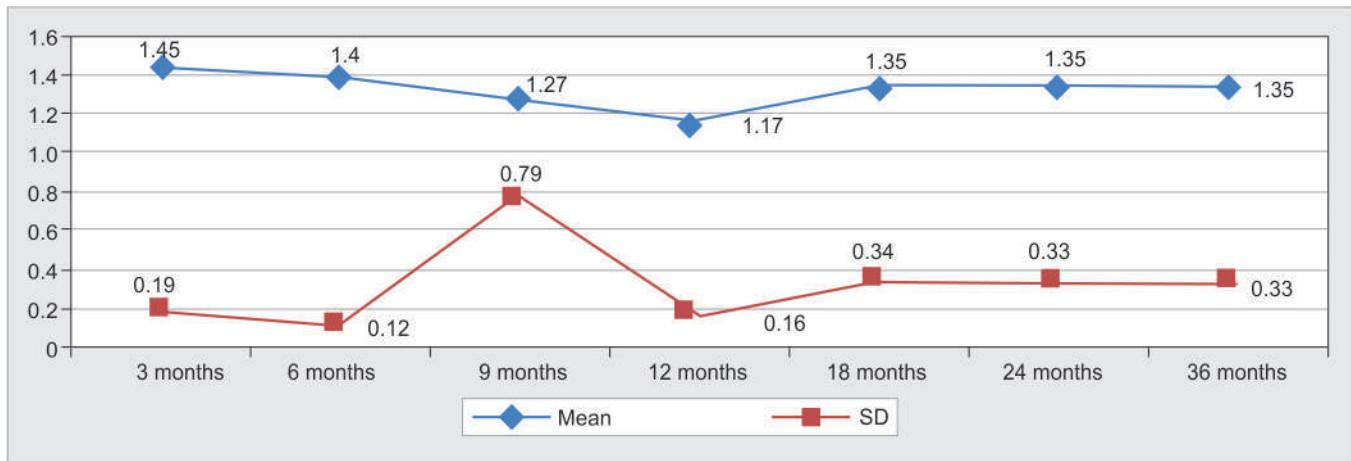


Fig. 7: Comparison of mean sulcus probing depth scores from baseline to 36 months with the follow-up intervals

Table 2: Comparison of bleeding on probing scores from baseline to 36 months (Wilcoxon matched pair test and Friedman test)

Interface	Timepoints	Mean rank	Test statistic	Z	p value
3 months	6 months	2.60	0.8	-1.300	0.3
	9 months	4.40	-1.0	-0.187	0.2
	12 months	4.25	-0.85	0.000	0.3
	18 months	3.90	-0.5	-0.431	0.5
	24 months	2.45	0.95	-1.633	0.2
	36 months	2.45	0.95	-1.633	0.2
6 months	9 months	4.40	-1.8	-1.508	0.03*
	12 months	4.25	-1.65	-1.318	0.04*
	18 months	3.90	-1.3	-1.265	0.1
	24 months	2.45	0.15	-0.447	0.8
	36 months	2.45	0.15	-0.447	0.8
9 months	12 months	4.25	0.15	0.000	0.8
	18 months	3.90	0.5	-1.414	0.5
	24 months	2.45	1.95	-2.646	0.02*
	36 months	2.45	1.95	-2.646	0.02*
12 months	18 months	3.90	0.35	-1.000	0.6
	24 months	2.45	1.8	-2.333	0.03*
	36 months	2.45	1.8	-2.333	0.03*
18 months	24 months	2.45	1.45	-2.236	0.08
	36 months	2.45	1.45	-2.236	0.08
24 months	36 months	2.45	—	—	—

p ≤ 0.05* considered as statistically significant

Radiological Observations

Peri-implant density scores were low at 3rd month compared to baseline and 6th month on the mesial side. The density scores were increased at the 6th month (mesial 113.4, distal 123.2) compared to 3rd month (mesial 100.0, distal 107.1) indicating bone formation and osseointegration (Fig. 8) (Supplementary Table 1). Results showed significant difference in bone formation from 12th to 36th month (*p* ≤ 0.05*). Mean crestal bone loss on the mesial and distal side of implant was 0.60 ± 0.29 mm at 3rd month, whereas it was increased to 1.17 ± 0.28 mm on the mesial side and 1.17 ± 0.28 mm on the distal side at 36th month during the follow-up period (Fig. 9) (Supplementary Table 2). The interobserver agreement was found to be good with a kappa value greater than 0.61.

INFERENCE

Clinical and radiological observations of the present study showed a significant increase in the bone density scores and stability values with minimal marginal bone loss, better soft tissue emergence with no bleeding on probing, and adequate keratinized gingiva. Hence, immediate loading of parallel conical connection (Nobel Biocare) implant is favorable in the maxillary esthetic zone with varying densities of alveolar bone.

DISCUSSION

The phase of restorative dentistry has been changed because of implants. The development of implant materials and implant design, with optimized surgical and prosthetic treatment protocols, has opened a wide array of treatment options for clinicians and patients.⁵ Implant-supported restoration provides better functional

stability of the prosthesis, which immensely improved the quality of life in edentulous patients.

Research and treatment evaluations have been shown to optimize the biomechanical design of superstructures and selection of patients for different treatment protocols, making oral implantology an even more predictable treatment option. Innovation, knowledge, and experience have led to improved implant designs and optimized treatment protocols.⁶ Thorough understanding of bone biology is an essential factor for adopting the proper treatment protocol.⁷ Different loading protocols have been proposed since the beginning of implant dentistry (Branemark Era). The changing scenario and patient's need for an immediate solution led to immediate loading protocols for the replacement of missing teeth. Immediate loading refers to an abutment connection and placement of restoration in occlusion at the time of surgery or within 48 hours.⁸ Better understanding of micromotion led to the development and evolution of the concept of immediate loading.⁹ A perceived psychological, economic, and functional advantage of shortened treatment periods has encouraged clinicians to challenge the conventional restoration with immediate temporization and/or early loading of dental implants.² So, the present study planned to assess hard and soft tissue changes after immediate loading of parallel conical connection (Nobel Biocare) implant with platform switch design for the restoration of single missing tooth in maxillary esthetic zone.

In the present study, stability of the implant was assessed using Osstell-resonance frequency analyzer (Osstell; Goteborg, Sweden) by attaching a standard transducer to the implant, immediately after implant placement and was repeated at follow-up intervals. All the implants showed mean primary stability values of 56.96 ± 2.96 ISQ which was significantly increased from baseline to 36 months indicating optimal osteointegration of the implant. In the present

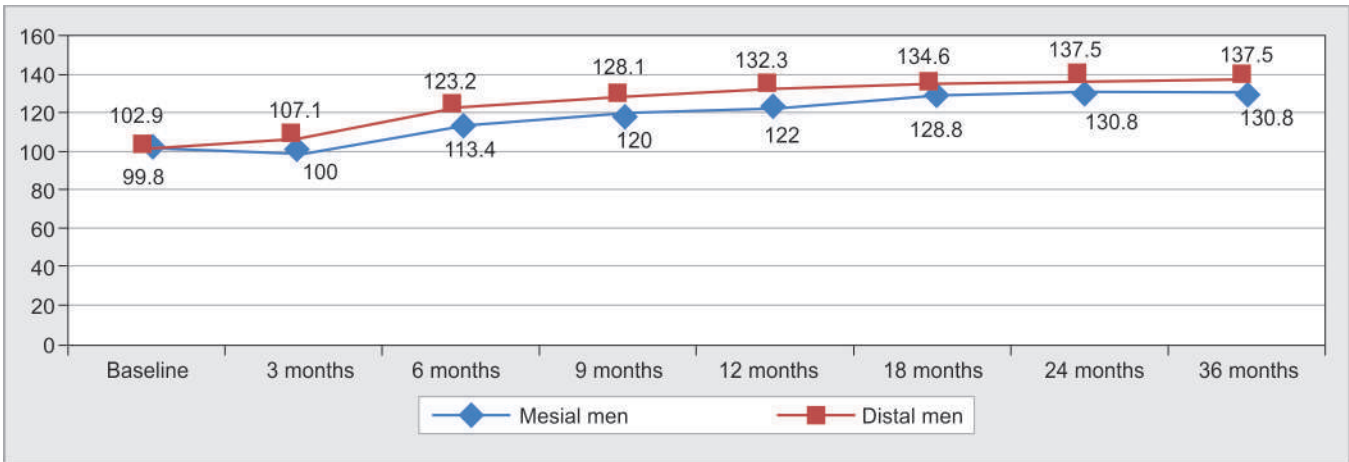


Fig. 8: Comparison of mean mesial and distal bone density scores from baseline to 36 months with the follow-up intervals

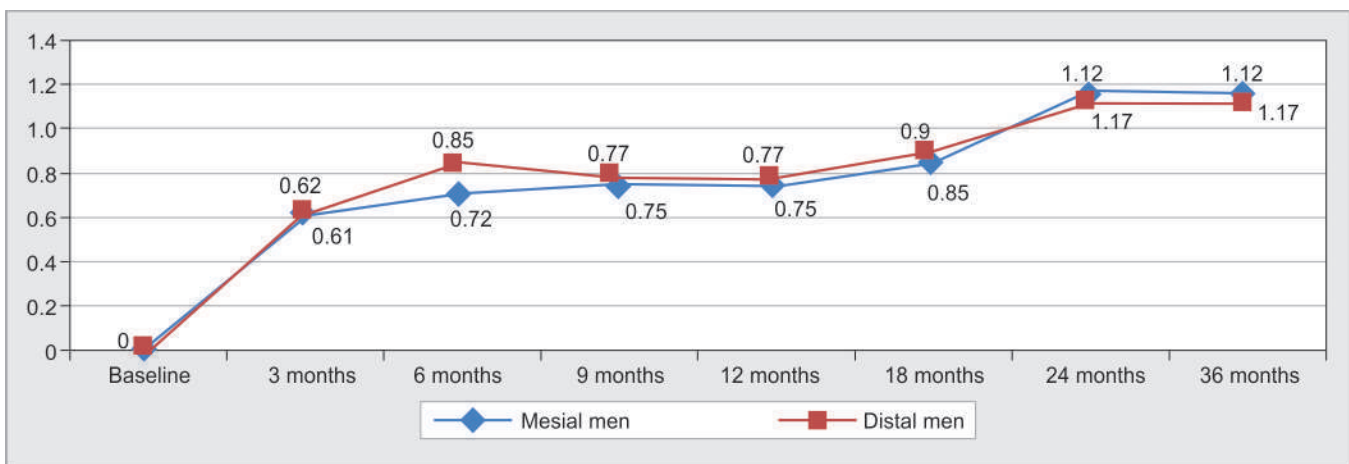


Fig. 9: Comparison of mean mesial and distal crestal bone loss scores from baseline to 36 months with the follow-up intervals

study, no mobility was observed indicating a 100% survival rate. This observation is in accordance with the study of Lorenzoni et al. indicating successful osseointegration.¹⁰ The resonance frequency analyzer findings of Fischer et al.¹¹ study showed little higher values than the present study at baseline (63.3 ± 6.1 ISQ) and 3rd month (64.3 ± 5.3 ISQ) whereas 6th-month values were similar (65.0 ± 4.6 ISQ). But these values were not from a single site and also Fischer et al.¹¹ have not standardized the implant size. But in the present study, implant size was standardized (3.75 mm diameter, 13 mm length) to overcome the bias associated with the length and diameter of the implant.

Peri-implant bone density values were measured using digital IOPAR with Digora software at baseline, thereafter at various follow-up time intervals till 36 months. Digora software measures density with a range of 0–255 pixels using the density tool. Mean bone density on the mesial side of the implant was decreased from baseline (102.90 ± 27.48) to 3rd month (100 ± 27.95) indicating early bone modulation, whereas on the distal side, (107.1 ± 41.12) it is increased. These changes were statistically insignificant. Later, at 36th-month density values had increased significantly (mesial 130.8 ± 18.9 , distal 137.5 ± 30.94) compared to 3rd and 6th months indicating increased bone mineralization and osteointegration. The density values were well correlated with the stability of the implant.

The crestal bone loss was measured using standard IOPAR with the help of an X-ray Grid. The X-ray Grid consists of one-millimeter graduations superimposed on IOPAR and was used for assessment. Crestal bone loss is an essential criterion for the evaluation of implant success. Schincaglia et al.¹² study showed an average radiographic change in bone level of 0.77 ± 0.38 mm in the immediate loading group and correlated these results to a hypothesis that micromovements caused by immediate loading have a positive effect on osteodeposition. This micromotion-assisted osteodeposition was earlier proved in an animal study by Vandamme et al.¹³ in which the results showed an increase of bone mineralization was significant around immediately loaded implants compared to unloaded implants. Salvi et al.¹⁴ showed 0.57 ± 0.49 mm of bone loss in an immediately loaded group whereas Kim et al.¹⁵ observed bone loss of 0.29 ± 0.19 mm after 6 months of crown placement. But in the present study, after the 3rd month of loading, the mean value of bone loss on the mesial and distal side was same (0.60 ± 0.29 mm). The differential measurements similar to the present study were not available for comparison in the published literature.

Further, mean bone loss of 1.17 ± 0.28 and 1.12 ± 0.27 mm on mesial and distal sides was observed, respectively, after 36 months of loading. These results were supported by the observations of

Schincaglia et al.,¹² Salvi,¹⁴ and Kim et al.¹⁵ The changes observed at 3rd and 36th months on both the mesial and distal sides were statistically significant ($p = 0.000$).

The soft tissue biotype surrounding the implant is one of the essential factors in the esthetic zone. The success of an implant depends on lesser biological complications, like peri-implantitis and gingival recession, which are crucial for aesthetic considerations. Patients with thin, highly scalloped gingiva are at risk of recession. So, it is advisable to ensure an adequate width of keratinized gingiva in the esthetic zone. The primary difference between the implant and tooth lies in the insertion of connective tissue at the neck of the implant (subcrestal in implant and supracrestal in the natural tooth).¹⁶ The present study showed an adequate width of gingiva at baseline and even during the follow-up period of 36 months. Minimal/no keratinized gingiva is still controversial for the development of peri-implantitis.¹⁷ But, published literature showed contrast reports regarding the width of keratinized gingiva and concluded that the width of keratinized gingiva has no role on the survival of the implant.^{18,19}

Mucosal thickness (MT) was measured using endodontic file number 20 with a rubber stopper. The file was inserted at a predetermined reference point. The distance between the rubber stopper and tip of the file was recorded using a digital caliper (to the nearest 0.1 mm) as mucosal thickness. The mucosal thickness in the present study at baseline was 3.44 ± 0.35 mm, whereas at 36th month, it is 3.47 ± 0.34 mm. Results showed no significant changes associated with mucosal thickness throughout the follow-up period with a $p = 0.343$.

The width of keratinized gingiva is measured using William's probe mid-facially from the mucogingival junction of the implant to the free gingival margin. The width of the attached gingiva is defined as the "portion of gingiva that extends from the base of the gingival crevice to the mucogingival junction." It is firm, resilient, and tightly bound to the underlying periosteum, through connective tissue. The presence of an adequate zone of gingiva is considered critical for the maintenance of marginal tissue health and the prevention of continuous loss of connective tissue attachment.^{20,21} Lang and Loe suggested that at least 2 mm of keratinized gingiva with 1 mm attached gingiva is adequate to maintain gingival health.²² Schrott et al.²³ studies showed that patients having good oral hygiene with keratinized tissues of less than 2 mm were not affected on the buccal side, but lingual sites demonstrated peri-implant inflammation because of difficulty in plaque control. However, recent evidence shows the opposite trend.²⁴⁻²⁶ The retrospective studies have shown an association of keratinized mucosa with peri-implant soft tissue health and stability.²⁴⁻²⁶

In the present study, no augmentation procedures were performed as the patients had an adequate width and thickness of gingiva. The width of keratinized gingiva in the present study remained the same over the 6-month follow-up. At baseline, width of keratinized gingiva was 4.105 ± 0.476 mm and is reduced to 2.46 ± 0.39 mm in the 36th month. The reduction is because of increased probing depth in few cases. Overall, the width of keratinized gingiva is within the limits of recommended minimum width. The change in the width might be attributed to patient oral hygiene factors.

In the present study, sulcus probing depth is measured using William's probe on mesial, distal line angle, and middle of buccal/lingual areas around the implant. The probing depth and radiographic measurements were considered for comprehensive

assessment of implant success as suggested by Degidi et al.²⁷ Giovanni et al.¹² study found a mean probing depth of 2.6 mm after 6 months of restoration in the immediate loading group, whereas Heydenrijk et al.²⁸ showed decreased mean probing depth from 3.6 mm (at first month) to 3.3 mm (at 6 months) in the immediate loading group. But in the present study, results showed 1.35 ± 0.34 mm of pocket depth which is lesser than Heydenrijk et al.²⁸ findings. This result substantiates the hypothesis of Schincaglia et al.¹² that osteodeposition is induced by mechanical strain in immediate loading compared to conventional, which led to reduced probing depth and radiographic changes in the bone level ($p < 0.05^*$).

In the present study, bleeding on probing was measured using pressure-sensitive probe at various follow-up intervals with a force of 0.25 N and scored as per the scoring criteria of modified sulcular bleeding index by Muhlemann and son. According to Lekholm et al.,²⁹ bleeding on probing is an earlier sign of inflammation and it may occur concurrently with increased probing depth and radiographic bone loss.³⁰ Salvi et al.¹⁴ observed 9.7% BOP in immediate loading group. In the present study, two patients showed bleeding on probing in the 3rd month which might be attributed to poor oral hygiene, and the count was decreased to one patient at 6th-month follow-up. Decrease in BOP might be attributed to effective oral hygiene instruction followed by the patients, and these results are in accordance with Salvi et al.¹⁴

Peri-implant radiolucency is defined as "radiographic evidence of progressive peri-implant bone loss." In the present study, peri-implant radiolucency is within the acceptable range (≤ 0.2 mm annually) and is in accordance with Degidi et al.²⁷ observations. The present study showed a 100% survival rate after 36 months of loading according to the success criteria of Albrektsson et al.¹⁹

In the present study, one patient showed BOP and was advised to follow strict oral hygiene instructions. Probing depth associated with peri-implant health in the present study remained within the normal limits ≤ 5.0 mm after 36 months of loading. The success of immediate loading requires careful and strict patient selection criteria aimed at achieving good primary stability, avoiding any excessive functional or non-functional loading.¹⁰

Studies indicate that ISQ values ≥ 60 demonstrated adequate primary stability where immediate loading is possible. Whereas ISQ values falling between 40 and 50 has to go for delayed loading and ISQ values less than 40 indicate potential risk of implant failure.³¹ But the present study showed primary stability of 56.96 ± 2.96 ISQ and is very near to the published literature value.³² Jawbone quality and primary implant stability are important prerequisites for a successful treatment outcome. In the present study, we considered ISQ values of more than 50 and insertion torque of more than 35 N/cm for immediate loading of the implant.

In the present study, a parallel conical connection implant (Nobel Biocare) with a unique TiUnite surface with grooves was used that maintained implant stability by faster bone formation that promoted long-term success. The combination of controlled titanium oxide texture and porosity makes bone growth onto the surface and into the grooves of implant threads.³³⁻³⁶ The application of immediate loading protocol is beneficial and is an alternative option to conventional loading for optimizing osseointegration, higher acceptance, patient satisfaction, and reduced treatment time. However, success is attributed to factors such as primary stability, marginal bone loss, implant design, osteotomy, and quality of the bone.³⁷

The parallel conical connection system has a limited number of drills that ensure straightforward surgical protocol and can be used flexibly in different bone densities. The design of the implant provides high primary stability and support for the immediate function/loading.^{38,39} The apex design allows bicortical anchorage to obtain high primary stability.⁴⁰ The design of the implant-abutment interface is an important factor as it provides a favorable connection for the growth of the gingival band around the abutment or neck of the implant. So platform switch has a unique advantage of better soft tissue adaptation around the implant neck and is essential in esthetic zone restorations. The recently introduced parallel conical connection (Nobel Biocare) implant with a platform switch offers a better clinical advantage over the conventional design.

LIMITATIONS

The results were obtained after 36 months of follow-up with a smaller sample size. The long-term studies with a large sample size are recommended for the predictability of success rate conclusively. The present study warrants multicenter studies using cone-beam computed tomographic evaluation based on bone density classification to provide guidelines for clinicians apart from ISQ or torque values for loading.

CONCLUSION

The present study showed promising results with 3-year follow-up duration. Marginal bone loss was minimal. Sulcus depth and bleeding on probing were confined to normal physiological limits indicating adequate soft tissue collar formation due to platform switch design. Increased bone density without any periapical or peri-implant radiolucency indicated 100% implant survival showing better osseointegration.

Author Contributions

HPK and TKM were involved in the planning, conceptualization, and investigation of the study. HPK, VSK, and RS carried out the data collection, analysis, and thesis write-up. HPK, TKM, YR, and CKI were responsible for the data analysis, photographs, and final reading approval.

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SUPPLEMENTARY MATERIALS

All the supplementary material from Supplementary Tables 1 and 2 are available online on the website of www.thejcdp.com.

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Assessment of Nicotine Dependence among Cigarette Smokers Seeking Oral Health Care Using Fagerström Test for Nicotine Dependence: A Cross-Sectional Study

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Abstract

Introduction: In the quest of articulating customized tobacco cessation strategies, evaluation of the level of nicotine dependence among participants is quintessential. **Objective:** This study was conducted to assess the levels of nicotine dependence and its association with age and socioeconomic status among patients seeking oral health care at a teaching dental institution in coastal Andhra Pradesh. **Materials and Methods:** This cross-sectional study was done among 199 participants with the habit of cigarette smoking who participated in the study. Nicotine dependence scores were assessed using Fagerström Test for Nicotine Dependence (FTND). IBM SPSS version 20 software was used for data analysis. The Chi-square tests and Kruskal–Wallis analyses of variance were done to analyze the study data. $P \leq 0.05$ was considered statistically significant. **Results:** The mean FTND score was 4.52 ± 2.1 . Significant differences in the mean nicotine dependence score were found between different age groups ($P < 0.001$) and participants belonging to different socioeconomic strata ($P < 0.001$). While a positive correlation was observed between age and FTND score, an inverse relation was noted between FTND score and socioeconomic status with participants from lower socioeconomic status demonstrating higher FTND scores. The majority of the participants reported smoking < 10 cigarettes per day. **Conclusion:** The study participants demonstrated moderate nicotine dependence. The assessment of nicotine dependence scores as a preliminary step in tobacco cessation counseling is essential to effectively articulate customized cessation strategies.

Keywords: Fagerström scale, nicotine dependence, smokers

INTRODUCTION

Tobacco consumption emerged as one of the most common and deleterious habits in recent decades.^[1] The global statistics reveal the ubiquitous nature of tobacco use.^[2] In India, the habit of tobacco consumption is very prevalent with 28.6% of all adults consuming tobacco in one form or the other.^[3] The contribution of tobacco toward a country's disease burden and the range of negative health outcomes tobacco could be responsible for led to the identification of tobacco consumption as a global epidemic.^[4] In the Indian context, a lot of efforts have been directed toward making people aware of the ill effects of tobacco. The Indian government has been making consistent efforts to bring down the growing consumption of tobacco which include the Cigarette Act 1975 and Cigarettes

and Other Tobacco Products Act, 2003. In 2007, National Tobacco Control Programme was launched and the National Tobacco Control Cell was constituted which organizes at the national, state, and district levels.^[5]

Although oral health-care professionals are well informed about the scope of the dental profession in identifying tobacco users and offering tobacco cessation counseling, it is seldom considered an integral part of the provision of oral health

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therapy at the dental office are evolving to be an effective means of achieving tobacco cessation among patients.^[7,8] These strategies not only provide the tobacco users with information about the quit process but also attempt to identify reasonable ways to identify motivational cues from previous quit attempts of the participants and draft customized strategies to counter the circumstances that preclude cessation of tobacco use.^[9] However, in the quest of articulating customized tobacco cessation strategies, evaluation of the level of nicotine dependence among participants is quintessential. The evaluation of nicotine dependence can be done in a multitude of ways which include the Diagnostic and Statistical Manual-IV substance dependence structured interviews,^[10] Fagerström Tolerance Questionnaire^[11] which is an eight-item scale, and the Fagerström Test for Nicotine Dependence (FTND)^[12] which is a six-item scale. There is a paucity of literature on the evaluation of nicotine dependence as a preliminary step in the provision of tobacco cessation counseling. With this background, the objective of this study was to evaluate nicotine dependence among cigarette-smoking dental patients using FTND.

MATERIALS AND METHODS

This cross-sectional study was conducted in a teaching dental institution in coastal Andhra Pradesh. The study was conducted from January 2017 to December 2017. Ethical approval for the study (SJDC/CEC/2015-2016) was obtained from the Institutional Ethical Committee of St. Joseph Dental College, Eluru, on December 22, 2015. Prior permission was obtained from the administrative authorities of the institution before the conduct of the study. The sample size for the study was determined to be 199 using G* power 3.1.9.2 software, (Kiel, Germany: Kiel University)^[13] (Test family: *t*-tests; Test: Means – difference from constant (one sample case); Tails: Two-tailed; α error probability – 0.05; power – 0.8; effect size – 0.2). One hundred and ninety-nine cigarette smokers were recruited for the study. All the participants were self-reported current cigarette smokers (participants who reported smoking at least 100 cigarettes during their lifetime and currently smoking) with no tobacco chewing habits. Informed consent was signed by all the study participants and the data collection was anonymous.

FTND is a six-item scale. Four items in the scale are dichotomous in nature, whereas the other two items are multichotomous. The scale score ranges between 0 and 10 with higher scores indicative of high nicotine dependence.^[12] The test was administered to all the study participants by a single trained interviewer. Details relating to age, gender, education, occupation of the head of the family, and monthly family income were also obtained from the study participants. Socioeconomic status was assessed using the modified Kuppuswamy scale.^[14] Data collection for each participant lasted for 3 min on average. The collected data were later intended to be used to articulate customized cessation strategies

of the institution. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 22 software (IBM SPSS, IBM, Armonk, NY, USA). Descriptive statistics, Chi-square tests, and Kruskal–Wallis analyses of variance were done to analyze the study data.

RESULTS

The mean age of the study participants was 42.89 ± 17.97 years and all the participants were males. Table 1 presents the age group-wise distribution of the participants' responses to each of the six items in the FTND. It was observed that participants who were 65 years and older prefer to smoke within 5 min after waking up in the morning at a comparatively higher frequency than the younger age groups. Although it was observed that the difficulty to refrain from smoking at forbidden places increased with increasing age, this difference between age groups was not statistically significant. The first cigarette in the morning was reported to be the most difficult one to give up by participants belonging to all the age groups considered in this study except ≤ 24 years. Most of the participants reported smoking 10 or lesser number of cigarettes per day. Among participants who were older than 64 years of age, 48.5% smoked 11–20 cigarettes per day. With increasing age, there was a clear transition from smoking cigarettes during the rest of the day to predominantly smoking in the 1st h after waking up in the morning. The majority of the participants reported that they would not smoke if they were so ill that they were on bed for most of the day.

The mean nicotine dependence score of the study sample was 4.52 ± 2.1 . Table 2 shows the differences in mean nicotine dependence scores based on the age group of the study participants. The highest mean scores were observed among participants aged 65 years and older. When the nicotine dependence scores were compared between participants belonging to different socioeconomic strata, a decreasing trend was observed from lower socioeconomic status to upper-middle socioeconomic status [Table 3].

DISCUSSION

The mean nicotine dependence score observed in this study reveals that there was moderate nicotine dependency among the study participants. To the best of our knowledge, this is the first study to preliminarily assess nicotine dependence scores in the quest of providing tobacco cessation counseling in the tobacco cessation clinics set up in teaching dental institutions as directed by the Ministry of Health and Family Welfare (MOHFW) and the Dental Council of India (DCI).^[15] Cigarette smoking is responsible for chronic diseases like lung cancer that tend to manifest in the later part of life besides compromising the quality of life owing to the other adverse effects on health in the short run. While the immediate impact of cigarette smoking on health is not life-threatening, the chronic conditions developed as a result of the habit can be fatal.^[16-18]

Table 1: Age group-wise distribution of item responses in the Fagerstrom test for nicotine dependence

Item	Category	Age group (years)						P
		≤24, n (%)	25-34, n (%)	35-44, n (%)	45-54, n (%)	55-64, n (%)	>64, n (%)	
How soon after you wake up do you smoke your first cigarette? (min)	31-60	29 (60.4)	14 (58.3)	9 (30)	7 (18.4)	8 (30.8)	3 (9.1)	<0.001*
	6-30	6 (12.5)	4 (16.7)	9 (30)	13 (34.2)	7 (26.9)	4 (12.1)	
	Within 5	13 (27.1)	6 (25)	12 (40)	18 (47.4)	11 (42.3)	26 (78.8)	
Do you find it difficult not to smoke in places where you shouldn't?	No	36 (75)	17 (70.8)	20 (66.7)	21 (55.3)	12 (46.2)	16 (48.5)	0.063
	Yes	12 (25)	7 (29.2)	10 (33.3)	17 (44.7)	14 (53.8)	17 (51.5)	
Which cigarette would you most hate to give up; Which cigarette do you treasure the most?	Any other	25 (52.1)	7 (29.2)	5 (16.7)	8 (21.1)	9 (34.6)	9 (27.3)	0.014*
	First one in morning	23 (47.9)	17 (70.8)	25 (83.3)	30 (78.9)	17 (65.4)	24 (72.7)	
How many cigarettes do you smoke each day?	10 or less	42 (87.5)	19 (79.2)	17 (56.7)	21 (55.3)	13 (50)	15 (45.5)	0.002*
	11-20	5 (10.4)	4 (16.7)	11 (36.7)	9 (23.7)	11 (42.3)	16 (48.5)	
	21-30	1 (2.1)	1 (4.2)	2 (6.7)	7 (18.4)	2 (7.7)	2 (6.1)	
	31 or more	0	0	0	1 (2.6)	0	0	
Do you smoke more during the first few hours after waking up than during the rest of the day?	No	30 (62.5)	12 (50)	4 (13.3)	3 (7.9)	4 (15.4)	3 (9.1)	0.001*
	Yes	18 (37.5)	12 (50)	26 (86.7)	35 (92.1)	22 (84.6)	30 (90.9)	
Do you still smoke if you are so sick that you are in bed most of the day or if you have a cold or the flu and have trouble breathing?	No	35 (72.9)	23 (95.8)	26 (86.7)	31 (81.6)	21 (80.8)	22 (66.7)	0.088
	Yes	13 (27.1)	1 (4.2)	4 (13.3)	7 (18.4)	5 (19.2)	11 (33.3)	

*Statistical significance. Chi-square test; P≤0.05 considered statistically significant

Table 2: Differences in nicotine dependence scores based on age

Age group (years)	n	Mean±SD	Mean rank	χ ² statistic	P
≤24	48	3.19±1.72	63.36	45.57	<0.001*
25-34	24	3.46±2.08	70.42		
35-44	30	4.77±1.94	105.53		
45-54	38	5.32±1.94	120.39		
55-64	26	4.92±1.87	113.98		
>64	33	5.79±1.79	135.27		

*Statistical significance. Kruskal–Wallis ANOVA; P≤0.05 considered statistically significant. ANOVA: Analysis of variance, SD: Standard deviation

Table 3: Differences in nicotine dependence scores based on socioeconomic status

Socioeconomic status	n	Mean±SD	Mean rank	χ ² statistic	P
Lower	35	5.71±2.5	128.64	31.05	<0.001*
Lower middle	71	4.7±1.58	106.03		
Middle	63	4.49±1.74	100.25		
Upper middle	30	2.77±2.22	51.78		

*Statistical significance. Kruskal–Wallis ANOVA; P≤0.05 considered statistically significant. ANOVA: Analysis of variance, SD: Standard deviation

The mean age of the participants in this study was comparable to the mean age reported in the studies conducted by Farooq *et al.*^[19] and Evins *et al.*^[20] All the study participants were males which is similar to other tobacco-related studies conducted by Farooq *et al.*,^[19] Park *et al.*,^[21] and Hill *et al.*^[22] While moderate nicotine dependency was identified in the present study, another study conducted by Goyal *et al.*^[23] among participants

attending dental outreach programs reported low nicotine dependency. Similarly, a study conducted by Chhabra *et al.*^[24] among patients attending a teaching dental institution reported low tobacco dependency. The mean FTND scores similar to those observed in this study were reported by Webb *et al.*^[25] and Malhi *et al.*^[26] Nearly, 64% of the study participants reported smoking <10 cigarettes per day in this study, which is in contrast with the study conducted by Raja *et al.*,^[27] where only 10% of the study participants smoked <10 cigarettes per day and another study conducted by Farooq *et al.*,^[19] where 75% of the participants reported smoking <10 cigarettes per day.

Participants from lower socioeconomic status were found to have higher FTND scores in the present study which is similar to the findings reported by Chen *et al.*^[28] and Siahpush *et al.*^[29] However, contrasting findings were observed in the study conducted by Ayo-Yusuf and Omole^[30] among adult smokers in South Africa. The possible reasons for higher dependence among participants from lower socioeconomic strata are low self-efficacy among participants and lack of intention to quit the habit which could be due to the stress arising from their social and financial disadvantages. A significant increase in FTND scores was observed with increasing age in this study. Li *et al.*^[31] reported increased dependence scores among middle-aged adults (45–64 years) compared to younger adults and older people. While the exact mechanism for increasing trend in nicotine dependence with increasing age is not completely discernible, it is postulated that desensitization of nicotine cholinergic receptors as a result of prolonged nicotine exposure with increasing age could be one of the primary reasons for this phenomenon.^[32] Furthermore, it is hypothesized that younger individuals are not as vulnerable as their older counterparts in responding to the psychological cues of the urge to smoke.^[33] The limitations of the present



the study results demonstrate that there is moderate tobacco dependency among cigarette smoking participants availing oral health care at dental institutions. Future studies can focus on obtaining data relating to dependency on smokeless tobacco in tandem with cigarette smoking.

CONCLUSION

The findings of this study provide an insight into the fact that there is moderate nicotine dependence among cigarette smokers availing care at oral health facilities, which is high among lower socioeconomic strata and older age groups. Therefore, the establishment of tobacco cessation centers in teaching dental institutions as directed by MOHFW and DCI, and making committed efforts toward drafting and delivering customized tobacco cessation strategies could be very effective.

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Conflicts of interest

There are no conflicts of interest.

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Evidence of improved bond strength of resin-based sealer with the use of natural antioxidants on hypochlorite treated dentin: an *in vitro* study

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Abstract

Aim. To evaluate the effect of natural antioxidants as final irrigants on the push-out bond strength of epoxy resin-based sealer to the hypochlorite treated dentin.

Methods. Eighty single-rooted human mandibular incisors were prepared using ProTaper Gold (Dentsply, TN, USA) and an irrigation protocol including 3% NaOCl, followed by ethylenediaminetetraacetic acid (EDTA), and 3% NaOCl. The groups (n =20) were divided according to the final irrigant used: Group 1: None (Control); Group 2: 5% sodium ascorbate, Group 3: 5% grape seed extract (GSE); and Group 4: 5% pine bark extract. The obturation of root canals was performed using gutta-percha and AH Plus (Dentsply DeTrey, Germany). Bond strength was evaluated using the push-out test under the universal testing machine at a crosshead speed of 0.5 mm/min, and statistical analyses were performed using one-way ANOVA. The p-value significance was evaluated by Tukey's post hoc test (p<0.01).

Results. Mean push-out bond strength values were compared in all the groups, and there was a statistically significant improvement in the experimental final irrigating groups. 5% pine bark extract had improved bond strength than the other groups, and the least bond strength was observed in the control group.

Conclusion. The irrigation protocols and naturally derived antioxidants affected the resin-based sealer's bond strength to root dentin. It was observed that the use of antioxidants effectively reversed the compromised bond strength of resin-based sealers to root dentin. 5% pine bark extract application showed better bond strength.

Keywords: antioxidant, epoxy resin-based sealers, grape seed extract, pine bark extract, sodium ascorbate

Introduction

Endodontic therapy success depends on the canal disinfection using proper irrigation solutions and subsequent fluid-tight seal between the obturation material and the root dentin. AH Plus is a hydrophobic, epoxy resin-based sealer that is widely used because of its physical properties [1]. The adhesive property of AH Plus to root dentin depends on covalent bonding between the exposed side-chain amine groups and the open

epoxide ring of the collagen network [2]. Dentin surface treated with different irrigation protocols predominantly causes alteration to dentin's collagen fibrils; this may compromise endodontic sealers' adhesiveness to the dentin surfaces [3].

AH Plus can bond to the organic components, principally the collagen network, of root dentin. An irrigation protocol using NaOCl as the final irrigant significantly reduced the bond strength of AH Plus to root dentin (Neelakantan et

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al.) [4]. Many other studies have demonstrated that the commonly used irrigating solution NaOCl reduces the bond strength between adhesive materials and dentin [5]. Moreover, it potentially reduces the bond strength of the AH Plus sealer. This may be attributed to residual oxygen species' presence on the dentin, which affects the adhesive material's setting time [6].

The compromised bond strength of NaOCl-treated dentin might be restored by applying antioxidants before the adhesive procedure [7]. These agents interact with the by-products of NaOCl, resulting in the reversal of the oxidizing effects of NaOCl and neutralization of the dentin surface [5-7].

However, Erhardt et al. [8] reported that some materials benefited from its protective effects, whereas others yielded negligible results. Naturally derived oxidizing agents can chemically modify collagen without damaging biological tissues and improve dentin matrix properties [9].

The aim of the study is to evaluate the effect of 5% sodium ascorbate (SA), 5% grape seed extract (GSE), and 5% pine bark extract (PBE) on the push-out bond strength of epoxy resin sealer to hypochlorite treated root dentin. The null hypothesis is that antioxidants, as a final irrigant, would not reverse the compromised bond strength of epoxy resin-based sealer and root dentin caused by NaOCl.

Methods

Specimen preparation

After obtaining ethical clearance from the institutional Review Board (238/IRB/SIBAR/2020), eighty non-carious permanent human mandibular incisors extracted due to periodontal reasons with fully developed root apices were selected. All the samples were investigated for the root canal morphology, and teeth with a single canal were included in the study. Each sample was decoronated at the level of cemento-enamel junction perpendicular to the tooth's long axis using a diamond disc at a low speed along with water coolant. The root lengths were standardized to 15 mm. Working length (WL) was established with a size 10 K-file and was set at 1 mm from the apex. A #25 K-file was used to standardize the foramina size. The roots were mounted on the self-cure acrylic resin.

Root canal preparation

ProTaper gold instruments (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA) were used in a 16:1 gear handpiece and torque-controlled electric motor (X-Smart Plus; Dentsply). According to the manufacturer's instructions, a consistent rotation speed of 300 rpm was used in a crown-down manner involving a gentle in-and-out motion. Initially, the orifice was enlarged using orifice opener SX. A shaping file (S1) was passed apically within 2 mm of the working length. Later S1 and S2 files were used till the full working length.

The finishing of the apical one third was done by passing the F1 and F2 till the entire working length was reached. Pecking motion was used for instrumentation, and flutes were regularly cleaned to remove debris.

Irrigation protocol

The irrigation protocols used for all the groups were:

1. 2 ml of 3% NaOCl (prime dental products Pvt Ltd, Maharashtra, India) after each instrument change.
2. Irrigation using 2 ml of 17% EDTA (MD-Cleanser, META Biomed, Korea) in all groups.
3. 1 ml of 0.9% of saline solution.
4. Irrigation for 3 minutes using 5ml of NaOCl and experimental antioxidant solutions as final irrigation.

The root samples were divided into four groups ($n = 20$ each) according to the final irrigation solution.

Group 1: None (Control).

Group 2: 5 ml of 5% sodium ascorbate solution.

Group 3: 5 ml of 5% of grape seed extract solution.

Group 4: 5 ml of 5% of pine bark extract solution.

Preparation of solutions

1.5% sodium ascorbate solution.

5 g of sodium ascorbate (All pure organics, India) was dissolved in 100 ml of distilled water to make a 5% sodium ascorbate solution.

2.5% grape seed extract solution.

5 g of grape seed extract (All pure organics, India) was dissolved in 100 ml of distilled water to make a 5% grape seed extract solution.

3.5% pine bark extract solution.

5 g of pine bark extract (All pure organics, India) was dissolved in 100 ml of distilled water to make a 5% pine bark extract solution.

Root filling procedures

The samples were dried using 25 size paper points (Dentsply Konstanz, Germany). After root canal preparation, all samples were obturated using the single cone technique with 25/.06 gutta-percha points (Dentsply Konstanz, Germany) and AH Plus sealer (Dentsply DeTrey, Konstanz, Germany) according to the manufacturer's instructions. The obturating material was allowed to set for 2 weeks.

Push-out test

A 2 mm slice was obtained from the middle third of the root using a microtome from each root. The push-out test was evaluated by applying a load at 0.5 mm/min, which was applied in an apical to coronal direction until the root filling was dislodged from the root slice. The push-out bond strength was measured under a universal testing machine (Instron Corp, Norwood, MA, USA).

Statistical analysis

The obtained results were statistically analyzed using computer software SPSS version 21.0. One-way analysis of variance (ANOVA) followed by Mann Tukey's Post hoc test was used to analyze the data. Significance was established at $p < 0.01$ level.

Table I. Comparison of mean push-out bond strength of different groups (MPa) using a one-way ANOVA test and Tukey's post hoc test.

Group	N	Minimum	Maximum	Mean	SD	Comparison	p-value
Group-1 (Control)	20	18.809	44.666	31.761	8.334	G-1 VS G-3, G-4	p<0.01*
Group-2 (SA)	20	32.142	45.762	41.116	4.203	G-2 VS G-1	p<0.05*
Group-3 (GSE)	20	31.843	54.456	42.251	8.077	G-3 VS G-4	p<0.05*
Group-4 (PBE)	20	41.559	60.929	51.572	6.452	G-4 VS G-2	p<0.01*

* P- value significant, SD- Standard deviation, N- no of samples.

Results

The one-way ANOVA revealed that the push-out bond strength was significantly affected by antioxidants as final irrigating solutions ($p<0.01$). The mean push-out bond strength values, standard deviation, and p-value significance are shown in table I and figure 1.

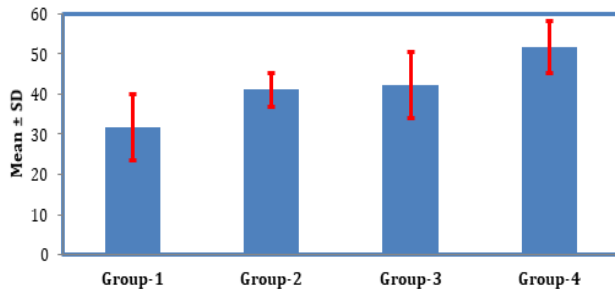


Figure 1. Graph representing the comparison of mean push-out bond strength among the groups (MPa).

When comparing the groups, Group 4 pine bark extract showed a statistically significant highest push-out bond strength compared to all other groups. The mean push-out bond strength value of Group 3 was statistically significant with that of Group 4 ($p<0.05$) and Group 1 ($p<0.01$) but not with that of Group 2 ($p>0.05$). The mean push-out bond strength value of Group 2 was statistically significant with that of Group 4 ($p<0.01$) and Group 1 ($p<0.05$) but not with that of Group 3 ($p>0.05$). Group 1 showed the least mean push-out bond strength values than all other groups and was statistically significant ($p<0.01$).

Discussion

Failure in the endodontic therapy occurs due to inadequate fluid-tight seal between the obturating material and the root dentin. Microleakage occurs due to bond failure between the epoxy resin-based sealer and the root dentin. Several studies described the adverse effects of NaOCl on dentin, which includes promoting structural changes in organic dentin components (mainly collagen) [10], and its impact on the mechanical properties, such as reducing the flexural strength and elastic modulus of dentin [11]. Similar reduction in the AH Plus sealer bond strength was

observed in Group 1 of the present study, which may be attributable to the release of residual oxygen species from NaOCl on the root dentin [6]. To overcome this problem, different techniques have been proposed to treat root dentin [12]. Antioxidants such as sodium ascorbate (SA) and oligomeric proanthocyanidin complexes (OPC) have been demonstrated to reverse oxidants' adverse effects and preserve resin-dentin bond integrity.

SA is a biocompatible and nontoxic antioxidant (salt of ascorbic acid-vitamin C) able to reduce a wide variety of oxidizing compounds, mainly free radicals [13]. In the present study, SA (Group 2) showed statistical significance compared to the control group. SA solution reduces the residual free radicals from the interior of the dentinal tubules [14] and dentin matrix [15]. SA reverses the denaturing effect of acid etching, NaOCl, or H_2O_2 on dentin collagen. It is a potent inhibitor of matrix metalloproteinases (MMPs). Hence, it protects against the long-term degradation of the resin-based materials and dentin interface [16]. Khoroushi et al. [17] also indicated that the treatment with SA solution improved the bond strength of fiber posts to dentin.

Group 2 specimens could not reverse the push-out bond strength as much as that of Group 4, which was in accordance with the studies that confirmed the antioxidant potential and the free radical scavenging ability of OPCs are 20 times greater than those of vitamin E and 50 times greater than those of vitamin C (Fine AM [18] and Stokes et al. [19]).

OPCs exhibited 78-81% inhibition of superoxide anion and hydroxyl radical. Under similar conditions, vitamin C inhibited these two oxygen free radicals by approximately 12-19%, while Vitamin E inhibited the two radicals by 36-44% (Bagchi et al. [20]). OPCs present in natural antioxidants like grape seed extract and pine bark extract have free radical scavenging activity [21]. OPCs are a class of polyphenolic bioflavonoids most commonly found in fruits and vegetables, free radical scavenging and antioxidant activity. They also have antibacterial, antiviral, anti-inflammatory, antiallergic, anticarcinogenic, and vasodilatory actions [18].

Grape seed extract consists of OPCs in the form of monomeric phenolic compounds such as catechin, epicatechin, and epicatechin-3-O-gallate and free flavanols

monomers [22]. Pine bark extract consists of phenolic compounds generally divided into monomers like catechin, epicatechin, taxifolin, and compacted flavonoids like oligomeric to polymeric proanthocyanidins [23]. Group 4 showed a statistically higher significant mean POBS value than Group 3. The difference in the antioxidant activity might be attributed to their different phenolic compositions, which is in accordance with a study conducted by Subramonian et al. [24]. OPCs (grape seed extract and pine bark extract) reacts with free radicals (e.g., oxygen) generated by the degradation of NaOCl, thereby neutralizing them within the dentin in which they are trapped. Oligomeric proanthocyanidin complex (OPC) contains multiple electron donor sites (hydroxyl sites) that bind to unstable molecules called free radicals by donating its hydrogen atoms [18]. The presence of gallic acid also increases the free radical scavenging activity by esterification of epicatechin [25].

Antioxidants can return the oxidized dentin substrate's redox potential, thereby facilitating the usual setting of the adhesive materials [26]. Furthermore, bioactive compounds derived from OPC (including GSE and PBE) improved the dentin matrix's mechanical properties, impaired biodegradation. It inhibited the action of proteases associated with extracellular matrix breakdown [9]. The interaction between OPC and collagen fibrils involves the formation of complexes that are predominantly stabilized by hydrogen bonding between carbonyl and hydroxyl functional groups of phenols and amide linkages [27].

The treatment of root dentin with OPC could reduce the demineralized dentin matrix's biodegradability and increase the durability between the resin-based sealer and root dentin. OPC rich extracts can also inhibit the activities of MMP and cysteine cathepsins to a greater extent [28]. Besides, the anti-inflammatory and antibacterial properties of OPC are favorable characteristics that could be beneficial in root canal therapies [29].

Limitations

As it was an *in vitro* study, the direct estimation of these results to the clinical application requires further *in vivo* studies.

Conclusion

Within the limitations of this *in vitro* study, it can be concluded that the use of antioxidants as final irrigating solutions can reverse the reduced bond strength of epoxy resin-based sealers to root dentin. The use of 5% pine bark extracts significantly increases epoxy resin-based sealers' bond strength to root dentin compared to no antioxidant therapy.

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Assessment of postoperative pain after single-visit root canal treatment using rotary and reciprocating file systems: an *in vivo* study

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Background: Various instrument kinematics used in single-visit endodontics influence the occurrence of pain after endodontic therapy. This study aimed to evaluate the occurrence of pain after mechanical instrumentation with Hyflex EDM (HEDM) and WaveOne Gold (WOG) during single-visit endodontic therapy.

Methods: Sixty patients diagnosed with asymptomatic irreversible pulpitis and normal apical tissues in mandibular premolar teeth were included in the study for single-visit root canal therapy. The patients were divided into two groups (n = 30) according to the rotary instrument used during root canal preparation (group A [HEDM] and group B [WOG]). Pain was evaluated after endodontic therapy at 8, 24, and 48 h intervals using the visual analog scale (VAS). Data obtained were analyzed using the chi-square test, independent t-test, MannWhitney U test, and Wilcoxon matched-pairs test.

Results: Statistically significant differences were observed between the two groups (P < 0.001) at 8, 24, and 48 h, with WOG exhibiting less pain than HEDM files.

Conclusion: Postoperative pain was lower in the WOG file system than in the HEDM file system after single-visit root canal therapy at 8, 24, and 48 h.

Keywords: Hyflex EDM; Postoperative Pain; Reciprocation; Root Canal Therapy; Rotary Instrumentation; WaveOne Gold.



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INTRODUCTION

Pain after endodontic therapy is a routine complication, reported to be 1.4-16% [1-3]. Post-endodontic pain is multifactorial [4]. One underlying cause is debris extrusion during chemomechanical preparation [5-7]. Proper irrigation protocols and aspiration techniques are used to limit instrumentation to the canal's confines, and the extrusion of debris can be minimized using different endodontic files with appropriate kinematics [8,9].

Current mechanical preparation of root canals uses rotary nickel-titanium (NiTi) instruments that employ either of the two kinematics (rotation or reciprocation). Rotary single-file systems, such as Hyflex EDM (HEDM), are produced by a distinctive process called “electric discharging machining.” This technique uses spark erosion, which amplifies the fracture resistance and cutting efficiency [10]. The cross-section of the file varies along the length of the file, with triangular, trapezoidal, and quadratic shapes in the coronal third, middle third, and apical third, respectively [10,11]. WaveOne Gold

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(WOG) files are reciprocating files based on M-wire alloy technology with a cross-section of an off-centered parallelogram. This thermal process alters the molecular geometry to increase the cyclic fatigue resistance and improve flexibility [12].

Systematic reviews of postoperative pain when rotary and reciprocating systems were compared after single-visit endodontic therapy have shown different results. Systematic reviews by Hou et al. and Sun et al. stated that rotary instruments result in less postoperative pain, while Martins et al. concluded that reciprocating instruments lead to less pain [13-15]. A systematic review by Spohr et al. stated that no clear conclusion could be drawn regarding the incidence of postoperative pain following the use of rotary and reciprocating instruments, and further well-designed studies are needed in this regard [16]. At this time, the current *in vivo* study was undertaken to assess episodes of pain following endodontic therapy using HEDM (rotary system) and WOG (reciprocating system). The null hypothesis was that there would be no significant difference in the severity of pain following the use of the HEDM and WOG instrumentation systems.

METHODS

This *in vivo* study was approved by the Institutional Ethics Committee (IEC) (14/IEC-SIBAR/CIR/18). Study participants were recruited from the Department of Conservative Dentistry and Endodontics between November 2018 and December 2019. The sample size calculation was performed using G* power 3.1.2. Sixty volunteer patients who met the inclusion criteria were randomized using the sequentially numbered, opaque, sealed envelope method and categorized into two groups of 30 each; group A (HEDM) and group B (WOG) by an endodontist who was blinded to the study protocols. The inclusion criteria were as follows: Patients aged 18-40 years, with single-rooted mandibular premolars diagnosed with asymptomatic irreversible pulpitis with

normal periapical tissue and patients who could understand the use of the pain scale.

The exclusion criteria were as follows: Patients with acute and chronic apical abscess or cellulitis; known allergies to opioids, non-opioids, NSAIDs, analgesics, lidocaine; pregnant or lactating mothers; teeth with complex root canal morphology; teeth with poor prognosis; patients with systemic diseases; unwilling to participate in the study and those receiving premedication with analgesics; and patients with active pain in other than the tooth to be tested.

A thorough treatment protocol was briefed to the participants and informed consent was obtained from all participants. Based on the group assigned to the sealed envelope paper, the respective treatments were performed as described below. The study protocol is shown in Fig. 1.

1. Treatment protocol

The entire clinical procedure was performed by a single surgeon. Local anesthesia was administered with 2% lignocaine (1:80,000 epinephrine) (Astra Zeneca Pharma India Limited, Bangalore, India), followed by reduction in occlusal. After the rubber dam application and access opening, a #10 stainless steel hand K-file was used to establish the glide path. The working length was obtained using the Root ZX apex locator (J. Morita, Kyoto, Japan) and was confirmed using an intraoral periapical radiograph. Mechanical preparation was performed up to size 20 using a stainless steel hand K-file.

Group A (n = 30): The canals were instrumented with HyflexEDM (25/~) (COLTENE/Whaledent AG, Switzerland) NiTi file with a gentle apical stroke and pecking movement with Endomotor X SMART[®] Plus (DentsplyMaillefer, Ballaigues, Switzerland) according to the manufacturer's instructions at 500 rpm and 2.5 Ncm torque.

Group B (n = 30): Mechanical instrumentation with WaveOne Gold primary file (25/0.07) (DENTSPLY Tulsa Dental Specialties, Tulsa, USA) with pecking in and out motion with Endomotor X SMART[®] Plus

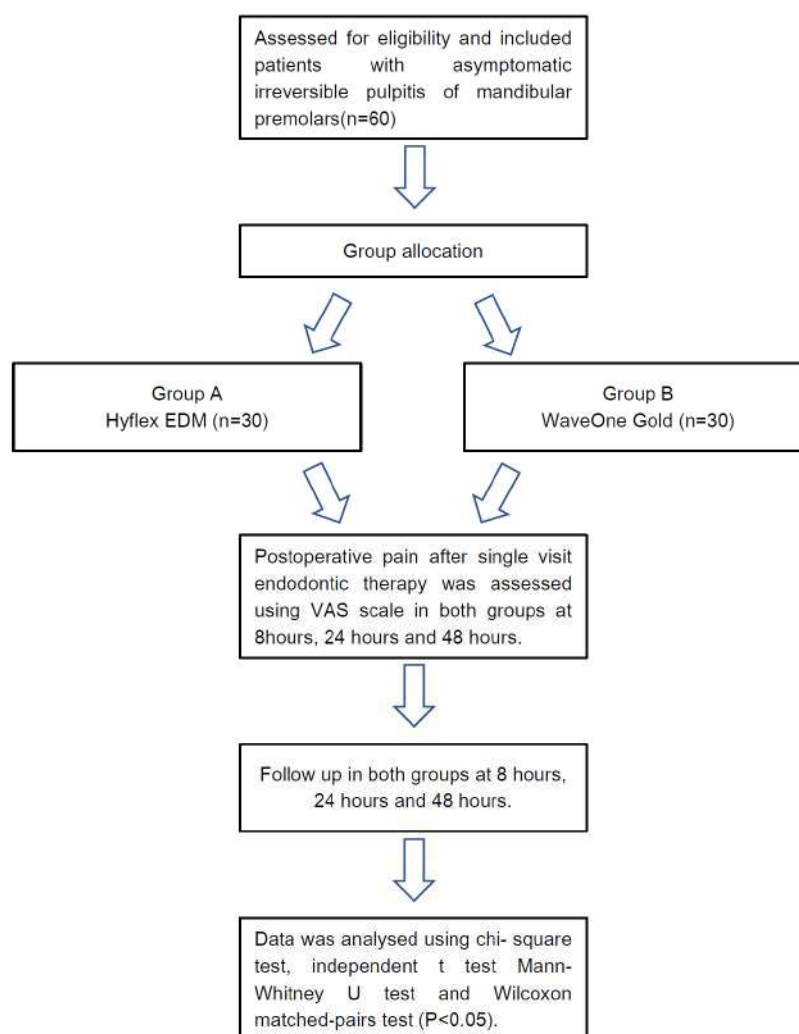


Fig. 1. Flowchart of the study.

(DentsplyMaillefer, Ballaigues, Switzerland) according to the manufacturer's instructions using WOG mode.

In both groups, irrigation was performed intermittently with a 30G side vented needle kept short of working length by 1 mm in the following sequence: 5 ml of 5.25% NaOCl followed by saline (NS, sodium chloride injection, 0.9% W/V) and followed by 5 ml of 17% EDTA solution. With each irrigation flush of NaOCl and EDTA, sonic agitation of the irrigant was performed using an EndoActivator (EA-A0913 022-025 Medium tip Dentsply Sirona, India) for 1 min. Distilled water (10 ml) was used as the final irrigant. Absorbent paper points were used to dry the canals, and the canals were obturated using the corresponding master gutta-percha cone, accessory cones, and a resin sealer (AH Plus, DentsplyMaillefer,

Ballaigues, Switzerland) using the cold lateral compaction technique. A nanohybrid composite resin (Filtek Z250 XT, 3M ESPE, Saint Paul, MN, USA) was used to seal the access openings. The patient's occlusion was evaluated to ensure that the restoration did not interfere with the occlusion. Representative intraoral X-rays of both groups are shown in Figs. 2 and 3.

A visual analog scale (VAS) was used to measure pain after endodontic therapy. Based on this scale, the pain level was numerically documented in the range of 0-100. The following VAS classifications were used: no pain, 0-24; mild pain, 25-49; moderate pain, 50-74; severe pain, 75-100. Pain scoring based on the VAS questionnaire was recorded at 8, 24, and 48 h by telephone inquiry. All patients were prescribed 400 mg of ibuprofen and asked

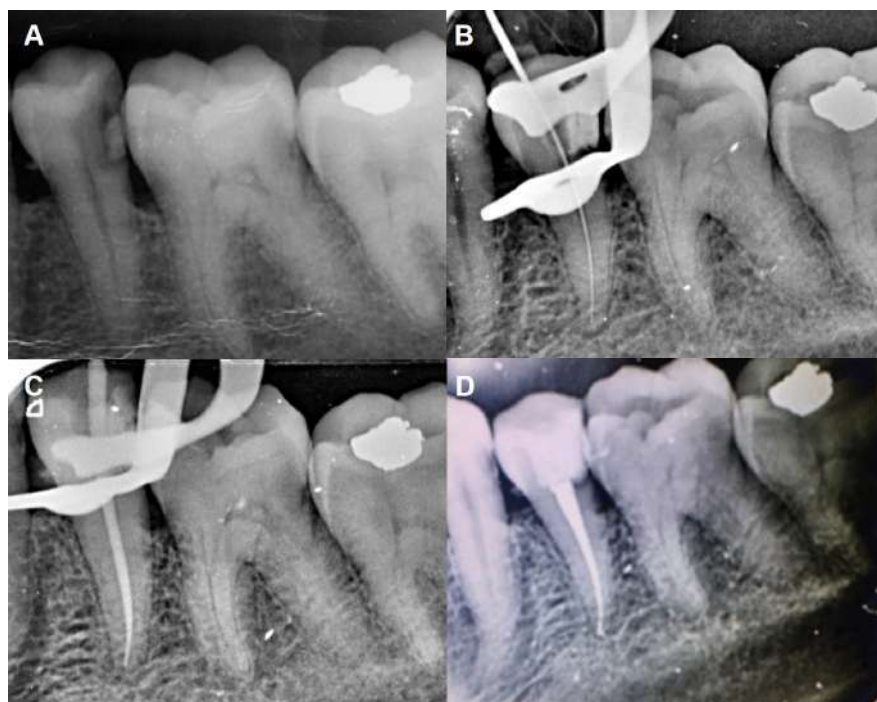


Fig. 2. Representative Intraoral Periapical radiographs of Hyflex EDM group. (A) Preoperative radiograph, (B) Working length determination, (C) Mastercone selection, (D) Postoperative radiograph.

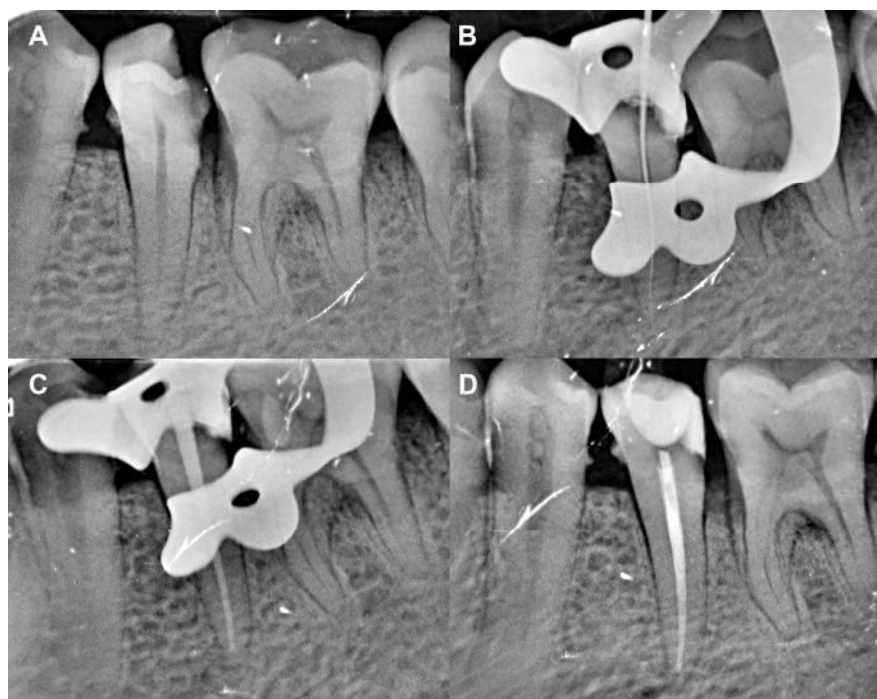


Fig. 3. Representative Intraoral Periapical radiographs of WaveOne Gold group. (A) Preoperative radiograph, (B) Working length determination, (C) Mastercone selection, (D) Postoperative radiograph.

to take it only for unbearable pain. They were advised not to take medications without the knowledge of the

investigator. The researcher conducted the telephone interview and performed the data entry, and the

Table 1. Comparison between group A (Hyflex EDM) and group B (WaveOne Gold) regarding sex

Gender	Group A	%	Group B	%	Total	%
Male	7	23.33	6	20.00	13	21.67
Female	23	76.67	24	80.00	47	78.33
Total	30	100.00	30	100.00	60	100.00

Chi-square = 0.0980, P = 0.7540

Independent t-test, P < 0.05 is considered statistically significant.

Table 2. Comparison between group A (Hyflex EDM) and group B (WaveOne Gold) regarding mean age

Groups	Mean	SD	SE	t-value	P-value
Group A	34.43	6.26	1.14	0.5398	0.5914
Group B	33.57	6.18	1.13		

Independent t-test, P < 0.05 is considered statistically significant. SD, standard deviation; SE, standard error.

Table 3. Comparison between group A (Hyflex EDM) and group B (WaveOne Gold) regarding tooth type

Tooth number	Group A	%	Group B	%	Total	%
34	3	10.00	3	10.00	6	10.00
35	10	33.33	12	40.00	22	36.67
44	1	3.33	1	3.33	2	3.33
45	16	53.33	14	46.67	30	50.00
Total	30	100.00	30	100.00	60	100.00

Chi-square = 0.3152, P = 0.9570.

Table 4. Comparison between group A (Hyflex EDM) and group B (WaveOne Gold) on postoperative pain using a 0-100 visual analog scale at 8, 24, and 48 h

Treatment time points	Group	N	Mean	SD	Mean rank	U value	Z value	P Value
8 hours	A	30	33.23	14.090	42.00	105	5.113	< 0.001*
	B	30	10.73	9.752	19.00			
24 hours	A	30	26.70	12.140	45.50	.000	6.69	< 0.001*
	B	30	3.03	2.606	15.50			
48 hours	A	30	23.73	8.212	45.50	.000	6.8	< 0.001*
	B	30	1.07	1.574	15.50			

*P < 0.05 is considered statistically significant. N, number; SD, standard deviation.

statistician was blinded to the type of intervention performed. The patients were recalled for definitive restoration after 2 weeks.

2. Statistical analysis

The data were entered into an Excel sheet and analyzed using SPSS version 16.0 (IBM Corporation, India). Data were analyzed using the chi-square test, independent t-test, Mann-Whitney U test, and Wilcoxon signed-rank test. The level of statistical significance was set at 0.05 (P < 0.05).

RESULTS

The sample distribution was revealed to be similar without any statistically significant differences between the two groups regarding sex, age, and tooth type (Tables 1, 2, and 3).

Table 4 shows the comparison of postoperative pain between group A (HEDM) and group B (WOG) at different time intervals (8, 24, and 48 h) using the Mann-Whitney U test. The results showed statistically significant differences between the two groups (P <

Table 5. Comparison of different treatment time points on postoperative pain in group A (Hyflex EDM)

Treatment time points	N	Mean	SD	Mean difference	Z-value	P-Value
8 hours	30	33.23	14.090	6.53	2.265	0.024*
24 hours	30	26.70	12.140			
8 hours	30	33.23	14.090	9.5	3.52	< 0.001*
48 hours	30	23.73	8.212			
24 hours	30	26.70	12.140	2.97	0.773	0.44
48 hours	30	23.73	8.212			

*P < 0.05 is considered statistically significant. N, number; SD, standard deviation.

Table 6. Comparison of different treatment time points on postoperative pain in group B (WaveOne Gold)

Treatment time points	N	Mean	SD	Mean difference	Z-value	P-Value
8 hours	30	10.73	9.752	7.70	3.458	0.001*
24 hours	30	3.03	2.606			
8 hours	30	10.73	9.752	9.66	4.214	< 0.001*
48 hours	30	1.07	1.574			
24 hours	30	3.03	2.606	1.96	3.057	0.002*
48 hours	30	1.07	1.574			

*P < 0.05 is considered statistically significant. N, number; SD, standard deviation.

0.001) at 8, 24, and 48 h.

Table 5 shows the comparison of different treatment time points with postoperative pain in group A (HEDM) using the Wilcoxon signed-rank test, which showed a gradual reduction in pain intensity with an increase in follow-up time. Group A (HEDM) was shown to have a statistically significant difference between 8 and 24 h ($P = 0.024$) and between 8 and 48 h ($P < 0.001$), but the comparison between 24 and 48 h did not show a significant difference ($P = 0.44$).

Table 6 shows a comparison of different treatment time points with postoperative pain in group B (WOG) using the Wilcoxon signed-rank test, which showed a reduction in postoperative pain with an increase in follow-up time. Group B (WOG) showed a statistically significant difference between 8 and 24 h ($P = 0.001$), 8 and 48 h ($P < 0.001$), and 24 and 48 h ($P = 0.002$).

DISCUSSION

Efficient chemomechanical preparation, three-dimensional filling of root canals, and the degree of postoperative discomfort will influence the success of

endodontic therapy. The subjective nature of pain is difficult to evaluate. Therefore, participants in this study received an adequate description of postoperative pain and VAS scores. This scale is considered a decisive and valid method for assessing pain [2,17].

Most participants interpreted the VAS scale smoothly and estimated their pain intensity appropriately. The two groups were comparable in terms of age, sex, tooth type, and pulpal and periapical status. In this study, a single operator performed endodontic therapy for all participants during a single visit to control the technique and operator-related variables. The only difference was the rotary files used for the mechanical preparation between the two groups.

The file systems used were similar in size to maintain standardization and rule out the effect of varying tapers on postoperative pain [18,19]. Both systems differ in kinematics. Previous studies have reported that postoperative pain is significantly influenced by preoperative pain and periapical status [4]. Therefore, this study admitted teeth with asymptomatic irreversible pulpitis with normal periapical status to evaluate pain after the completion of endodontic therapy.

Single-visit endodontic procedures are preferred to

minimize the effects of related variables. Su et al. reported that the occurrence of pain after endodontic therapy in a single visit was minimal compared with multiple-visit endodontic treatment [20].

In this study, an activator was used in both groups to agitate NaOCl and EDTA for 1 min. EndoActivators can break bacterial biofilms by generating bubbles that expand and collapse, producing miniature shockwaves, thus reducing bacterial stagnation. A study by Vishwakarma et al. reported an improved efficiency in reducing postoperative pain at 8, 24, and 48 h in the EndoActivator group compared to the group with an open-ended needle [21]. Ramamoorthi et al. also found that the EndoActivator group experienced significantly less postoperative pain than the conventional needle protocol at 8, 24, and 48 h [22]. Therefore, activation of irrigants using an EndoActivator can be considered an effective method of reducing postoperative pain.

In the present study, the cold lateral compaction technique was used, as this technique has been shown to cause less postoperative pain than the warm compaction technique [23]. The teeth of both groups were relieved of occlusal contact, as trauma from occlusion would impact the results.

The results of this study revealed significantly less pain in the WOG group than in the HEDM group at 8 h, which was subsequently reduced at 24 h and 48 h. When comparing the VAS between HEDM and WOG in three different time slots, it was found that WOG had higher success than HEDM among participants in terms of the least post-endodontic pain. Therefore, this study showed that WOG was better at minimizing postoperative pain than HEDM.

A systematic review by Pak et al. stated that, during the initial phase, pain after single-visit endodontic treatment was found to be preponderant. According to this review, the mean posttreatment pain severity was greater at 24 h. After 7 days, the severity of the pain gradually reduced [4]. In the present study, the incidence of pain tracked a similar curve, which was greater at 8 h in both groups, followed by a significant reduction in

pain at 24 and 48 h.

A routine prescription of analgesics was not provided in this study, as this would have affected the outcome. Analgesics were advised only on demand. None of the patients in this study required the use of analgesics, as none of them experienced severe pain.

Differences in cleaning and shaping procedures, immune response to extruded root canal debris, irrigants, instrumentation beyond the apex, or foreign body reactions to obturating materials have been reported to cause postoperative pain [19,20]. Therefore, in this study, great care was taken to avoid the influence of these factors.

Superior results were observed for the WOG system compared to the HEDM system. Similarly, the WOG system showed better results than the Protaper system in a study by Saha et al. [24].

The reasons for the lower postoperative pain in the WOG group could be attributed to the following:

1. The WOG has fixed and progressively decreased tapers in D1-D3 and D4-D16, respectively, conserving dentin and maintaining space for more debris to collect and push coronally [25].
2. The off-centric design of WOG with one point of contact with the canal wall results in adequate space for debris collection and removal [26,27].
3. HEDM uses continuous rotational motion with a variable cross-section design, reducing the space for debris clearance [28].

There are various etiological factors for post-endodontic pain, of which the amount of apically extruded debris is the most contributing factor [29,30]. Rotary files have also been shown to cause a greater accumulation of proinflammatory mediators at the periapex than reciprocating files. This could also have contributed to higher pain scores in the HEDM group. The limitations of this study include the sample size and the subjective nature of pain, which are difficult to quantify. The use of hand instruments before the rotary and reciprocating instruments could also be attributed to debris extrusion, resulting in pain. Therefore, more large-scale studies are needed to compare this two

kinematics, focusing on their limitations.

In conclusion, pain after single-visit endodontic treatment was less in patients treated with the WOG system than with the HEDM system. Higher pain scores were observed at 8 h, which gradually decreased in intensity after 24 and 48 h. Since the present *in vivo* study revealed the influence of geometric characteristics of the files on postoperative pain following single-visit endodontic therapy, the findings may help in clinical decision-making in selecting the NiTi instruments and providing a pain-free outcome.

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Effect of the different chromatic filters of the dental operating microscope on the volumetric shrinkage, surface hardness, and depth of cure of bulk fill composite

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Abstract

Aim. Evaluate the effect of different chromatic filters of the dental operating microscope on the volumetric shrinkage, surface micro-hardness, and depth of cure of bulk-fill composite.

Methods. Bulk fill composite specimens of 4 mm depth, 4 mm width, and 4 mm length were prepared. Five groups were designed based on a light source under which composite samples were condensed. Group 1: dental chair light without filter, Group 2: microscopic light without filter, Group 3: microscopic light with a yellow filter, Group 4: microscopic light with a green filter, Group 5: dental chair light with a red filter. After condensation, the samples were subjected to curing using Bluephase NM curing light. The parameters like volumetric shrinkage, surface hardness, and depth of cure were measured for all the samples. The results were subjected to statistical analysis using one-way ANOVA, followed by post-hoc Tukey tests.

Results. Group-2 showed the highest mean volumetric shrinkage (14.514%), surface micro-hardness (58.065 kg/m²), depth of cure (0.831%), whereas group-5 showed the least volumetric shrinkage (7.386%), surface micro-hardness (46.536 kg/m²), and depth of cure (0.789%). Working time was 40 seconds for group-2, whereas the remaining groups were allowed to complete 1-minute condensation. A statistically significant difference was shown between groups 1 and 5 (p=0.050), 2 and 5 (p=0.007) for volumetric shrinkage. Regarding surface micro-hardness, a statistically significant difference was observed between all the groups (p=0.001) except group-3, group-4 (p=0.100). There was no statistically significant difference between all the groups except group 2 and 5 for depth of cure (p=0.016).

Conclusion. Microscope light without filter showed the highest surface hardness and depth of cure. However, the highest volumetric shrinkage and lesser working time were also observed, which are undesirable. Use of filters during composite manipulation showed less detrimental effects on depth of cure, volumetric shrinkage and working time. Overall, composite condensed under filters showed acceptable properties.

Keywords: bulk fill composite, intensity, microscopic light filters, surface hardness, volumetric shrinkage

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Introduction

Today's dentistry is more focused on the attention to detail. Micro restorative dentistry is an emerging trend in this perspective. Many anterior as well as posterior esthetic restorations, warrant the use of magnification [1]. Dental Operating microscopes (DOM) are provided with different chromatic filters for versatile applications. Literature suggests that the mechanical properties of composite resin can be influenced by different light source [2,3]. There is a linear relationship between light intensity and polymerization shrinkage, i.e., the higher the intensity of light, the higher the polymerization shrinkage [4-6]. Also, the degree of conversion (DC) of composite resin can determine the mechanical properties, chemical stability, and longevity of a restoration. Efficient polymerization is important for obtaining the optimal physical properties of the material and achieving improved clinical performance of resin composite restorations [7]. The hardness of the light-cured resin composites depends on several factors: the composition of the organic matrix, the type and amount of filler particles, and the degree of polymerization. An examination of the resin composite micro-hardness was used to evaluate the rate of polymerization [8,9].

While the relative degree of cure of the external surface of a restoration can usually be evaluated with simple techniques, the cure of the inner layers of resin is not similarly accessible to evaluation. It was recognized that, unlike chemically activated resins, an adequate cure of the entire visible light activated restoration cannot be assumed, based on external surface properties [10]. It has been shown that inadequate polymerization would result in a reduction in physical properties [11]. A number of different techniques have been employed to measure the properties of the polymerized resin composite which include scraping away the unset material and measuring the remaining specimen, measuring top and bottom hardness and measuring top and bottom degree of conversion of double bonds in the polymer [12,13]. Micro-hardness (MH) is an indirect measure of a material's depth of cure (DOC). It provides valuable information on the DOC when measured on the top and bottom surfaces of a specimen. The depth at which a composite resin achieves 80% of its surface hardness is generally considered the maximum depth at which the composite should be used [14,15].

Despite advances in the composite resin itself, compensation for volumetric shrinkage (VS), post-cure density, and surface hardness are the considerable challenges that play a significant role in durability. It is claimed that bulk-fill composites can obtain an optimal degree of conversion even at the bottom of the cavities [16,17]. But little is known about the effect of microscope filters on the properties of the bulk-fill composite. The study aims to evaluate the effect of microscopic filters on the surface hardness, depth of cure, volumetric shrinkage of the bulk-fill composite.

Methods

Forty molds of 4 mm width, 4 mm length, 4 mm depth were used to condense the Tetric-N-Ceram bulk fill (IvoclarVivadent, shade A2) composite. Manipulation of composite specimens was carried under respective microscopic filters (Magna, Labomed) in a dark environment to prevent the deleterious effects caused by the daylight and then cured with Bluephase NM curing light (Ivoclar, Vivadent).

Based on the type of light source under which composite specimens were prepared, five groups were designed.

Group 1: composite condensed under dental chair light.

Group 2: composite condensed under microscopic light without the filter (Figure 1).

Group 3: composite condensed under microscopic light with a yellow filter (Figure 2).

Group 4: composite condensed under microscopic light with green filter (Figure 3).

Group 5: composite condensed under dental chair light with a red filter (Figure 4).

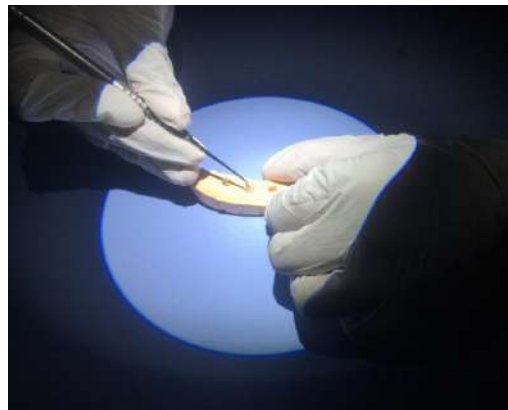


Figure 1. Composite condensed under microscopic light without the filter.

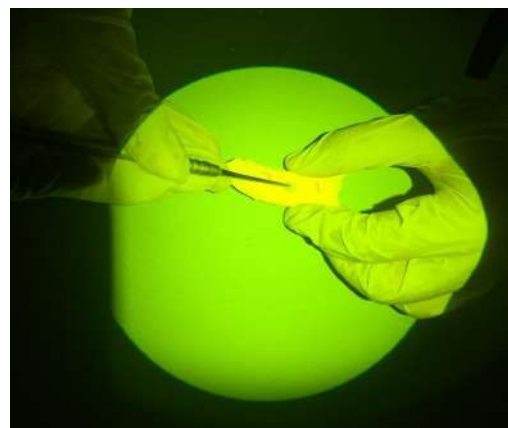
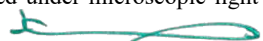


Figure 2. Composite condensed under microscopic light with a yellow filter.



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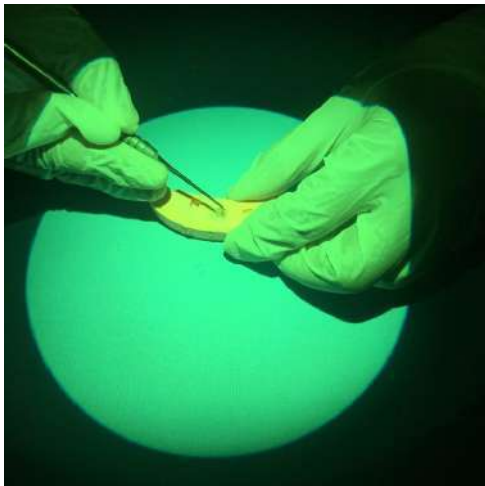


Figure 3. Composite condensed under microscopic light with green filter.



Figure 4. Composite condensed under dental chair light with a red filter.

Each group contains a total of 8 specimens. The distance between the illuminant and sample was maintained at 30 cm and was kept constant. After condensation for 1 minute with a plastic filling instrument, we covered the composite specimen surface with a mylar strip and placed the curing light upon it. The size of the illuminated area was 8cm. The irradiance on the surface of the specimen with microscopic light was 1 lakh lux, and with the placement of filters, it was 75,000 lux. Under the chair light, irradiance on the sample's surface was 30,000 lux, and with a red filter, it reduced up to 20%. Bluephase NM (Ivoclar) was used to cure the samples with an intensity of 800 mW/cm² and a wavelength range of 430-490 nm for 15 sec. Later, each specimen was removed from the mold, stored in artificial saliva for seven days, and placed in an amber bottle. Amber bottles were stored in a humidifier until testing. Initially, we calculated the volumetric shrinkage using a micrometer. Later all the samples were

tested for top and bottom hardness using Vickers micro-hardness tester and calculated the depth of cure.

Measurement of volumetric shrinkage

Three sides of the post-cured composite specimen (length, width, height) were measured using a micrometer. All three values multiplied to obtain the volume of the post-cured composite specimen. The difference in the mold and post cured composite specimen volume was calculated, and that value was taken as volumetric shrinkage.

Measurement of micro-hardness

The top and bottom surface hardness of each 4-mm increment specimen were measured using the Vickers micro-hardness tester (Daksh quality system, India). The measuring indenter, the Vickers pyramid, was pressed to the composite sample using a load of 100 grams for 20 sec. The surface Vickers hardness was measured at three points of each specimen to minimize measurement errors within a sample.

The average of the three micro-hardness values was taken to obtain a single value of Vickers micro-hardness.

Equation 1: Vickers hardness of the material (VHN) = (1.8544P)/D²

In which VHN represents Vickers's hardness of the material (kg/m²), P is the predetermined load applied on the sample (kg) and D is the average diagonal distance (mm) of the square resulting from the indentation of the pyramid tip of Vickers hardness tester.

Depth of cure measurement

After determining the top and bottom micro-hardness, the depth of cure of each sample was calculated according to Equation 2.

Equation 2: Depth of cure = bottom micro-hardness/top micro-hardness

Working time was measured simultaneously using a stopwatch while condensing the composite into the molds until resistance to condensation was observed.

Statistical analysis

The collected data were subjected to descriptive analysis and followed by Mann-Whitney u test to measure the outcome variables in different groups. One way ANOVA was performed to know the mean comparison within and between the groups of outcome variables followed by Tukey post hoc test for multiple group comparisons. The statistical significance level was set as p<0.05. The analysis was performed using SPSS software version 20.0 IBM (NY, USA).

Results

Group-2 showed the highest mean volumetric shrinkage (14.514%), whereas group-5 showed the lowest (7.386%) among all the groups (Table I). A statistically significant difference is demonstrated between group 1 and 5 (p=0.050), 2 and 5 (p=0.007) (Table II).

Table I. Comparison of mean values of volumetric shrinkage, surface hardness, depth of cure of all the groups.

Group	Mean Volumetric Shrinkage (%)	Mean Surface Micro-Hardness (kg/m ²)	Mean Depth of Cure (%) (Bottom/Top Surface Hardness)
Group-1	12.868	51.994	0.811
Group-2	14.514	58.065	0.831
Group-3	12.055	50.966	0.800
Group-4	10.842	50.434	0.803
Group-5	7.386	46.536	0.789

Table II. Multigroup comparison of volumetric shrinkage.

Group		VS (%)	P-value
		Mean Difference	
1	2	-1.646	.914
	3	0.813	.993
	4	2.026	.834
	5	5.483	.050*
2	3	2.459	.714
	4	3.672	.342
	5	7.128	.007*
3	4	1.213	.970
	5	4.670	.139
4	5	3.457	.402

VS-volumetric shrinkage; *-significant difference; %-percentage

The mean Surface micro-hardness (SH) of group-2 was highest (58.065 kg/m²). The least values were observed in group-5 (46.536 kg/m²) (Table I). A statistically significant difference was observed between all the groups (p=0.001) except group-3, group-4 (p=0.100) (Table III).

Table III. Multigroup comparison of surface micro-hardness.

Group		SH (kg/m ²)	P-value
		Mean Difference	
1	2	-11.529	<0.001*
	3	-4.430	<0.001*
	4	-3.898	<0.001*
	5	-5.458	<0.001*
2	3	7.099	<0.001*
	4	7.631	<0.001*
	5	6.071	<0.001*
3	4	0.532	.100
	5	-1.028	<0.001*
4	5	-1.560	<0.001*

SH-surface micro-hardness; *-significant difference; kg/m²-kilogram/meter²

The highest mean DOC was obtained for group-2 - 0.831% (Table I). All the groups showed a mean DOC of 0.80 or more, whereas group-5 showed the lowest value of 0.789%. But there was no statistically significant difference between all the groups except groups 2 and 5 (p=0.016). (Table IV).

Table IV. Multigroup comparison of depth of cure.

Group		DOC (%)	P-value
		Mean Difference	
1	2	-.020	.524
	3	.011	.900
	4	.009	.958
	5	.023	.406
2	3	.031	.124
	4	.029	.182
	5	.043	.016*
3	4	-.003	1.000
	5	.011	.900
4	5	.014	.815

DOC-mean depth of cure; *-significant difference; %-percentage

For group-2 samples, the average working time recorded was 40 sec, whereas the remaining groups showed 1 minute working time.

Discussion

Bulk-filling techniques are popularized due to the introduction of materials with improved curing, controlled polymerization contraction stresses, and reduced cuspal deflection [18-22]. By this approach, the number of increments required to fill a cavity is decreased compared to traditional incremental techniques. In contrast to the 2-mm incremental technique for conventional composites, manufacturers recommend 4-5-mm increments of the bulk-fill resin composites. The bulk-fill method undoubtedly simplifies the restorative procedure and saves clinical time in cases of deep, wide cavities.

The incident light influences the properties of these bulk-fill composites. The use of a microscope, combined with co-axial illumination (MLMCI), improves the dentist's ability to prepare, bond, restore, and adjust composite restorations, compared to use unaided vision and non-co-axial, shadow forming overhead lighting [23,24]. A co-axial light axis is coincident with the visual axis of the dentist's eyes, resulting in shadow-free illumination [25,26]. DOM are provided with different types of filters and are used for observation and photomicroscopy. Each microscope filter placed in the light path serves a different purpose and is placed either over the illuminator or in a filter slot that lies in the light path. The dental operating microscope used in

this study has inbuilt yellow and green filters. We added a red filter to the dental chair to compare the effects of the different lights (dental chair light and microscopic light) with and without filters.

The higher the light intensity, the higher the degree of conversion, and higher the polymerization shrinkage [27]. High polymerization shrinkage in group 2 could be because of the high intensity of DOM light without filter while condensing, followed by curing light intensity. Similarly, low polymerization shrinkage in group 5 could be because of the low intensity of dental chair light with a filter. Groups without filters showed higher volumetric shrinkage values when compared to groups with filters.

Micro-hardness is an essential parameter for the physical and mechanical behavior of composite resin restorations. The hardness of the light-cured resin composites depends on several factors: the composition of the organic matrix, the type and amount of filler particles, and the degree of polymerization and distance between the composite and tip of curing light [28]. Since all the factors except the degree of polymerization are made constant, and it could be the only factor that changed properties. The degree of polymerization, in turn, depends upon the light source and its intensity. Greater SH in Group-2 is because of the higher intensity of the light source, which increased the DC.

Similarly, Group-5 specimens were subjected to a lower intensity of light, giving lower SH values. Similar results were shown by Kassim et al., where the DOC and SH increased with an increase in light intensity up to 1500 mW/cm² [29]. There is no statistically significant difference between groups 3 and 4, which could be because of manipulation under similar conditions except for the change in filters. A significant difference was observed for group 2 (without filter) with group 3 and group 4 (both with filters); this might be because of a 35% decrease in the microscopic light intensity. According to the manufacturer's specifications, the microscopic light intensity is nearly 1 lakh lux, which decreased to 75,000 lux approximately with the filters.

SH is not an adequate indicator of complete material polymerization. The hardness of the bottom surface should be close to the hardness of the top surface. A value of over 0.80 in the bottom to top surface micro-hardness indicates adequate DOC. The DOC is the depth up to which the light can cure the material. The presence of unreacted monomer within the resin-based composite (RBC) bulk may also attenuate the irradiating light, preventing the formation of free radicals and thereby reducing the DOC. All the groups showed a considerable DOC (>0.8) except group-5 (0.78), which is because of lower hardness values in the case of Group 5. The chemical composition of the filler and matrix can have significant effects on the degree of conversion of RBCs. Tetric N Ceram bulk-fill composite has prepolymerized fillers (PPF) containing barium glass

and silica minerals. New types of photo-initiators such as Ivocerin and Benzoyl Germanium are used in the composition of PPF of bulk-fill RBCs instead of the standard type camphor quinone (CQ) [30]. The higher capability of these materials in creating free radicals per molecule unit can improve the light sensitivity of RBCs [31]. These changes have a positive effect on the light absorbance ability and DC of RBCs.

All the parameters were lower for group 5 when compared with group 1. Groups 1 and 5 showed significant differences for SH and VH. The difference could be because of the reduction of the intensity of the chair light after placing the filter. The working time is less in Group 2 because of the high intensity of light, causing the rapid formation of a highly cross linked polymeric network in surface layers of composite resin. The remaining groups showed an adequate working time.

Conclusion

Microscope light without filter showed the highest surface hardness and depth of cure. However the highest volumetric shrinkage and lesser working time were also observed, which are undesirable. Use of filters during composite manipulation showed less detrimental effects on depth of cure, volumetric shrinkage and working time. Overall, composite condensed under filters showed acceptable properties.

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Original Article

Effect of nonthermal atmospheric plasma, grape seed extract, and bromelain on immediate bonding of composite to bleached and microabraded surfaces

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Abstract

Background: Tooth whitening procedures such as bleaching and microabrasion alters the enamel surface and thus reduce the composites' bond strength. Hence, various surface treatments were introduced to overcome this problem.

Aim: To assess the effect of nonthermal atmospheric plasma (NTAP), grape seed extract (GSE) and bromelain on the shear bond strength (SBS) of the composite after bleaching and microabrasion.

Materials and Methods: Eighty extracted maxillary anterior teeth were distributed into two groups. Group 1: bleaching and Group 2: Microabrasion and further subdivided into four subgroups ($n = 10$) based on the form of surface treatment employed. Group A: no surface treatment, Group B: bromelain, Group C: GSE, Group D: NTAP. Composite resin was bonded to the labial surface and placed in artificial saliva for 24 h. SBS testing was done for all the samples.

Statistical Analysis: Analysis was performed using two-way analysis of variance and *post hoc* Tukey's test with $P < 0.05$ considered statistically significant.

Results: Group 1D revealed the highest bond strength (35.4 Mpa) and Group 1A showed the least bond strength values (15.7 Mpa). Among the bleaching groups, significant difference was observed between all the subgroups except Group 1A and 1B ($P = 0.972$). In microabrasion group, significant difference was observed between Group 2A and 2D ($P = 0.0001$), Group 2B and 2D ($P = 0.0010$), and Group 2C and 2D ($P = 0.0001$).

Conclusions: Following bleaching, NTAP application and GSE significantly improved the SBS of composite resin. Application of NTAP to microabraded surfaces increased the bond strength of composite resin.

Keywords: Bleaching; bromelain; grape seed extract; microabrasion; nonthermal atmospheric plasma

INTRODUCTION

Intrinsic enamel defects, such as opacities and brown pigmentations can adversely affect the color, translucency of enamel. Depending on the severity of the

defects, various treatment modalities such as bleaching, microabrasion, veneers, and crowns are performed.^[1] However, bleaching remains the mainstay of treatment for these intrinsic stains. The bleaching agent slowly interacts with stains located within the superficial layers of the tooth and thus lightens the tooth surface.^[2] Hydrogen peroxide (H_2O_2) has side effects such as tooth sensitivity, external cervical resorption, and reduction of composites' bond strength when bonded immediately

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to the bleached surface. The standard method to overcome this problem is to delay the bonding for up to 4 weeks following bleaching.^[3] A variety of methods have been proposed to overcome this problem, which includes superficial tooth reduction, alcohol application before bonding, using acetone-based adhesives, and antioxidants such as alpha-tocopherol and sodium ascorbate.^[4]

Grape seed extract (GSE) is a natural antioxidant that contains 98% of oligomeric proanthocyanidin complexes (OPCs). OPCs contain electron donor sites, i.e., hydroxyl sites, which binds to the free radicals. They neutralize the free radicals trapped in the enamel following the degradation of H₂O₂. There is a need to compare the effect of applying these antioxidants to bleached surfaces with other surface treatment procedures.^[4]

Espinosa *et al.* stated that Enamel deproteinization before etching increases the retentive surface of the enamel to 94.47%.^[5] Deproteinizing enzymes like bromelain remove the unsupported collagen, thereby increasing the dentin permeability and enhancing the diffusion of monomers. Bromelain was reported to decrease nanoleakage following the removal of collagen.^[6] However, there are no studies evaluating the efficacy of bromelain following bleaching on the shear bond strength (SBS) of composite to the tooth surface.

In recent times, plasma has gained considerable importance in the medical field. Plasma is a partially ionized gas and known as the fourth state of matter. Nonthermal atmospheric plasma (NTAP) is a low-temperature artificially generated gas, which does not use a vacuum system.^[7] Various studies have reported the increase in composite resin's bond strength to the tooth surface using proteolytic enzymes, antioxidants, and NTAP. However, limited literature is available regarding the effect of bromelain, and NTAP on the bond strength of composites immediately following the tooth whitening systems.

Microabrasion is a simultaneous procedure of abrasion and erosion, reducing the superficial enamel resulting in a compact, prism-free layer. It comprises dense mineralized tissue in an organic matrix, which is replaced by a densely compacted prismless layer. Thus, by altering the enamel surface, it reduces the composites' bond strength.^[8] Opinya *et al.* observed that removal of the compact mineralized layer can improve the composites' bond strength.^[9] Hence, there is a need to study the effect of various surface treatments on microabraded surfaces. The present work aimed to assess the impact of bromelain, GSE, and NTAP on the SBS of composite resin following bleaching and microabrasion.

MATERIALS AND METHODS

After obtaining ethical clearance from the institutional ethics committee (Pr. 139/IEC/SIBAR/2018), eighty human maxillary anterior teeth ($n = 80$), extracted for various reasons, were selected for the study. The samples were thoroughly cleaned and were mounted in plastic molds with self-cure acrylic resin until the cemento-enamel junction. The facial tooth surfaces were cleaned with pumice slurry. The samples were allocated into two groups based on the teeth whitening systems ($n = 40$ each).

Treating with tooth whitening systems

Group 1 ($n = 40$): the labial tooth surfaces were dried. Pola Office syringe (SDI limited, Australia) is taken, the plunger is pulled back to release pressure and extrude the contents into the pot supplied by the manufacturer. Immediately mixing is done using an applicator tip until a homogeneous mixture is obtained. Generous amount of this gel is applied on the labial surface for 8 min. The bleaching gel is then rinsed and blot dried.

In Group 2 ($n = 40$): a fine grit diamond bur with water coolant is used to slightly remove the labial surface superficially to initiate micro reduction. A plastic mac tip supplied by the manufacturer was attached to the Opalustre (Ultradent, USA) syringe. 1 mm thick layer is applied uniformly on the tooth's labial surface. A micromotor handpiece with Opal prophyl cups was used at RPM of 400, along on the enamel surface for 120 s. Following this, the teeth were rinsed with distilled water.

The groups were subdivided into four subgroups: A, B, C, and D, respectively, based on the type of surface treatment performed.

Subgroup A ($n = 10$): No surface treatment is done (control group).

Subgroup B ($n = 10$): 5% bromelain solution for 10 min and then rinsed with distilled water.

Subgroup C ($n = 10$): 5% GSE solution for 10 min and then rinsed with distilled water.

Subgroup D ($n = 10$): NTAP exposed for 15 s.

Preparation of solutions

1. Proteolytic enzyme– 5% bromelain solution-5 gm of bromelain (Meteoric Exim Pvt. Ltd., India) dissolved in 100 ml of distilled water
2. Antioxidant– 5% GSE solution-5 g of GSE (All pure organics, India) dissolved in 100 ml of distilled water.

Nonthermal atmospheric plasma application

To generate the plasma jet, compressed He gas with a pulsing head of 2 GHz at 3.25 standard liters was utilized.

Banu, *et al.*: Surface treatments on bleached and microabraded surfaces

The NTAP was applied at a pressure <2 Pascal's and a power of 2 Watt [Figure 1]. The application was carried out in an isolated closed chamber at a distance of 5–6 mm from the tooth surface for 15 s for each sample with an alternating cycle of activation and resting phase of 5 s each. The temperature on the tooth surface was also examined using a thermocouple which ranged from 27 to 29.4°C, that is endured by pulpal tissues.

Placement of composite restoration

For all the groups, the composite placement was carried out immediately after surface treatment. Self-etch adhesive (Solare Universal bond, GC, India) was applied to the labial surfaces of the tooth. A cylindrical plastic matrix of 3 mm diameter and 3 mm height was prepared. The matrix was placed on the labial surface and the composite resin (Spectrum, Dentsply, USA) was condensed incrementally. It was then photopolymerized in all directions using light-emitting diode light-curing unit (Orikam Eighteenth Curing Pen, India) for 60 s. The matrix was removed carefully following polymerization. The samples were then placed in artificial saliva for 24 h at 37°C. SBS testing was performed for all the samples using Universal Testing Machine (UTM) (Instron, Model 8801). Each sample was secured in UTM in such a way that the leading edge of the plunger is targeted at the interface between composite and labial surface at 1 mm/min speed. The force needed to dislodge the composite material was noted. The SBS was calculated using N/mm² (peak load value/composite base area).

Statistical analysis

The data obtained were analyzed using version 20.0. SPSS software (Statistical Package for the Social science, IBM Corp. Ltd., Armonk, NY, USA). All the groups were analyzed for overall significance values using two-way analysis of variance test, whereas, comparison within the group and intergroup comparisons were made utilizing *post hoc*

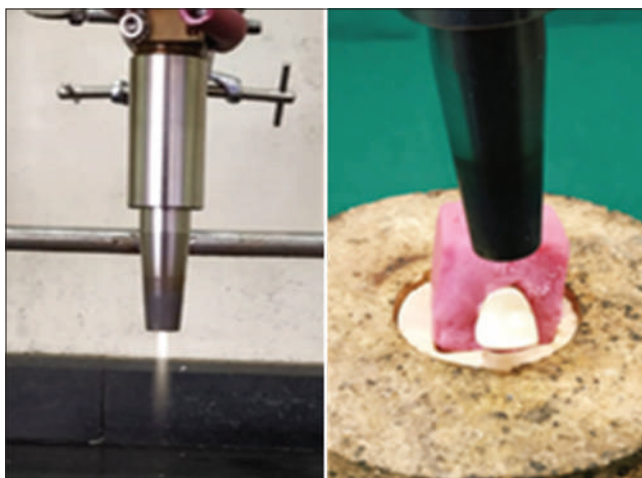


Figure 1: Application of nonthermal atmospheric plasma

Tukey's tests with $P < 0.05$ was considered as statistically significant.

RESULTS

The results showed that Group 1D had higher bond strength (35.4 Mpa) out of all the groups, and Group 1A with least bond strength values (15.7 Mpa). In the bleaching groups, significant differences were observed between all the subgroups except Group 1A and 1B ($P = 0.972$) [Table 1]. In microabrasion group, significant differences were observed between Group 2A and 2D ($P = 0.0001$), Group 2B and 2D ($P = 0.0010$), and Group 2C and 2D ($P = 0.0001$) [Table 2].

DISCUSSION

The bleaching procedure might exhibit definite antagonistic effects on the enamel. These include a decrease in the surface microhardness, morphological as well as compositional changes.^[10] These changes may affect the bonding of restorations to enamel when applied immediately postbleaching. According to the literature, tooth bleaching may reduce composites' bond strength applied to bleached enamel. Smith *et al.* stated significant surface structural changes in bleached enamel. According to them, fundamental changes may be the cause of the reduction in bonding strength.^[11] Several reports have been reported regarding the association between bleaching agents and changes in the chemical or morphological structure of enamel.^[12] These modifications include loss of calcium and regular prism margins, change

Table 1: Comparison of interactions between the four subgroups (A, B, C, and D) with mean shear bond strength using Tukey's multiple *post hoc* test in Group 1

Interactions	Group 1A	Group 1B	Group 1C	Group 1D
Mean	15.7793	18.0374	25.1041	35.4856
SD	2.9509	6.2325	2.9104	5.6861
Group 1A	-			
Group 1B (P)	0.9721	-		
Group 1C (P)	0.0022*	0.0466*	-	
Group 1D (P)	0.0001*	0.0001*	0.0005*	-

*Statistically significant. SD: Standard deviation

Table 2: Comparison of interactions between the four subgroups (A, B, C, and D) with mean shear bond strength using Tukey's multiple *post hoc* test in Group 2

Interactions	Group 2A	Group 2B	Group 2C	Group 2D
Mean	18.4271	21.4822	18.5608	31.3464
SD	4.9409	3.7010	4.9608	7.0988
Group 2A	-			
Group 2B (P)	0.8714	-		
Group 2C (P)	0.9999	0.8954	-	
Group 2D (P)	0.0001*	0.0010*	0.0001*	-

*Statistically significant. SD: Standard deviation

in the ratio of the organic and inorganic constituents of the tooth.

In the present study, bleaching followed by immediate bonding of composite resin with no surface treatments showed the least bond strength. This finding is similar to the previous investigation by Nimet *et al.* who reported a drop in the mean bond strength value of composite by half due to the application of 35% H₂O₂.^[13] Physical alterations in the tooth structure after bleaching were the probable reason for the decrease in enamel bond strength. Similar decrease in bond strength was reported by Cinthia *et al.*, Khamverdi *et al.*^[14,15] this decrease in bond strength may be due to scanty, small, and poorly distinct resin tags bonded to bleached teeth. The eminence of these resin tags, their frequency, and their depth of penetration effect the bond strength of composite to the enamel, as mentioned in the research done by Titley *et al.*^[11] Similar short, sparse, structurally incomplete, or complete absent resin tags in bleached enamel was reported by Dishman *et al.*^[16]

Torneck *et al.* showed a substantial decrease in composite resin's adhesive bond strength following exposure of enamel to H₂O₂.^[17] The residual peroxides on the enamel surface are responsible for the bond failure that happened at the resin-enamel interface. Rinsing leads to the residual H₂O₂ washout only on the superficial enamel (0.5–5 nm).^[18] However, the residual free radicals trapped deep inside the enamel could have attributed to the compromised bond strength. Reversal of the reduced bond strength can be achieved in two ways. The first one is the time-dependent delay of composite placement, while the latter is by surface treatments performed on the bleached enamel using alcohol, acetone-based adhesives, and antioxidants.

In the current study, bromelain was used as an experimental surface treatment agent. Bromelain is commonly used in medical supplements. The pH of the bromelain ranges from 3 to 6.5. At this pH, the bromelain components are stable, and it disrupts adhered proteins causing stains. According to Aarti *et al.* there is a significant increase in microleakage after bleaching with 35% H₂O₂ and effectively reduced when the bleached teeth were treated with bromelain.^[19] However, their methodology involved class V cavity preparation on the cervical third of the teeth. Better results for bromelain group in their study could be due to dentin involvement while cavity preparation, thereby exposing the collagen substrate for the bromelain to act. In the present study, bromelain showed no statistical significance when compared to the control group. This could be because the bromelain acts on the protein content, which is less in enamel.

To overcome the failure of immediate bonding to bleached enamel, Vidya *et al.* studied the effectiveness of antioxidants in the reversal of decreased adhesion to the bleached

surface.^[4] Nair *et al.* and Rose *et al.* studied that antioxidant agents have the potential to eliminate free radicals, thereby minimizing the interference with the polymerization of adhesives, which in turn can improve the bond strength of composites to bleached surface.^[20]

GSE is a natural antioxidant that contains 98% of OPCs.^[20] When compared to control, GSE significantly improved the bond strength. OPCs contain electron donor sites, i.e., hydroxyl sites, which binds to the free radicals. They neutralize the free radicals trapped in the enamel following the degradation of H₂O₂. This potential of OPCs is said to be 50 times superior than other antioxidants.^[4,6]

In the microabrasion group, control samples showed the least bond strength. Microabrasion increases the roughness of the enamel surface and is also related to the reduction in the enamel microhardness. The abrasion of enamel rods causes the formation of by-products which get compacted on the micro abraded surface. This results in a surface which is impervious to etching.^[8] Group 2B showed no improvement in mean SBS values, which could be because the bromelain acts on the protein content, which is less in enamel. As there is no free radical production, even the application of antioxidants did not show improved bond strength in Group 2C.

Plasma is the fourth state of matter, an extremely reactive material comprises charged particles, and a strong electric field. Plasma treatment increased hydrophilicity of the dental materials on the dentin surface, thereby reducing the interface defects/voids and improve the interfacial quality.^[21] In the current study, NTAP application to both bleached and microabraded surfaces, i.e., Groups 1D, 2D significantly improved the bond strength. This is in accordance with several studies which have proven NTAP to increase bond strength at the interface of tooth and composite by 60%.^[22] This bonding at the interface helps in improving durability, longevity, and performance of the composite resin.^[23]

Plasma application increases the wettability, hydrophilicity of the substrate and modifies its chemical structure by provisionally exposing and partially dispersing the functional groups. This results in improved infiltration of the bonding agent into the enamel and enhances the adhesive bond strength producing bonds that depend on surface chemistry rather than surface porosity, which is in accordance with Cho *et al.*^[24] Even though the resin tags do not significantly contribute to the bond strength in the case of self-etch adhesives, the enhanced resin tag formation by NTAP in the present study could have contributed to the rise in initial bond strength.^[7,21]

NTAP causes molecular alterations such as reducing the molecules containing nitrogen and carbon and exposing the

Banu, *et al.*: Surface treatments on bleached and microabraded surfaces

hydroxyapatite crystals, which is considered a significant improvement in the wettability of enamel. Short exposure periods of enamel surfaces to NTAP for only 30 s lead to the formation of super hydrophilic surfaces.^[25] There appears to be a need for further clinical studies to compare and gather more details of plasma effects on the organic components of enamel, analyzing the different periods of plasma application and its implications on adhesive properties to the bleached and micro abraded enamel to support the present hypothesis.

Limitations of the study

1. In the present study, even though plasma has shown better results of increased bond strength, the use of the same in the clinical scenario is practically difficult
2. The results have to be confirmed with large-scale and *in vivo* studies.

CONCLUSIONS

- Composite resin application immediately following bleaching and microabrasion showed decreased mean SBS values
- Following bleaching, NTAP, and GSE application significantly improved the SBS of composite resin
- Following microabrasion, NTAP significantly increased the SBS of composite resin.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Comparison of sealing ability of mineral trioxide aggregate, biodentine with and without bioactive glass as furcation repair materials: An ultraviolet spectrophotometric analysis

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ABSTRACT

Aim: The aim of this study was to evaluate the sealing ability of mineral trioxide aggregate (MTA), Biodentine with and without Bioactive glass (BG) as furcation repair materials by ultraviolet (UV) spectrophotometric analysis.

Materials and Methods: Forty extracted human maxillary molars ($n = 40$) were selected and decoronated 3 mm above the cemento-enamel junction and 3 mm below it and a defect in furcation was created. The samples were then divided into 4 groups of 10 ($n = 10$) each, Group I: MTA, Group II: Biodentine, Group III: BG + Biodentine and Group IV: BG + MTA and the defect was treated with respective furcation repair material. All the samples were then immersed in 2% methylene blue solution for 24 h and later stored in 65% nitric acid solution. The solution obtained was subjected to centrifuge at 3500 rpm for 5 min. From this solution, 100 μ l of the supernatant was collected, analyzed in UV spectrophotometer at 550 nm with nitric acid as the blank and readings were recorded as absorbance units.

Results: All four groups exhibited a significant difference in dye absorbance values ($P < 0.01$). Group I, i.e., MTA showed the least dye absorbance values when compared with the other three groups. Data were analyzed using one-way analysis of variance and *post hoc* Tukey tests. The level of statistical significance was set at $P < 0.05$.

Conclusion: Within the limitations of the study, it can be concluded that MTA had superior sealing ability than Biodentine, whereas BG + Biodentine showed better sealing ability when compared with BG + MTA.

Keywords: Calcium silicate cement, furcation, mineral trioxide aggregate

INTRODUCTION

The physiological dentition serves as a defense against pathologies. On the other version, it also harbors diverse microbiota, which leads to the pathologies and failure of treatment progress. Endodontic mishaps occur because of iatrogenic causes. However, repair of these mishaps plays a pivotal role in the procedure's success. Furcation perforation is one such mishap.^[1]

Antecedently, amalgam, gutta-percha, calcium hydroxide, calcium sulfate-based materials have been used as furcation repair materials. These materials exhibited some potential risk factors leading to the destruction of the periodontium. Some of these materials are no longer preferred as a furcation

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repair material. Nowadays, Glass Ionomer Cement, mineral trioxide aggregate (MTA), Biodentine, Bioactive Glass (BG), Demineralized Freeze-Dried Bone, Tricalcium Phosphate, and Dentine Chips are the most commonly used perforation repair materials.^[1]

The advancements in the furcation repair material were majorly to overcome the sealing ability of the material with the tooth structure, biocompatible to promote the healing of the underlying periodontal tissue, and control of the repair material to avoid its extrusion into the periodontal tissue, antibacterial property. Furcation repair material should induce healing, bone formation, be radiopaque, induce mineralization, cementogenesis, comfortable in manipulation and placement.^[2]

MTA is one of the materials of choice to repair the furcation perforation because of its superior quality of marginal adaption, sealing ability, antibacterial effects, biocompatibility, and bioactivity, which may induce the regeneration of periodontal tissue. MTA's major disadvantage is its delayed setting time, which compromises initial setting time when in contact with oral fluids and diminishes the adaptation of the MTA to tooth structure.^[3] Biodentine is a calcium silicate-based material with a polycarboxylate-based hydro-soluble polymer system described as a water-reducing agent, reducing the mix's overall water content, along with calcium chloride as the setting accelerator.^[4]

BG is a potentially new repair material introduced in dentistry as a root-end filling material. BG is a type of bioactive ceramic consisting of SiO₂, CaO₅, Na₂O, P₂O₅. It is well suited as a repair material for a variety of endodontic treatments. BG has adequate strength and load-bearing capacity, good handling, and working properties.^[5,6] It has a faster setting time of around 15 min,^[7,8] tolerates moist environment very well, good marginal adaptation, low cytotoxicity comparable to MTA.^[8]

In the present *in vitro* study, BG is attempted by modifying the polymer/powder part of bone cement with MTA or Biodentine. All the favorable properties of BG are retained and the potential disadvantages faced with MTA or Biodentine are overcome. The purpose of the current study was to evaluate the sealing ability of MTA, Biodentine, BG + Biodentine, BG + MTA as furcation repair materials using the dye extraction method.

MATERIALS AND METHODS

Forty freshly extracted human maxillary molars ($n = 40$) with nonfused and well-developed roots were collected for the

study and stored in 0.2% thymol solution until use. The sample size was calculated using G*power 3.1.9.4 software (Heinrich Heine University, Düsseldorf, North Rhine-Westphalia) at an effective size of 1.39, error probability of 5% and 95% power, a minimum sample of 16 with 4 samples in each of the four study groups is required. The teeth were made free from any calculus, soft tissue, and debris with an ultrasonic scaler. Each tooth was then decoronated 3 mm above the cemento-enamel junction (CEJ) and 3 mm below the furcation. The sectioned surface and root canal were covered with sticky wax on the external root surface, in the orifice and then two layers of varnish were applied over them, a black marker pen was used to mark the location of the defect. Artificial perforation of 1 mm in diameter was created from the external surface of the tooth with the number a #2 round carbide bur (Mani Inc., Japan) mounted on a high-speed handpiece with air-water coolant. For standardization purposes, the perforation was created in the floor of the pulp chamber from the external tooth surface to ensure that each perforation is to be centered between the roots with standard dimensions, without any access deviations for all samples. The chamber and perforation were flushed with water and dried. This methodology was similar to the one that was followed by Balachandran and Gurucharan.^[9]

The samples ($n = 40$) were then divided into four groups ($n = 10$) based on the furcation perforation repair material used.

Group I ($n = 10$): MTA: Ten molars in which perforations were repaired with Pro Root MTA (Dentsply Malleifer, USA)

Group II ($n = 10$): Biodentine: Ten molars in which perforations were repaired with Biodentine (Septodont, Saint Maur des Fosses, France)

Group III ($n = 10$): BG + Biodentine = Bioactive bone cement: Ten molars in which perforations were repaired with Bioactive bone cement.

Group IV ($n = 10$): BG + MTA = Bioactive bone cement: Ten molars in which perforations were repaired with Bioactive bone cement.

All the materials were manipulated according to the manufacturer's recommendations and placed incrementally with the help of a plastic filling instrument. The material condensation was done with the cylindrical end of the plastic filling instrument until the repair material was extruded out of the tooth surface and extruded repair material was removed with the flat end of the plastic filling instrument.

Preparation of bioactive bone cement

To prepare the bioactive bone cement powder and liquid of both the MTA and Biodentine were modified. The ratio for MTA/Biodentine: BG was 60:40 for powder, and for liquid, it is 1 ml of monomer liquid: 1 drop of saline coupling agent. The mixing of the cement was carried out in Amalgamator (Ultram 2, SDI, Australia). The modified powder and liquid were mixed together in the ratio of 2:1. Each sample was then repaired with the respective material in each group and stored for 24 h.

Evaluation of microleakage method

All the samples in each group were placed in separate Petri dishes containing 2% methylene blue. The teeth were immersed in dye up to the CEJ for retrograde dye challenge and dye was added to the access chamber of each tooth so that it was filled for orthograde dye challenge. All samples were stored similarly for 48 h.

All the samples were rinsed under tap water for 30 min and varnish was removed with a polishing disc. Each tooth was stored in a vial containing 5 ml of concentrated (65 wt %) nitric acid for 3 days. The solution thus obtained was subjected to centrifuge at 3500 rpm for 5 min. From this solution, 100 µl of the supernatant was collected, then analyzed in an ultraviolet spectrophotometer at 550 nm with nitric acid as the blank, and readings were recorded as absorbance units. The results thus obtained were subjected to statistical analysis. One-way ANOVA, followed by *post hoc*-Tukey tests were done using the statistical package SPSS (Statistical Package for Social Science, version 21, IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).

RESULTS

Mean spectrophotometric dye absorbance values of four different experimental groups.

Meanspectrophotometric dye absorbance values of four different experimental groups have been presented in Table 1. The least absorbance value was obtained for MTA (Group I - 0.484), followed by Biodentine (Group II- 0.555). The highestabsorbance values were obtained for Bioactive glass + MTA (Group IV- 0.890), when compared with Bioactive glass + Biodentine (Group III- 0.706). The meandye absorbance values were comparatively higher in the Bioactive bone cement groups i.e., Group III, Group IV when compared to the MTA (Group I) and Biodentine (Group II) alone. There was no significant difference between Group I and Group II. Group III showed absorbance values slightly higher than Group I and Group II.

DISCUSSION

Endodontic mishaps consist of various procedural accidents, perforation located in any tooth region is one such accident that may be accessed cavity preparation related. Irrespective of their situation, these perforations may serve as a connection between the external and internal tooth surface, leading to a decrease in the endodontic treatment's successful outcome. Repairing these perforations immediately with biocompatible material will increase the chances of the success of the endodontic treatment.

In this *in vitro* study, the sealing ability was assessed by the dye extraction method, which according to Camps and Pashley, gives similar results to the fluid filtration technique; both are based on quantitative measurement of liquid passage within the interfaces.^[10] In the dye extraction method, the absorbance or reflectance in the visible range directly affects the perceived color of the chemicals involved.^[10]

The present study revealed the highest sealing ability for Group I, i.e., the MTA group; these observations agree with previous studies. Jeevani *et al.* reported that MTA resulted in superior furcation sealing ability compared to Biodentine. MTA's excellent, unique property is its ability to promote cementum regeneration, thus facilitating the periodontal apparatus's regeneration and excellent marginal adaptation to the perforation sites' external borders.^[11]

In the present study, Biodentine showed sealing ability comparative to MTA. Sinkar *et al.* reported that MTA and Biodentine showed better sealing ability than MTA.^[12] Kokate and Pawar conducted a study to compare the microleakage of glass ionomer cement, MTA, and Biodentine as retrograde filling materials and concluded that Biodentine exhibited the least microleakage when compared to other materials used.^[13]

In the current study, Bioactive bone cement's sealing ability in sealing was equally effective as MTA. This property is because the BG in the presence of simulated body fluid forms a layer of apatite crystals, which nucleate and grow and occupy the space present between the bone cement and dentinal wall at the microscopic level.^[10] From their study results, Demir *et al.* concluded that BG had shown osteostimulatory and osteoconductive properties.^[14] It has been reported that it had an antibacterial effect against subgingival, supragingival bacteria. Sculean *et al.* stated that this material has good clinical manageability and specific hemostatic properties. They assumed that BG might be an ideal matrix in furcal perforations by considering its barrier-like properties.^[15]

Table 1: Mean spectrophotometric dye absorbance values of four different experimental groups

Group	n	Mean	SD	Minimum	Maximum
MTA	10	0.484	0.153	0.264	0.665
BIODENTINE	10	0.555	0.212	0.265	0.966
BIOACTIVE GLASS + BIODENTINE	10	0.686	0.164	0.396	0.976
BIOACTIVE GLASS + MTA	10	0.890	0.060	0.804	0.985

F=12.89; P<0.001

Balachandran and Gurucharan stated that combining two biocompatible materials could produce minimal cytotoxicity and maximum biocompatibility.^[9] In the present study, MTA and Biodentine modified BG to provide a track record favoring factors of repair material. Tests done by various authors^[16,17] for cytotoxicity revealed that fibroblast cells were unaffected by bone cement and biocompatibility of bone cement was similar to MTA. According to Miyazaki *et al.*, bioactivity can be induced in a biomaterial by incorporating silanol (Si-OH) groups and calcium (Ca⁺²) salts.^[18] The Ca⁺² salts start triggering hydroxyapatite formation, while the silane coupling agent may provide a Si-OH group after exposure to simulated body fluid.^[19] The reason for the addition of a silane coupling agent (MPS) was to accelerate apatite formation, maintain mechanical properties and increase the compressive strength.^[18]

The marginal sealing ability of calcium silicate-based materials is attributed to their ability to produce surface apatite crystals when in contact with the phosphates available in tissue fluids.^[20] Han and Okiji compared calcium and silicon uptake by adjacent root canal dentine in the presence of phosphate-buffered saline using Biodentine and ProRoot MTA. The results showed that both materials formed a tag-like structure composed of the material itself or calcium-or phosphate-rich crystalline deposits. The thickness of the calcium and silicon-rich layers increased over time. The thickness of the calcium and silicon-rich layer was significantly larger in Biodentine than MTA after 30 and 90 days, concluding that the dentine element uptake was greater for Biodentine than for MTA. These findings lead to the notion that apatite formation contributes to leakage reduction by filling the gap with the interface and dentine interactions such as intrafibrillar apatite deposition.^[21]

The setting time and handling properties for Biodentine + BG were superior compared with MTA + BG. The possible reason for the reduced sealing ability of BG + MTA might be modifying the bioactive bone cement, which affected the cement's setting time and handling properties of the cement. The exothermic reaction of polymethylmethacrylate bone

cement during its setting has adverse effects. The cement required for perforation repair is significantly less and produces a smaller exothermic reaction much-reduced amount of monomer. A study conducted by Mehrvarzfar *et al.* stated that BG interferes with the adaptation or bonding of the MTA to the tooth structure, leading to the MTA's reduced sealing ability.^[3] This explains the reason for reducing the sealing ability of the MTA bone cement in our study.

Though there are various studies on MTA, Biodentine as furcation perforation repair, our research is unique in assessing these materials in combination with BG as furcation repair materials. Additional *in vitro*, *in vivo* tests and clinical trials, are desirable to elucidate the perforation repair materials' effectiveness.

CONCLUSION

Within the limitations of this study, it can be concluded that MTA had the superior sealing ability when compared with Biodentine, whereas BG with Biodentine showed superior sealing ability when compared with BG with MTA.

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Conflicts of interest

There are no conflicts of interest.

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Effect of Calcium Hydroxide (CH), Triple Antibiotic Paste (TAP), Nano-sized CH and Nano-sized TAP as Intracanal Medicament on the Push-out Bond Strength of Biodentine: An In-vitro Study

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ABSTRACT

Introduction: Root perforations are one of the common procedural errors faced in endodontic procedures. The ideal root repair material must be capable of bonding to the dentin. Calcium Hydroxide (CH) and Triple Antibiotic Paste (TAP) are mostly used for root canal disinfection.

Aim: To evaluate the effect of calcium hydroxide, triple antibiotic paste, nano-sized calcium hydroxide and nano-sized triple antibiotic paste as intracanal medicament on the push-out bond strength of biodentine.

Materials and Methods: The present in-vitro study was conducted in Department of Conservative Dentistry and Endodontics, Sibar Institute of Dental Sciences, Guntur for a period of 1 month (March 2020). A total of fifty maxillary central incisors (n=50) were decoronated, instrumented with ProTaper rotary NiTi instrument followed by peesoreamers up to number 3, and were divided into five groups (n=10). Group I: CH, Group II: Nano CH, Group III: TAP, Group IV: Nano TAP and Group V: Control group. The respective

medicaments were mixed with propylene glycol and placed to the working length. After one week, medicaments were removed using Passive Ultrasonic Irrigation (PUI). Later, samples were sectioned into slices of 2 mm thickness using hard tissue microtome, and biodentine root repair material was placed into lumen of the slices. All the samples were stored for one week at 37°C, then subjected to push-out bond strength test. Data were analysed using one-way ANOVA, Tuckey, Dunnett's Post-hoc test (p-value <0.05)

Results: The highest push-out bond strength values were observed in the group IV (p-value <0.001) and least in the control group. Biodentine showed significantly higher resistance to displacement among the group II (p-value=0.431) and group IV (p-value=0.074) when compared with their conventional medicaments.

Conclusion: Nano Triple Antibiotic Paste and Nano Calcium Hydroxide enhanced the push-out bond strength values of biodentine when compared to their conventional intracanal medicaments.

Keywords: Calcium silicate cement, Endodontic procedure, Nanoparticles, Passive ultrasonic irrigation

INTRODUCTION

The primary rationale behind intracanal medicament is to kill the bacteria inside the root canal and to avert reinfection. Chemomechanical preparation cannot eliminate the bacteria residing deep in the dentinal tubules (Bystrom A and Sundqvist G) [1]. If the root canal is not appropriately dressed with antiseptic medicaments between the visits, the residual bacteria may increase in the same number as it was in the starting stage of the endodontic treatment. Thus the use of effective intracanal medication for disinfection of root canal is necessitated [2].

Nanotechnology provides the advantage of reducing the size of the particles as well as increasing the efficacy of materials with their specific physicochemical characteristics, such as ultrasmall sizes, increased surface area/mass ratio, bactericidal, increased chemical reactivity and bioavailability [3,4]. Nanoparticle size is <100 nm and can reach the complex anatomy of the root canal system. The use of nanoparticulate intracanal medicaments are proven as an effective way to eliminate resistant bacterial strains [4].

Several intracanal medicaments are used to disinfect the root canal during the inter appointment period. Calcium hydroxide is widely used in clinical procedures due to its high alkalinity, better antimicrobial properties, ability to denature the bacterial lipopolysaccharide and tissue dissolving property (Siqueira Jr JF and Lopes HP) [5]. However,

it is ineffective in the elimination of *Enterococcus fecalis*, the most important bacteria in persistent infections and candidiasis infection. It also has disadvantages like prolonged treatment, alteration in the hardness, and demineralisation of dentine. Nano Calcium Hydroxide (NCH) particles are reported for higher levels of antimicrobial activity in comparison with conventional CH and deeper penetration into the dentinal tubules [6,7].

The TAP is a mixture of ciprofloxacin, metronidazole, and minocycline. Initially, its use was recommended by Sato I et al., the proportions of the three antibiotics were recommended in a ratio of 1:1:1 by Hoshino E et al., 3:3:1 (Takushige T et al.) and a 3 Mix MP paste using propylene glycol and macrogol as a vehicle in the ration 7:1; however, the ideal guideline to which to adhere remains a controversy [8-10]. Triple Antibiotic Paste (TAP) also has some of the demerits, like causing tooth discolouration due to the presence of minocycline [11,12]. Karczewski A et al., reported the synthesis of clindamycin modified triple antibiotic nanofibres. In this study, the preparation of nano TAP was done by particle reduction using a ball miller [13].

Endodontic treatment can be complicated and challenging. Some of the iatrogenic errors complicate the outcome of endodontic treatment. Of those mishaps, one of the most commonly encountered procedural errors is a perforation of the root, which

affects the prognosis of the tooth. Ideally, root perforations should immediately be repaired with a biocompatible material to seal the communication with the periradicular tissues. An ideal perforation repair material should provide an adequate seal, be biocompatible, not affected by blood contamination, not be extruded during condensation, able to resist masticatory forces under function, bactericidal, induce bone formation and healing, radio-opaque, induce mineralisation, cementogenesis and easily manipulated for placement [14,15].

Calcium hydroxide, glass ionomer cement, Mineral Trioxide Aggregate (MTA), Hydroxyapatite, etc., are commonly used to manage perforation defects. Among these calcium silicate cements like MTA and Biodentine are emerging in contemporary practice because of their biomimetic properties and ability to withstand moist conditions. Biodentine (Septodont, Saint Maur des Fosses, France) has several advantages over MTA because of better handling properties, increased strength and hardness close to that of natural dentine, smaller particle size, increased micromechanical retention, ability to complete single appointment procedure [16-19].

Interim medicaments should be removed entirely from the root canals to maintain the sealing efficacy of root canal sealers and filling materials. The literature to date suggests that no method was able to remove the intracanal medicament placed in the root canals completely. The frequency of the ultrasonic device is in the range of 20-40 kHz. It has advanced fluid dynamics by the formation of cavitation and acoustic microstreaming that can remove the debris even from the complex anatomy of the root canal system (Goodman A et al.,) [20].

To date, many studies have been attempted to evaluate the effect of different intracanal medicaments on the bonding ability of calcium silicate-based cements [21-23]. None of them were based on the effect of nanoparticle ranged intracanal medicaments. The purpose of this study was to evaluate the effect of conventional TAP, CH, and their nano modified intracanal medicaments, i.e., nano TAP, NCH on the push-out bond strength of biodentine root repair material.

MATERIALS AND METHODS

This in-vitro study was conducted in March 2020 for a period of one month in the Department of Conservative Dentistry and Endodontics at Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India. The study protocol was approved by the Institutional Ethical Committee (IEC) (Pr.228/IEC/SIBAR/2020).

Inclusion criteria: Maxillary central incisors that were freshly extracted for periodontal reasons also with complete root formation and single canal with $<10^\circ$ curvature, no visible caries, fracture, cracks, resorption, calcifications. The degree of curvature was calculated using the methodology described by Schneider SW [24]. Canal morphology was assessed by buccolingual and mesiodistal radiographs.

Exclusion criteria: Open apex tooth, teeth having more than one canal and $>10^\circ$ curvature, teeth that were having fracture, crack lines, resorptive defects and calcification.

Preparation of nano medicaments: Nano medicaments are prepared mechanically by particle size reduction attained through a ball miller (top-down approach) similar to Zhang W et al., [25]. The average particle size of nano modified intracanal medicaments were analysed using the dynamic laser scattering method as done by Che L et al., in Zetasizer nano Ver.7.12 (Malvern Instrument Co., Southborough, UK) equipped with a He-Ne laser of wavelength 632.8 nm [26].

Specimen Preparation

Fifty maxillary central incisors (n=50) that were under inclusion criteria were selected and disinfected in 0.1% thymol solution for 24 hours and stored in saline until use. All the samples were decoronated

upto cemento-enamel junction using a diamond disc and the apical patencies were checked with a 10 k file (Mani.Inc., Tochigi, Japan). The working lengths were confirmed with radiographs. Root canals of all samples were prepared to the working length by using Protaper (Dentsply Maillefer, Ballaigues, Switzerland) NiTi rotary system to a size F3 using 17% Ethylenediaminetetraacetic acid (EDTA) (Desmear, Anabond Stedman Pharma Research Ltd, India). The canals were irrigated with 2 mL of 3% Sodium hypochlorite (NaOCl) (Prime Dental Products Pvt., Ltd.) after each instrumentation. The samples were then prepared with peesoreamer (Mani, Inc. Ltd Japan) upto a size of number 3 to attain a standardised internal diameter of 1.3 mm. Final irrigation was done with 3% of NaOCl, followed by 17% EDTA to remove the smear layer. The samples were finally irrigated with 2 mL of saline to avoid harmful effects of NaOCl and EDTA and dried with paper points. The samples (n=50) were divided into five groups, as follows:

Group I: Calcium Hydroxide paste (CH) (n=10)

Group II: Nano Calcium Hydroxide paste (NCH) (n=10)

Group III: Triple Antibiotic Paste (n=10)

Group IV: Nano Triple Antibiotic Paste (n=10)

Group V: Control group (n=10)

The respective medicaments in Groups I-IV will be mixed with propylene glycol and placed to working length using a lentulospiral (Dentsply, Konstanz, Germany). No medicament was placed in the control group. The coronal orifices were sealed with a cotton pellet and temporary filling material (Cavit; 3M ESPE, Seefeld, Germany), and the samples were stored for one week in an incubator at 37°C and 100% relative humidity. After one week, the medicaments from group I-IV were removed from the root canals using Passive Ultrasonic Irrigation (PUI). A 15 U file was used, which was kept 2 mm short of working length and agitated for 30 seconds duration at a power setting of 6. Distilled water was used as an irrigant in PUI followed by 5 mL of NaOCl irrigation and a final flush of 5 mL of distilled water. The samples were sectioned perpendicular to the long axis to obtain 2 mm slices from the middle third of the root from each sample using hard tissue microtome (Isomet; Beuhler Ltd., NY, USA) so that from each group a total of 10 slices were taken from 10 samples.

The biodentine root repair material was mixed according to the manufacturer's instructions, i.e., liquid from a single-dose container was emptied into a powder-containing capsule and mixed for 30 seconds at 4,000-4,200 rpm. It was incrementally placed into canal spaces of dentine slices being placed on a glass slab and condensed with a hand plugger. Excess material was removed from the surface of samples with a plastic instrument. The specimens were stored in an incubator at 37°C in 100% relative humidity for one week.

Push-Out Test

After one week, the samples were subjected to push-out bond strength analysis using Instron AutoGraph AG15 universal testing machine (Shimadzu, Japan) [Table/Fig-1]. Samples were placed on a custom made metal slab with a central hole. It was aligned with the centre of the test sample, which allowed the stainless steel stylus of the machine to pass freely once the bond between the test material and tooth was broken. The plunger was placed such that it had only minimum clearance with the sample to ensure contact with test material only. A stainless steel needle with 1 mm diameter was used to apply the compressive load with downward pressure on the surface of test material at a speed of 1 mm/min. The maximum force at the time of dislodgement was recorded in Newtons. The push-out bond strength was calculated in MPa by using the formula Push-out Bond strength (MPa)=debonding force (N)/surface area ($2p \times r \times h$) in mm^2 , where 'p' is the constant (3.14), 'r' is the root canal radius, and 'h' is the thickness of the root dentine slice in millimetres [27].



[Table/Fig-1]: Push-out test.

STATISTICAL ANALYSIS

Data were entered in MS-Excel and analysed in Statistical Package for the Social Sciences (SPSS) version 21.0. Shapiro Wilk test was applied to find normality. Descriptive statistics were represented by Mean with Standard Deviation (SD). A 95% confidence intervals were calculated. One-way ANOVA, Tuckey, Dunnet post-hoc test were applied to find significance. p-value <0.05 was considered statistically significant.

RESULTS

[Table/Fig-2] represents the mean values and standard deviation of push-out bond strength (MPa) of all groups. The mean highest push-out bond strength values were observed in group IV i.e., 12.33 MPa (p-value <0.001) and least in the group V i.e., 3.32 MPa. Biodentine showed significantly higher resistance to displacement among the group II (p-value=0.431) and group IV (p-value=0.074) when compared with their conventional medicaments [Table/Fig-3].

Group	Minimum	Maximum	Mean	SD	Median	IQR	p-value
Group I	2.34	12.03	6.54	3.13	6.25	4.28	<0.001
Group II	6.09	13.13	8.65	2.39	7.81	3.95	
Group III	5.63	15.78	9.03	2.88	7.88	3.18	
Group IV	6.88	15.01	12.33	2.95	12.97	4.09	
Group V	3.25	3.41	3.32	0.07	3.31	0.12	

[Table/Fig-2]: Mean push-out bond strength values and Standard Deviations (SD) of all test groups.

(One-way ANOVA, Tuckey, Dunnet post-hoc test were applied to find significance. p-value <0.05 was considered statistically significant)

Intergroup comparison		p-value
Group I	II	0.431
	III	0.269
	IV	<0.001
	V	0.292
Group II	I	0.431
	III	0.998
	IV	0.036
	V	0.017

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Group III	I	0.269
	II	0.998
	IV	0.074
	V	0.009
Group IV	I	<0.001
	II	0.036
	III	0.074
	V	<0.001
Group V	I	0.292
	II	0.017
	III	0.009
	IV	<0.001

[Table/Fig-3]: Table showing intergroup comparisons between the experimental groups and p-value.

(One-way ANOVA, Tuckey, Dunnet post-hoc test were applied to find significance. p-value <0.05 was considered statistically significant)

DISCUSSION

Root repair materials should adhere well to root canal dentine and offer significant resistance during the function in the oral cavity. There are various bond strength tests like tensile, shear, push-out bond strength to study the adhesive property of root repair materials to the dentine. In the present study, the push-out bond strength test was used, as it is a reliable, efficient, and practical method [27-29].

Chou K et al., and Berkhoff JA et al., reported that removing intracanal medicaments entirely is a challenge because of their penetration and binding to root canal dentine [30,31]. Calt S and Serper A reported that the residual intracanal medicament affects the penetration of obturating material into the root canal dentine [32]. Margelos J et al., confirmed the interaction of residual intracanal medicaments with zinc oxide based sealers affecting the quality of the sealer and Ozturk TY et al., reported formation of more voids during the marginal adaptation of biodentine with root canal wall [33,34].

In the present study, PUI with distilled water, NaOCl as irrigants using stainless steel 15 U file keeping 2 mm short of working length was performed to remove the intracanal medicaments. This method enables better acoustic streaming of the irrigants producing micro cavitation bubbles within the root canals (Van der Sluis LW et al.) [35]. The effect of intracanal medicaments on the bond strength of root repair materials are critical because they affect the penetration ability of calcium silicate-based cements into the root canal dentine and formation of tag like structures and mineral infiltration zone by hindering calcium silicon ion exchange with the dentine [16].

In this study, the push-out bond strength values of Biodentine were higher in the nano TAP (group IV), followed by TAP (group III) when compared to other groups. Nano medicament groups showed significantly higher bond strength values when compared to their conventional counterparts. The results of the present study were in accordance with Tulumbaci F et al., who reported that the binding of residual minocycline to the calcium via chelation might have increased the push-out bond strength values of TAP [36]. On the contrary, Shaheen NA et al., found that intracanal medicaments have no significant effect on the bond strength values of biodentine. TAP causes excessive demineralisation of the dentinal surface, which might be the reason for the significant difference in the TAP group causing better penetration of the biodentine in our study [37]. The residues of intracanal medicaments shown to decrease the micromechanical interlocking properties of these cements. In the present study, the least mean pushout bond strength in the control group (saline) might be due to the interaction of residual NaOCl with the biodentine affecting the adhesion of calcium silicate based biodentine [38]. Pereira AC et al., found no significant effect of intracanal medicament on bond strength of biodentine when compared with chlorhexidine, TAP, distilled water and control groups [39]. However, they detected small amounts of phosphorous ions in the triple antibiotic group

at their interface. The higher bond strength of biodentine was due to the small particle size and high flowability of biodentine, which might have improved the penetration of the material into the dentinal tubules, increasing bond strength when compared to MTA group in their study. The powder of biodentine is composed of tricalcium silicate, calcium carbonate, zirconium oxide and the liquid part contains water and calcium chloride as a setting accelerator and a hydrosoluble polymer as water reducing agent, which improves the physical and handling properties of this material [40].

In the present study, the NCH (group II) had shown better push-out bond strength values when compared with the CH (group I); however, these were inferior when compared to group III and IV. Propylene glycol was used as a vehicle in the present study, which might have negatively affected the carbonation reaction of CH with the environment, study by Cruz EV et al., [41]. The results of the present study are in accordance with Guiotti FA et al., who reported that CH remnants reduces the bond strengths of calcium silicate-based cements [42]. Yassen GH et al., reported that disruption of dentinal collagen occurs due to the high alkaline pH of CH [43]. This affects the bond between hydroxyapatite and collagen fibrils of dentine, which in turn decreases the push-out bond strength of biodentine. It might be the possible reason for the lesser push-out bond strength values of CH when compared to nano and conventional TAP. The study done by Nagas E et al., reported a higher bond strength of biodentine with CH group when compared with TAP [23]. It was due to the smaller particle size of biodentine and better penetration ability.

The possible reason for better push-out bond strength values of nano modified medicaments in the present study when compared with the conventional groups might be due to the size of nano medicaments less than 100 nm. Nano medicaments might be easily dislodged on the ultrasonic activation leading to better bonding with the exposed dentinal tubules in the root canal wall. So lesser remnants cause better exposure of dentinal surface, enhancing the tag like structures of biodentine which might have increased resistance to dislodgement study by Aggarwal V et al., [15].

The difference in bond strengths of biodentine might also be due to experimenting maxillary incisors, usage of the viscous vehicle for the medicaments, and experimental design, affecting the bond strength values of biodentine when compared with previous studies by Alsubait S et al., Nagas E et al., Tulumbaci F et al., [21,23,36].

Limitation(s)

Although it is not always possible to generalise the results of in-vitro studies in the clinical scenarios. In the present study, the authors have considered the application of ultrasonics in comparing the effect of residual intracanal medicaments on push-out bond strength of biodentine. Further studies are necessary to substantiate the findings of this research and also the removal efficacy of ultrasonic agitation on nano-sized intracanal medicaments and highlight their importance in endodontic treatment.

CONCLUSION(S)

Within the limitations of the present study it can be concluded that nano-sized medicaments can be efficiently used as intracanal medicaments as alternative to conventional medicaments because of improvement in the push-out bond strength values of biodentine. CH, NCH, TAP and nano TAP have enhanced the push-out bond strength values of biodentine. Nano TAP had shown highest mean pushout bond strength values when compared to other groups. NCH and nano TAP had significantly improved the push-out bond strength values when compared to CH and nano TAP respectively.

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REVIEW ARTICLE

Antioxidants: The counterstriking immunomodulators in therapeutics of oral lesions

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ABSTRACT

Antioxidants are a group of natural phytochemicals found in dietary ingredients that can be utilised to treat oral lesions and diseases. They are employed as chemical compound alternatives since they have less adverse effects. The dietary antioxidants found in fruits and vegetables reduce the damage by modulating detoxification enzymes, increasing immune system, and hormone metabolism. Scientists were drawn to antioxidants because of their ability to modify cell cycle controls, apoptosis, invasion, angiogenesis, and metastasis. They have demonstrated significant success as single treatments or in combination with chemo-preventive medicines for oral lesions.

Inside this article, we will look at the immuno-modulating effects of antioxidants, which aid in the treatments and even sometimes avoidance of numerous oral and maxillofacial conditions that contribute to morbidity, such as autoimmune lesions, and death, such as oral cancer. Antioxidants are chemicals that considerably impede or decrease the degradation of a living matter and defend the organism from oxidative harm. As a result, the sickness will be significantly reduced.

Keywords: Antioxidants; Phytochemicals; Phenolics; Beta-carotene; Tocopherol; Cyanins; 13-Cis-retinoic Acid (Isotretinoin); Apoptosis; Autoschizis; EGCG (Epigallocatechin Gallate)

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1. Antioxidants classification: First classification

1.1 Antioxidants enzymatic

Superoxide dismutase, catalase, glutathione peroxidases, malonaldehyde, glutathione reductase, and glutathione transferase.

1.2 Antioxidants non-enzymatic

1) Nutrient—alpha tocopherol, vitamin A, beta carotene, vitamin E, ascorbate, glutathione, selenium.

2) Non-nutrient—ceruloplasmin, transferrin, uric acid, peptides, camosine.

2. Second classification

Antioxidants found in nature—can be distributed into the following categories:

1) Enzymes, such as superoxide, hydroxyl, and glutathione peroxidase.

2) Low molecular weight antioxidants—these are additionally classified as:

- a) Tocopherol, carotenoids, bilirubin, and certain polyphenyl antioxidants are examples of lipid-soluble antioxidants.
- b) Water-soluble antioxidants such as ascorbic acid, uric acid, and polyphenyls.

3. Phenolics

Phenolics occur by far the most abundant phytochemical class and are found throughout the plant world. Flavonoids, phenolic acids, and polyphenols are the three major classes of nutritional phenolics. These were all hydroxyl group (-OH) containing chemical substances in which the (-OH) is directly connected to an aromatic hydrocarbon chain. In the category of natural substances, phenol (C₆H₅OH) is considered a basic class. They are plant's secondary metabolites that play a vital function as defence chemicals. Phenols have various beneficial qualities for beings, and their antioxidant capabilities are significant in identifying their role as anti-free radical-mediated disease processes. Flavonoids are the most well-known and biggest category of plant phenols. Phenolics are also classified as a broad category that includes the commonly utilized hydroxyl-benzoic and hydroxyl-cinnamic acids. Phenolic polymers, often referred as tannins, are high-molecular-weight substances classified in two main types: hydrolysable tannins and condensed tannins.

Flavonoids are polyphenolic chemicals found throughout ecosystems. Over 4,000 flavonoids have been identified, several of which are found in veggies, fruits, and consumables such as teas, coffee, and juice drinks. Those seems to have played a great effect in medical achievements from ancient times. They can be found in vascular vegetation as glycones, glucosides, and methylated compounds. Additionally, 4,000 flavonoids have been encountered in plant components ingested by mankind, with roughly 1,030 flavanols and 650 flavones being widely recognised. Flavanoids like as luteolin and catechins are more powerful antioxidants than nu-

trients such as vitamin C, vitamin E, and beta-carotene^[1].

Many therapeutic qualities have been claimed for them, including enzyme inhibition, anti-inflammatory activities, antimicrobial activities, anti-allergic activities, estrogenic action, powerful antioxidant action, vascular function, and cytotoxic anticancer activity. They are a diverse group of chemicals that play a crucial role in shielding raw mechanisms against the damaging effects of oxidation process on critical supermolecules such as carbs, proteins, lipids, and Genetic material.

4. Terpenoids (Terpenes)

Terpenes, commonly referred as isoprenoids, are the most abundant phytonutrient class in plant foods, soy plants, and grain. The significance of such terpenes to plants derives to their need for carbon via photosynthetic activity involving photosensitizing compounds. They are oligomers and polymers centered on photo-reactive chemistry. Terpenes were indeed tanins with just a heterogeneous group of high molecular mass polyphenolic molecules and the ability to form mutable and irrevocable developments with proteins (primarily), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids, and natural resources, among other things. Based on its structural and chemical properties, tannins may be classified into four distinct categories: gallo tannins, ellagitannins, complicated tannins, and condensed tannins. Tannin-containing plants are often used as astringents, diuretics, and stomach and duodenal remedies.

5. Beta-carotene

Spinach, carrot, sweet potato, mangoes, papaya, and oranges are high in beta-carotene, a natural vitamin A precursor.

Beta-carotene's actions include:

- Antioxidant free radical reduction/elimination.
- Immuno-modulation-promotion of a rise in cell-reconciled immune reaction (T-helper, NK cells, and cells with IL-2 receptors) as a result of enhanced monocytes expression and tumour necrosis

factor-alpha activity.

- Mutagenesis inhibition.
- Cancer cell growth inhibition^[2].

In places with low oxygen concentrations, beta-carotene is also employed to scavenge able radicals like peroxy and hydroxyl radicals. Serum beta-carotene concentrations have been demonstrated to decline in several oral premalignant lesions and disorders, and due to its supplementation (30 mg/day) has resulted in lesions reduction. As per Liede *et al.*^[3], a beta-carotene-enriched diet can help avoid alterations in the oral mucosa, particularly in smokers, who have lower blood amount of vitamin C and beta-carotene than non-smokers. In one trial, 23 individuals with oral leukoplakia have been given beta-carotene in oral dosages of 90 mg/day for three months. A third of the 18 individuals who finished the research had a full clinical recovery. No details or clinical signs of poisonousness were observed in several of the patients.

5.1 Adverse effects

Portion of the beta-carotene stored in fatty tissue serves as the reserve form of vitamin A and can be split into this vitamin in the event of a deficiency. To maximise benefits and minimise damage, dose evaluation in future research must be based on known knowledge. Two large-scale trials found deleterious effects when beta-carotene dosages exceeded 20 mg/day. The content of many other antioxidant in the organism is likewise affected by selective carotene supplementation. High dosages of carotene increase plasma concentrations of beta-carotene, lycopene, and vitamin E while decreasing lutein and zeaxanthin levels.

6. Retinoic acid (vitamin A)

Retinoic acid is found in carotene and poultry meat, milk, and eggs. Furthermore, retinoic acid is processed into retinal in the gut, and retinol and hypervitaminosis develop while ingestion surpasses the liver's capacity to retain retinoids. Vitamin A is essential for the correct differentiation of epithelial cells. Retinoids' impacts on gene expression have been shown to alter the proliferation and development of normal cells and cancer cells *in vitro*.

Because retinoids promote apoptosis, which leads to proper cell maturation, and prevent carcinogenesis, they have been studied in the regulation and control of carcinogenesis. It has also been shown that epithelial cells grow slower after being exposed to retinoids^[1].

Changes in enzyme activities and DNA alterations caused by free radical reactions increase the chance of establishing cancerous cell lines. Reducing free radicals with antioxidants like vitamin A may help to avoid such cellular alterations. A link has been shown between vitamin A deficiency and an increased susceptibility to carcinogenesis, with an increased chance of developing various epithelial carcinomas of the lungs, colon, pharynx, larynx, and oesophagus. Isotretinoin (13-cis retinoic acid) may inhibit the formation of leading squamous cell carcinoma. Treatment with 13-cis retinoic acid (Isotrenitin) can restore the production of retinoic acid receptors (RAR) mRNA, which itself is lost in pre-malignancy, indicating a hopeful response to the treatment^[2].

- Inhibits keratinization and epidermal cell terminal differentiation; improves cellular immunity.
- Stops or slows the development of leukoplakia.
- Causes cancer cells to be cytotoxic and cytostatic.
- Has an impact on DNA, RNA, and gene function^[3].

It was recommended that individuals with a large premalignant oral lesion start with 50 mg of 13 RA/d. Shah *et al.*^[4] cured 16 affected individuals having oral leukoplakia given topical dosages of 13-cis retinoic acid (Isotrenitin) ranging from 3 to 10 mg/day administered as lozenges for six months. 5 (31.2%) of the individuals discontinued participation due to adverse effects, while two of the three patients who demonstrated complete clinical remission experienced relapses within 5 weeks after stopping the drug. In oral leukoplakia, Stich *et al.*^[5] investigated combination beta carotene and retinol, beta-carotene alone, and palliative, yielding full response rates of 27.5 percent, 14.8 percent, and 3.0 percent, respectively.

6.1 Adverse effects

Toxicities at preventive dosages include skin rash, nasal mucosal dryness and bleeding, conjunctivitis, oral mucositis, cheilitis, hyper-tri-glycerinemia, and teratogenic consequences. Topical treatment provides the potential benefit of delivering a high local dosage with less systemic exposure, reducing the risk of systemic adverse effects. The topical treatment, on the other hand, necessitates patient motivation and the capability to cooperate with repetitive local applications. Furthermore, topical administration may be problematic in some areas, and the drug may well be weakened by saliva. Because of the acid medium of the topical preparations or the base in which the drug is put, local tissue irritation in the form of tissue sensitivities and burning may occur with current topical administrations^[2].

7. Lycopene

It is a red, fat-soluble pigment produced by plants and microbes. Millard found it in 1876 and extracted it from other red fruits and vegetables, such as rose grapefruits, melons, pink guavas, and apricots. Apart from these, it is found in fungi and algae. It is the non-cyclic isomer of beta-carotene, which is essentially a carotenoid. It is among the most powerful antioxidants available. Lycopene, unlike most of the other carotenoids, does not exhibit pro-vitamin A activity. In a three-month double-blind, placebo-controlled randomised controlled experiment undertaken by Singh *et al.*^[6], lycopene supplementation at 4 mg and/or 8 mg per day reduced hyperkeratosis in 80 percent of patients. Complete clearance of the lesions was observed in 55% of individuals receiving 8 mg/day and 25% of those receiving 4 mg/day. Lycopene was identified as a viable therapy option in a comprehensive evaluation of antioxidants in the cure of different leukoplakia. Gupta *et al.*^[7] discovered that eating tomato—the primary source of lycopene—has a preventive effect on oral leukoplakia when researching the link of certain nutrients and dietary products with oral precancerous lesions. It has happened proven to limit the growth of KB-1 human being oral tumour cells by regulating connex-

in-43 (gap junction proteins) levels, resulting in increased gap-junctional communication. The average daily consumption of lycopene is calculated to be 3.7 mg^[1].

A three-month randomised controlled experiment done by Zakrzewska *et al.*^[8] indicated clinical and functional improvement in individuals with oral leukoplakia treated with 4 mg and/or 8 mg lycopene per day. Positive histological alterations were also seen in individuals receiving 8 mg of lycopene per day^[8].

7.1 Adverse effects

Large amounts of dietetic consumption have no negative impact on an individual's wellbeing. To present, there is no evidence of lycopene treatment-related adverse effects or systemic toxicity. According to numerous safety investigations, no negative effects were observed at the greatest consumption amount, 5 g/kg/day^[8].

8. L-ascorbic acid (vitamin C)

Citrus fruits including kiwi, strawberries, papayas, and mangoes contain L-ascorbic acid (L-AA), often recognized as vitamin C. Adults in the United States should consume about 100 and 120 milligrammes of ascorbic acid per day. In the event of smokers, a daily consumption of almost 140 mg/day may lower L-AA concentrations in serum white cells. L-AA has had an anti-oxidizing function and interacts with superoxide generated by the cells' regular metabolic activities; this superoxide inactivation reduces the synthesis of nitrosamines throughout protein breakdown and helps to avoid damage to cellular DNA and cellular proteins. Other than being an antioxidant, L-AA does have the following influences:

- Slows the deterioration of vitamin E.
- Increases the activity of chemotaxis, phagocytosis, and collagen formation.
- Prevents the production of nitrosamines.
- Improves detoxifying via cytochrome P450.
- Prevents the production of fecal mutagens.
- Lowers oncogene expression^[4].

9. Alpha-tocopherol

Vitamin E refers to a trio of organic compounds that are substantially linked to alpha-tocopherol. Natural forms of vitamin E include four tocopherols, alpha, beta, gamma, and delta tocopherols, and four tocotrienols, alpha-, beta-, gamma-, and delta- tocotrienols. The most prevalent and effective form of vitamin E is alpha-tocopherol (AT). It can be found in vegetable oil, margarine, and green plants. The daily advised limit rates for male adults are 10.00 mg/day and 8.00 mg/day for grownup females^[4].

Ribeiro *et al.*^[9] investigated the toxicity and effectiveness of vitamin E in 43 individuals with OL using 400 IU twice each day for 6 months and discovered that ten patients (23%) had detailed clinical remission of lesion and 10 patients (23%) had a moderate clinical response. Tocopherol is an efficient antioxidant at elevated oxygen levels, shielding cellular membranes from lipid peroxidation. A dose of 800 IU/day for 6 to 9 months was regarded acceptable for chemoprevention of oral leukoplakia. AT's primary actions are as follows:

- Scavenging of free radicals.
- Membrane integrity and immune function regulation.
- Cancer cell progression/differentiation inhibition.
- Cytotoxic activity.
- Preventing mutagenicity and the generation of nitrosamines.
- Preventing the synthesis of DNA, RNA, and proteins in cancer cells.

Vitamin E has been recommended as an antioxidant in oral lesions by Chandra Mouli *et al.*^[10] Tobacco-specific nitrosamine (carcinogens) undergo a unique activation and detoxifying mechanism, which vitamin E can block. It has the potential to avoid oral cancer at an experimental stage, in premalignant lesions and premalignant situations. Many previous research show that antioxidants (vitamin E) can be used to treat oral mucosal diseases such as oral leukoplakia, oral lichen planus, oral submucous fibrosis, and oral cancer. In animal models, vitamin E shows synergistic inhibitory action against carcinogenesis and may have some

therapeutic benefits in humans. Many research employing vitamin E in oral leukoplakia found a good clinical response in 46% of 43 patients after 24 weeks, as well as a histological response with no major side effects. Another study discovered a substantial reduction for oral leukoplakia after combining vitamin E, retinol, and beta-carotene therapy^[8]. As a result, it shows promise for the treatment of leukoplakia.

9.1 Adverse effects

Synthetic vitamin E dosages utilised in antioxidant treatment may raise legitimate concerns, because in the United States, for example, 300 mg/d is the highest acceptable daily dosage of this supplement, and 2 g/d is already deemed hazardous. In comparison to vitamins A and D, vitamin E is only ostensibly harmless, i.e. somewhat non-toxic. When used in high doses, it can cause adverse effects (most notably during injections) by inhibiting 5-lipoxygenase in blood platelets and leucocytes (resulting in inadequate synthesis of thromboxane and leukotrienes), diminished blood coagulability (due to excessive suppression of platelet activation), and interruption of granulocyte and phagocyte anti-infective function relying on responsive oxidants.

10. Vitamin K

It is a fat-soluble 2-methyl-1, 4-naphthoquinone family that includes phyloquinone (K1), menaquinones (K2), and menadione (K3). One study discovered that vit K1 doses of 100–300 ng/ml inhibited cell proliferation. Vitamin K2 has been demonstrated to function at the cell cycle level, inhibiting the cell growth and initiating differentiation by acting on cyclins. The cytotoxic activity of vitamins C and K3 is characterised by autschizis, a cell death that is anatomically different from apoptosis and necrosis.

11. Green tea

Green tea polyphenols' anti-carcinogenic properties, primarily EGCG (epigallocatechin-3-gallate), the most biologically active catechin, are likely inhibiting the activity of tumor for-

mation and advancement, induced apoptosis, and inhibition of cell reproduction rates, all of which slow tumour growth and development. Green tea polyphenols' antioxidant capacity is directly tied to the aromatic rings and hydroxyl molecules that comprise their structure, and is a result of the hydroxyl groups' binding and neutralisation of free radicals. Epigallocatechin-3-gallate arrests cells in the G0-G1 stage, downregulates cyclin D1, increases p14ARF and/or p16 protein content, therefore stabilising p53 and regulating apoptosis, and inhibits angiogenesis by lowering VEGF phosphorylation and inhibiting VEGF release by tumour cells. Green tea extract used topically has been demonstrated to diminish the severity of oral leukoplakia^[4].

11.1 Adverse effects

Green tea has positive effects, however, excessive use of green tea has been linked to possible negative effects. Caffeine is a primary component, and consuming over than 5 cups of green tea per day may result in sleeplessness, restlessness, and stomach distress. It has been noticed that green tea is used for weight loss, therefore high quantities of caffeine may induce significant negative effects. Green tea should be used with caution for this purpose. Tannins found in green tea, such as catechin and epicatechin, bond with non-heme iron in human blood. This inhibits absorption of iron, which can result in iron deficiency anaemia. Iron deficiency anaemia can induce fatigue, breathlessness, irritability, headaches, and irregular heartbeat. It also includes vitamin K, which is why excessive use of green tea is discouraged, and individuals on warfarin, aspirin, or anticoagulants may prevent blood clotting^[11].

12. Neem

Gallic acids, catechin, and epicatechin are phytochemicals linked to oral cancer that include glutathione, a carcinogen-detoxifying enzyme. Catechin can block the formation of metalloproteinases, limiting cancerous cells invasion and migration and triggering death. It has anti-inflammatory properties by inhibiting the activity of nuclear factor-b

(NF-b), which causes tumor cells to die. It is antibiotic, antifungal, antihelminthic, anticancer, anti-inflammatory, and neuroprotective. It is used in mouthwashes to treat aphthous ulcers. Neem leaf extract has been linked to the treatment of oral cancer. It is available in both dry and oil forms^[12].

13. Curcumin

Curcumin is a bright yellow phenolic compound derived from turmeric. It is a perennial herb of the Zingiberaceae family. It has been utilised for generations in Indian conventional medical treatment for its anti-inflammatory properties. Its principal ingredients are three curcuminoids, including curcumin (the vital element and the one accountable for its yellow coloring and anti-inflammatory properties due to the compounds demethoxycurcumin and bis demethoxycurcumin). It is well-known for its anti-carcinogenic as well as other curative potential. Turmeric's active ingredients are the flavonoid curcumin (diferuloylmethane) and several volatile oils such as tumerone, atlantone, and zingiberone. Sweeteners, proteins, and resins are some of the other components.

Curcumin, which makes about 0.3–5.4 percent of raw turmeric, is the most well explored active ingredient. It has anticancer effect in the oral cavity, inhibiting cell proliferation and induction of apoptosis in oral tumor cells. It is also linked to notch-1, nuclear factor b (NF-b), and cyclooxygenase-2 (COX2), liquid oxygen (LOX), iNOS (inhibitory nitric oxide synthase), matrices metalloproteinase 9 (MMP-9), tumour necrosis response (TNF), chemokines, various cell-surface adhesion molecules, and cyclin D1 expression. It can boost the effectiveness of cancer therapy with cancer necrotic factor apoptosis-inducing ligand (TRAIL). Curcumin applied twice weekly towards the buccal pouches of Syrian golden hamsters inhibits DMBA-induced oral carcinogenesis. A daily dosage of up to 10 g is indicated to decrease tumour development, progression, and metastasis. Because oxidative stress can play a role in the pathophysiology of Oral Lichen Planus, and Oral Lichen Planus is a chronic inflammatory illness, herbs with both anti-inflammatory and antioxidants qualities may be

effective in controlling Oral Lichen Planus^[13].

13.1 Adverse effects

Researchers have identified unfavourable side effects connected with this polyphenol. Lao *et al.*^[14] performed a dose-escalation research in 34 healthy volunteers to establish the maximum tolerated dosage and tolerability of a sole oral dose of curcumin. The participants were given escalating dosages of curcumin varying from 500 to 12,000 mg, and their safety was evaluated 72 hours later. The experiment had twenty-four participants, seven of whom had mild toxicities that did not show up to be dose-related. These seven subjects particularly reported diarrhea, headache, rashes, and yellow stool. Curcumin at dosages varying from 0.45 to 3.6 g/day for 1 to 4 months was related with nausea and diarrhea, as well as an increase in blood alkaline phosphatase and lactate dehydrogenase levels in humans, according to another research. Curcumin dosages more than 8 g/day were unacceptably high in individuals with higher or premalignant lesions due to the bulky size of the pills. As a result, further research is needed to assess the long-term toxicity of curcumin before it could be licenced for human usage. It is generally regarded as harmless, however it may induce gastric irritation, stomach discomfort, nausea, diarrhea, allergic skin response, and anti-thrombosis activity that interferes with blood clot formation.

14. Cyanidins from grapes

Cyanidin is a pigment extract found in red berries such grapes, blackberries, cranberries, raspberry, apple, plum, red cabbage, and red onion. It has antioxidants and radical scavenging properties that may lessen the risk of cancer. It has been shown to decrease cell growth as well as the expression of the iNOS and COX-2 genes in colon carcinoma cells. Another research confirms this. According to Elattar *et al.*^[15], resveratrol in concentrations comparable to those found in red wines is an efficient regulator of oral squamous cell cancer cell growth and proliferation, hence contributing to its anti-tumor impact. Casto *et al.*^[16] investigated the efficacy of berry extracts to suppress the reproduction of

human tumour cells. According to research done by Selvendiran *et al.*, piperine has greatly extended its chemopreventive action by modifying lipid peroxidation and increasing antioxidant defence^[17].

15. Gingerol from gingers

Gingerol is the essential constituent of fresh ginger that gives it its characteristic spiciness. Ginger contains 1%–4% essential oils and an oleoresin, as well as zingiberene, curcumin, sesquiphellandrene, bisabolene, monoterpenoid aldehydes, and alcohols. It is antibacterial, anti-inflammatory, and analgesic. It has been examined for its anti-cancer properties in tumours of the colon, breast, ovary, and pancreas. Oyagbemi *et al.*^[18] have outlined the processes behind gingerol's medicinal actions. It reduces iNOS and TNF-alpha production by inhibiting IκBα phosphorylation and NF-κB nuclear translocation. When K562 and MOLT4 cells were treated with gingerol, the levels of ROS were much greater than in the control groups, triggering leukaemia cell death via the mitochondrial route. Gingerol and 6-shogaol were discovered to have anti-invasive effect against hepatoma cells by control of MMP-9 and TIMP-1, and 6-shogaol also controlled urokinase-type plasminogen activity in human hepatocarcinoma cells. Single dose of 6-shogaol, other active component of ginger, is more efficient than 6-gingerol and curcumin in suppressing TPA-induced expression of iNOS and COX-2 transcriptional activity in mouse skin, suggesting that additional *in vitro* and *in vivo* research is required. It is used to treat oral thrush as a sialogogue and to reduce toothache. Ginger may lessen the chemotherapeutic agent's toxicity^[13].

15.1 Adverse effects

Although the plant extracts seem to be quite safe, the most common adverse effects that have been recorded include headache, dizzy, agitation, nauseous, vomiting, diarrhoea, and cutaneous sensitivity. Ginkgo has been shown to suppress platelet-activating factors and to shorten bleeding times. As a result, caution was suggested among persons or individuals on anticoagulant treatment. This should not be taken during pregnancy or in

people suffering from biliary illness. Since ginger can interact with blood coagulation, it should be taken with caution in individuals taking anticoagulants like Coumadin or heparin^[12].

16. Capsaicin

Other compounds obtained from plants for spices, in addition to curcumin, have anti-cancer properties. Capsaicin, a spicy ingredient of pepper plants, is widely acknowledged to have anti-cancer characteristics, reducing growth and induction of apoptosis in a broad variety of human tumor cell lines, however, its genotoxic and cancerous capabilities have also been described in multiple papers. Capsaicin has been shown to decrease TPA-induced NF-B activation by inhibiting IB breakdown and consequent nuclear translocation of NF-B/p65. It also inhibits TNF- and TPA-induced adherence of AP-1 and NF-B to DNA binding sites in sentient leukaemia cells and causes apoptosis in human mammary epithelial cells by inducing JNK1 and p38 and depressing ERK. Furthermore, capsaicin therapy inhibits VEGF-stimulated angiogenesis via inhibiting VEGF-induced p38 MAPK and Akt stimulation. In a rodent study, capsaicin administration significantly increases the activity of phase II enzymes such as glutathione S-transferase and quinone reductase in the liver and colon of experimental F344 rats and reduces the advancement of azoxymethane (AOM)-induced colon pre-malignant lesions, aberrant crypt foci (ACF), as well as adenocarcinoma. Tanaka *et al.*^[19] also demonstrated that capsaicin supplementation inhibits chemically induced tongue tumorigenesis in male F344 rats. Capsaicin is a painkiller used in topical application to treat neuropathic pain due to its analgesic action. Capsaicin thereby desensitises stimuli produced by heat, chemical, and mechanical sources. Capsaicin has been proven in studies to be beneficial in people suffering from burning mouth syndrome.

16.1 Adverse effects

Capsaicin has an impact on skin (irritant, sensitizer), eye (allergen), ingestion, and inhaling (lung irritant, lung sensitizer). In mice, the LD50 is 47.2 mg/kg. Painful capsaicin-containing pepper expo-

sure are one of the most regular plant-related exposures reported to poison control centres. They induce searing or stinging discomfort to the skin and can cause nausea, vomiting, stomach pain, and blistering diarrhoea if consumed in large doses by adults or tiny amounts by children. Excessive tears, discomfort, conjunctivitis, and blepharospasm result with eye exposure.

17. Garlic

Garlic is a biological active ingredient that is utilised in Ayurveda to treat a variety of ailments. Allicin, the main component of garlic, is thought to have anti-inflammatory and immunomodulatory activities. Allicin has been shown to significantly reduce inflammatory product release, neutrophil migration, and bacterial and viral inhibition. They also inhibit oxidation and play an important role in immunological regulation. According to one study, allicin can be an efficient pain reliever, promote ulcer healing, and prevent the relapse of recurrent aphthous stomatitis.

17.1 Adverse effects

It has been observed that garlic extract causes a burning feeling in the gastrointestinal system, nausea, diaphoresis, and light-headedness. These extracts may also induce contact dermatitis, and excessive garlic consumption has indeed been linked to severe spontaneous spinal epidural hematoma.

18. Aloe vera

For millennia, people have recognised and used the *A. vera* plant for its medical and cosmetic qualities. The term *A. vera* is derived from the Arabic term *alloe*, which means bright bitter material, and *vera*, which means truthful in Latin. *A. vera*, the wonder plant, has been shown to be advantageous in a variety of health functions. Vitamins, enzymes, minerals, carbohydrates, lignin, saponins, salicylic acids, and amino acids are among the 75 potentially active ingredients. Vitamins A (beta-carotene), C, and E are present and act as antioxidants, helping to neutralise free radicals.

18.1 Adverse effects

In sensitive people, the detrimental consequences of aloe on a topical treatment may produce redness, burning, stinging, and, in rare cases, widespread dermatitis. Anthrax quinones including such aloin and barbaloin cause the majority of allergic responses. It is important to try it on a tiny area initially to rule out any allergic responses. Abdominal pains, diarrhoea, crimson urine, hepatitis, dependence, or deepening of constipation result with systemic ingestion of aloe vera. Long-term usage has been linked to an increased risk of colorectal tumor. Electrolyte imbalances may occur as a result of the laxative impact (low potassium levels).

19. Conclusion

Antioxidants are crucial in the treatment of numerous oral lesions and disorders, acting as a key adjuvant in halting the malignant potential of these illnesses. Thorough understanding of these phytochemicals is essential for clinicians in order to avoid the chronicity of lesions and hence reduce morbidity and death in patients with any recognised oral illness.

Conflict of interest

The authors declare no potential conflicts of interest.

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Evaluation of relationship between stress and periodontal disease in different professional college students

ABSTRACT

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Background: To evaluate the association between stress and periodontitis in different professional college students. **Subjects and Methods:** The study was conducted in 360 professional college students which includes Medical, Dental, Pharmacy, Nursing, Engineering, and Chartered Accountancy. From each group, 60 students were included in the study. Self-reported Depression, Anxiety, and Stress Scale questionnaire was given to all the students and clinical examination was conducted in all the participants to assess the Oral hygiene index - simplified (OHI-S), clinical attachment level (CAL), and Gingival Index Simplified (GI-S). **Statistical Analysis:** Statistics was performed using the ANOVA test, *post hoc* test, and Pearson correlation test to compare the psychological parameter of stress with periodontal parameters among six groups of students. $P < 0.05$ was considered to be statistically significant. **Results:** Group I showed higher mean stress scores (10.78 ± 0.76) compared to other groups. The mean OHI-S (1.61 ± 0.15), mean CAL (3.68 ± 0.79), and mean GI-S (1.43 ± 0.15) scores also increased with elevated stress levels among medical students. Followed by Group VI and Group II showed almost similar results. **Conclusion:** The present study showed strong association between stress and periodontal disease and have an adverse effect over oral hygiene factors among the students.

Keywords: Depression, Anxiety, Stress scale, oral hygiene status, periodontitis, stress

Periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession, or both.^[1]

College students often experience stress due to various reasons, of which educational demands also play a role.^[2] Stress is subjective and relative; something that may be stressful for one person may not be stressful for another.^[3] It is a state of physiological or psychological strain caused by adverse stimuli, physical, mental, or emotional, internal

or external, that tend to disturb an organism's functioning and which the organism naturally desires to avoid.^[4] The stress which is experienced by students may adversely affect their academic achievement, personal well-being, and long-term professional capabilities. Furthermore, acceleration of mental distress takes place, and it can have a negative impact on their cognitive functioning and learning.^[5]

In response to various stressful stimuli, a sequence of events is initiated. This stress can result in the deregulation

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of the immune system, leading to periodontitis by two mechanisms stated by Genco are based on physiological response mediated by the HPA axis (Hypothalamo-Pituitary Axis), sympathetic-adrenal medullary axis, and others by the person's psychological behavior. An increased concentration of corticotropin-releasing hormone from the hypothalamus occurs following the HPA axis activation due to stress that in turn, acts on the adrenal cortex releases cortisol into the circulation, which produces a multitude of effects throughout the body. The second major pathway to be activated is the sympathetic nervous system, which releases catecholamines results in the adrenal medulla's activation for the secretion of norepinephrine and epinephrine, which results in a range of effects that may act to modulate immune responses, which shows flight or fight response to potentially harmful stimuli.^[4]

Stress impairs the balance between pro-inflammatory and anti-inflammatory responses. Alterations in the levels of Gingival Crevicular Fluid, interleukin-1, interleukin-6 were mediated between stress and periodontal diseases which causes reduction in polymorphonuclear leukocyte chemotaxis, phagocytosis, reduced proliferation of lymphocytes.^[6] Acute necrotizing ulcerative gingivitis, aggressive periodontitis, and latent herpes virus infection are the three conditions associated with periodontium that is severely affected by stress.^[7]

Due to the various factors such as tight academic schedule, competition among the students who are designated as different professional students and they also suffer from the burden of achieving various targets between them contributing to psychological stress development. This excessive stress, in turn, increases the prevalence of psychological factors such as depression, anxiety. Literature suggests that psychological stress has a detrimental effect on oral health.^[8] Hence, the Depression, Anxiety, and Stress Scale was designed to fill the gap in detecting common mental disorders.^[9] One practical benefit of the Depression, Anxiety, Stress Scale-21 (DASS-21) is that it was designed as a single instrument to measure symptoms of depression, anxiety, and stress given by Lovibond and Lovibond.^[10,11] Hence, this study was aimed to evaluate the association between stress and periodontitis in different professional college students.

SUBJECTS AND METHODS

This study was approved by the Institutional Ethical Committee. The present study was conducted on 360 professional college students and the sample size was calculated using an effect size of 0.25 (small), alpha error-5%; power-95%; the number of groups-6. These 360 students were classified into six groups,

each group consisting of 60 students. These students were taken from 6 different colleges in and around Guntur. The six groups included were Group 1-Medical, Group 2-Dental, Group 3-Pharmacy, Group 4-Nursing, Group 5-Engineering, Group 6-Chartered Accountancy.

The primary objective of this study remains to be evaluation of stress and its association with periodontal disease among students in professional colleges, the choice of study subjects from different academic disciplines was made to develop a preliminary insight into potential differences in stress, which is the exposure variable in this study, between academic groups and also its differential association with periodontal disease should there be any.

Criteria for selection of students

Students who were systemically healthy and pursuing their final year in the age group of between 20 and 30 years were included in this study. Students who received periodontal treatment in the last 6 months, had a habit of smoking and alcohol consumption, who used any medication (antibiotics, anxiolytics, anti-depressants) in the previous 6 months were excluded from the study.

Questionnaire

The DASS is the only self-report scale that measures all three negative emotional states, i.e. Depression, Anxiety, and Stress altogether. It is designed to maximize discrimination between the three components it measures. In clinical and community samples, DAS scale had shown high reliability that is compatible with allocating the items to subscales, exhibiting high validity with other measures of anxiety and depression. In the present study, we have used stress questionnaire in DASS-21, a short form of the DASS-42, and had many advantages over the full-length version, which includes it takes less time to complete, more consistent, the questions retained from the original version are generally superior to those excluded.^[12]

Each component of the DAS scales contains seven items. So, only seven questions that were related to stress were selected. It also estimates difficulty relaxing, nervous arousal, and being easily agitated, irritable, and impatient. When completing the DASS-21, the respondent is required to present a symptom over the previous week. Each question has four specified alternative answers giving scores ranging from 0 to 3: Score 0-“never applied to me,” score 1 - “sometimes applied to me,” score 2 - “often applied to me,” and score 3 - “applied almost always to me.” Higher scores indicate greater stress levels in individuals.

Periodontal parameters

Along with the questionnaire, periodontal parameters like Oral hygiene index - simplified (OHI-S), clinical attachment level (CAL), GI-S were also assessed.

Procedure

A questionnaire was given to all the students included in the study, followed by clinical examination, wherein OHI-S, CAL, GI-S were assessed. The questionnaire duly filled by the participants should be given to the investigator in return once the clinical examination was completed.

Referral cards were issued to all the participating students who were found to have periodontitis to avail of oral health care at the corresponding teaching dental institutions.

Statistical analysis

Data were collected and entered into an MS Excel sheet, and results were analyzed using SPSS version software. Statistical analysis was performed using One-way ANOVA and Tukey's *post hoc* test with a value of $P < 0.05$ was considered to be statistically significant. One-way ANOVA test is used to test the differences in mean scores of DAS scale, OHI-S, CAL, GI-S between the groups. Tukey's Post-hoc test is a single-step multiple comparison test. It is used to find means that are significantly different from each other. Pearson's correlation test was performed to measure the linear correlation between two variables and has a value between +1 and -1.

RESULTS

The total number of participants who were subjected to analysis was 360. Data were collected from 6 academic streams, namely, Medical, Dental, Pharmacy, Nursing, Engineering, and Chartered Accountancy. The students who responded to the questionnaire were currently in their final year undergraduate with no gender discrimination. All the clinical parameters (OHI-S, CAL, GI-S) were evaluated with each group. The obtained data were subjected to appropriate statistical analysis, and results are discussed. The differences in the study parameters between the groups are shown in Table 1

Significant differences were observed between the study groups concerning mean stress, OHI-S, CAL, and GI-S scores ($P \leq 0.05$). As significant differences between groups were found for stress, OHI-S, CAL, and GI-S, Tukey's *post hoc* tests were done.

Stress

The mean score of stress is found to be highest in Group 1 (10.78 ± 0.76), followed by Group 6 (8.6 ± 0.49); Group 2 (8.4 ± 0.49), and the least score was found in Group 4 (3.35 ± 1.84). According to the interpretation values by Lovibond and Lovibond,^[10,11] it was found that the medical students fall under the category of having a moderate level of stress and that of dental and Accountancy group students fall under the category of

Table 1: Differences in the study parameters between groups

Parameter	Group	n	Mean±SD	F	P
Stress	1	60	10.78±0.76	291.75	0.001*
	2	60	8.4±0.49		
	3	60	4.1±2.03		
	4	60	3.35±1.84		
	5	60	3.4±2.03		
	6	60	8.6±0.49		
OHI-S	1	60	1.61±0.15	421.19	0.001*
	2	60	0.58±0.06		
	3	60	0.31±0.17		
	4	60	0.38±0.27		
	5	60	0.25±0.17		
	6	60	0.77±0.23		
CAL	1	60	3.68±0.79	386.18	0.001*
	2	60	3.1±0.7		
	3	60	0.63±0.39		
	4	60	0.82±0.57		
	5	60	0.6±0.46		
	6	60	3.3±0.47		
GI-S	1	60	1.43±0.15	421.94	0.001*
	2	60	0.55±0.15		
	3	60	0.31±0.17		
	4	60	0.32±0.18		
	5	60	0.27±0.18		
	6	60	0.6±0.15		

*Denotes statistical significance. One way analysis of variance (ANOVA); $P \leq 0.05$ considered statistically significant. ANOVA – Analysis of variance; OHI-S – Oral hygiene index - simplified; CAL – Clinical attachment level; GI-S – Gingival index - simplified

having mild stress. Other groups of academic students show normal stress [Table 1 and Figure 1].

OHI-S: The mean score of OHI-S is found to be highest in Group 1 (1.61 ± 0.15), followed by Group 6 (0.77 ± 0.23); group 2 (0.58 ± 0.06), and the least score was found in Group 5 (0.25 ± 0.17). Using interpretation values given by Green and Vermillion for OHI-S,^[13] medical students showed fair oral hygiene scores while other groups showed good oral hygiene status in terms of debris and calculus deposits on teeth [Table 1].

Clinical attachment level

The mean score of CAL is found to be highest in Group 1 (3.68 ± 0.79), followed by Group 6 (3.3 ± 0.47); Group 2 (3.1 ± 0.7) and the least score was found in group 5 (0.6 ± 0.46). Using CAL scores,^[14] Medical, Accountancy, and Dental students show stage II periodontitis, whereas other groups show no signs of periodontitis [Table 1].

GI-S: The mean score of GI-S is found to be highest in Group 1 (1.43 ± 0.15), followed by Group 6 (0.6 ± 0.15); Group 2 (0.55 ± 0.15), and the least score was found in group 5 (0.27 ± 0.18). In terms of GI-S scores,

using interpretation values given by Loe,^[15] moderate gingivitis was observed in medical students. In contrast, other groups showed mild gingivitis status of gingival conditions [Table 1].

The correlation and association between clinical parameters were shown in Table 2 by performing Pearson's correlation test. The correlation between stress and OHI-S showed a 0.86, and that of stress and CAL was found to be 0.92, with GI-S, 0.84 was observed. The correlation between OHI-S, and CAL, GI-S was found to be 0.77, 0.95, respectively, and that of CAL and GI-S was 0.77. All the scores showed a statistically significant strong correlation with a $P \leq 0.05$.

The scattered plot in Figure 2 shows the correlation between stress and OHI-S with $R^2=0.743$, indicating that 74.3% of the variance in OHI-S was predicted by stress. Figure 3 shows the scattered plot representing the correlation between stress and CAL with $R^2=0.847$, indicating that only 84.7% of CAL variance was predicted by stress.

DISCUSSION

Periodontitis is a chronic inflammatory disease in the periodontal tissue mediated by the host-associated plaque biofilms, leading to progressive destruction of tooth-supporting structures and loss of periodontal attachment. The bacteria present in the subgingival microbiota results in upregulated host immune-inflammatory response in the periodontal tissues characterized by increased production of inflammatory cytokines like interleukin, tumor necrosis factor-alpha, prostanoids like PGE2 and enzymes including MMP's. These upregulated pro-inflammatory mediators are responsible for majority of periodontal tissue breakdown that occurs including

alveolar bone resorption by the activation of osteoclasts. These changes result in the development of clinical signs of periodontal disease. The pathogenesis of the periodontal disease is modified mainly by local factors such as the

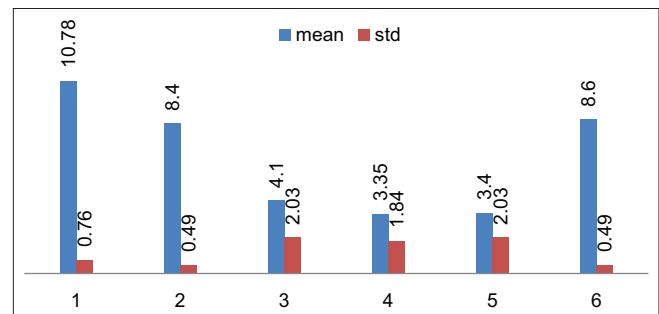


Figure 1: Bar chart showing differences in mean stress scores between the study groups

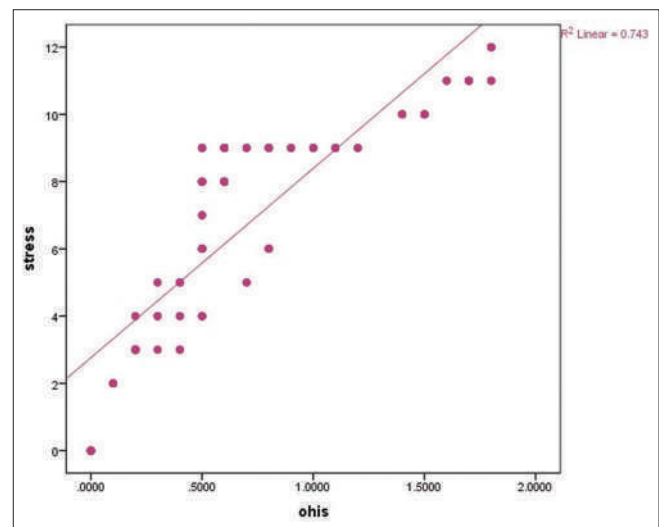


Figure 2: Scatter plot showing the correlation between stress and Oral hygiene index - simplified scores

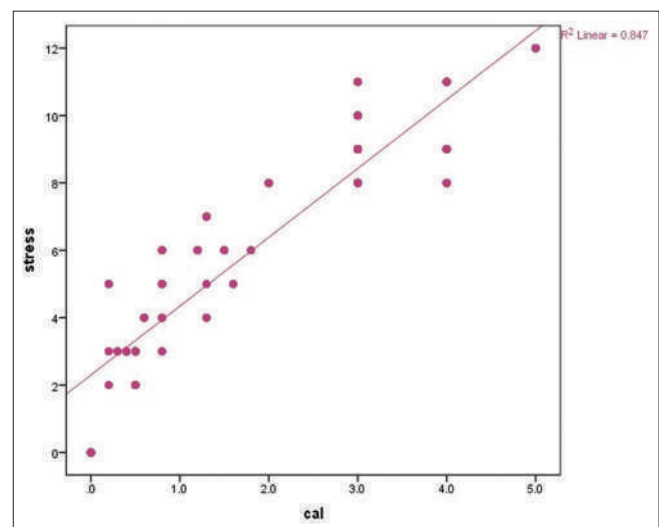


Figure 3: Scatter plot showing the correlation between stress and Clinical attachment level scores

Table 2: Correlation between the study parameters

	Stress	OHI-S	CAL	GI-S
Stress				
Pearson correlation		0.86*	0.92*	0.84*
Significance (two-tailed)		0.002	0.001	0.033
OHI-S				
Pearson correlation	0.86*		0.77*	0.95*
Significance (two-tailed)	0.002		0.000	0.000
CAL				
Pearson correlation	0.92*	0.77*		0.77*
Significance (two-tailed)	0.001	0.000		0.000
GI-S				
Pearson correlation	0.84*	0.95*	0.77*	
Significance (two-tailed)	0.033	0.000	0.000	

*Denotes statistical significance. Pearson's correlation test; $P \leq 0.05$ considered statistically significant. OHI-S – Oral hygiene index - simplified; CAL – Clinical attachment level; GI-S – Gingival index - simplified

presence of plaque and calculus, systemic diseases, and also environmental factors such as smoking and by genetic susceptibility.^[1]

In the present study, the relationship between stress and periodontal disease has been evaluated using the DAS scale's stress component. The results of our study suggest that the psychological property of stress in DAS scale is more related to group 1 (Medical students) when compared to other groups and these results are in accordance with a study conducted by Moayedi *et al.*, where they evaluated stress levels among medical, dental, pharmacy, and nursing students.^[16]

Our study is following other studies conducted by Behere *et al.*, where nursing students were least stressed compared to medical and engineering students;^[17] Mayildurai *et al.* evaluated the stress among only engineering students and stated that they are distressed in a significant level.^[18] Hou *et al.* stated that stress among accounting students might be due to 5 factors such as assistance, assessment, assignment, course difficulty, and presentation and career.^[19] Nor *et al.* stated that 92.4% of accountancy students experience stress.^[20] A study conducted by Kamble and Minchekar showed a positive correlation observed between stress and depression among college students.^[21]

Study conducted by Murphy *et al.* showed that dental students experience more stress than medical students except in professional identity.^[22] Taneja *et al.* stated that emotional distress is common among medical students.^[23] Among dental students, clinical year students were more stressful than nonclinical year students was stated by Sravani *et al.*^[12] Singh and Singh stated that professional students are more stressful than nonprofessional students.^[24] Noureen *et al.* showed that stress in nursing students might be due to anxiety, fear of handling patient, solicitude, fear of distinction by teachers and staff.^[25]

It has been stated that stress negatively influences oral health status.^[26,27] The present study revealed that OHIS, CAL, and GIS values were higher among medical students than in other groups. This increase in periodontal parameters was observed with increasing stress values. An increase in OHIS, CAL, GIS values was also evident, indicating that the students have poor oral hygiene practice under stressful conditions. A few studies suggest that students generally neglect their oral health care under conditions of stress, thereby showing poor oral status leading to periodontitis.^[28,29] These findings are in coincidence with the findings of our study. Similarly, another study conducted by Mahmood *et al.* suggested that there is strong evidence of an association between examination stress and periodontal parameters.^[30]

The present study evaluated the relationship between stress and periodontitis by observing the correlation of stress with periodontal parameters, which was strong. In this study, even though there is an increased level of awareness of oral hygiene practices among dental students compared to other students, there was no difference in the periodontal parameters between them.

The role of potential confounders such as oral hygiene practices and deleterious habits cannot be ignored while attempting to ascertain such direct association. However, it can be hypothesized that oral hygiene practices and acquisition of deleterious habits can be directly influenced by the stress levels of the subjects and these factors have a mediating role to play in the association between stress and periodontitis.

Certain limitations of the present study include no clear demarcation on the type of stress that the student is facing. A self-reported questionnaire that the responders may or may not reveal their true experience of stress, heterogeneous sex distribution.

CONCLUSION

Of the six groups, medical students had shown higher values of both stress and periodontal parameters than other groups. The accountancy group and dental group showed almost similar results regarding stress and the evaluated clinical parameters. Therefore, it was concluded that psychological factors have an adverse effect on the student's oral hygiene factors. The worsening of periodontal parameters could be due to the student's negligence because of stressful conditions. Hence, education of students regarding the relationship between the stress-periodontal disease (oral hygiene), thereby avoiding the stress-induced factor behind the negligence of oral hygiene.

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Conflicts of interest

There are no conflicts of interest.

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Perceptions and Preparedness of Dental Professionals toward COVID-19-Related Oral Manifestations in India

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Abstract

Background: With the reports of oral manifestations observed in coronavirus disease 2019 (COVID-19) patients snowballing day-by-day, it calls for the attention of dental professionals to keep themselves updated regarding these manifestations and how to prevent and manage them in COVID-infected patients. **Aims and Objectives:** The aim of this study is to assess the perceptions and preparedness of dental professionals in India toward the COVID-19-related oral manifestations. **Materials and Methods:** A cross-sectional, web-based survey was conducted on dental professionals using a pretested and validated questionnaire. Six hundred and twenty-three responses obtained from January 23, 2021 to February 15, 2021 were included in the study. **Results:** Mean knowledge scores regarding COVID-19-related oral manifestations were noted to be significantly high among males (13.5 ± 4.9), having a PhD (18.3 ± 5.8), belonging to the specialty of oral medicine and radiology (15.6 ± 4.7), with more than 15 years of clinical experience (15.4 ± 4.7) and practicing in metropolitan areas (13.7 ± 5.03). Tele-consultation and advising palliative care (65.5%) were the most preferred ways of managing COVID-19-related oral manifestations. Ninety-one percentage of the participants felt that inclusion of dentists in the intensive care unit multiprofessional teams, would contribute toward early diagnosis and management of oral manifestations. **Conclusion:** Study noted lower knowledge scores pertaining to COVID-19-related oral manifestations among BDS graduates with <5 years of clinical experience calling for the implementation of continuing dental education on the oral manifestations occurring in COVID-19 patients.

Keywords: Coronavirus disease 2019, coronavirus disease tongue, dental professionals, oral manifestations

INTRODUCTION

As the pandemic hit the world, it immensely shook everyone's lives affecting almost every sector including and mainly healthcare. Oblivious to what has befallen, researchers across the world delved into the whys and wherefores of the pandemic. Intriguingly, the research published in recent times disclosed the occurrence of oral manifestations in coronavirus disease 2019 (COVID-19)-infected patients. Chaux-Bodard *et al.*^[1] has first published a case of tongue ulceration noticed in a 45-year-old COVID patient. Zarch and Hosseinzadeh^[2] found dry mouth as a common oral manifestation of COVID-19 followed by dysgeusia and candidiasis after reviewing 170 cases in 17 studies. Favia *et al.*^[3] has categorized the oral lesions seen in COVID-19 patients into four groups based on features of the lesions, timing of their occurrence, and the treatments administered: (a) Probably, preexisting conditions (b) severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-related lesions

(c) Treatment-related lesions (d) Lesions related to poor oral hygiene.

The possible mechanisms for the occurrence of these oral manifestations in COVID patients are still ambiguous. Nonetheless, the oral cavity could be a potential target organ of SARS-CoV-2 owing to the expression of angiotensin-converting enzyme 2 and furin in oral tissues, which play a pivotal role in SARS-CoV-2 invasion of host cells.^[4] Hence, it is essential for oral health care providers to be aware of how COVID-19 and its treatments affect oral health. In this context, the present study

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aimed to assess the perceptions and preparedness of dental professionals toward COVID-19-related oral manifestations.

MATERIALS AND METHODS

Study design and population

A cross-sectional web-based study was conducted using an online questionnaire to assess the perceptions of dental professionals including dentists, postgraduates, and interns in India regarding the oral manifestations reported among COVID-19 patients and their preparedness to prevent and manage them. The dental professionals who were involved in patient care during the COVID pandemic were included in the study. The study was carried out from January 23, 2021 to February 15, 2021.

Questionnaire development and pretesting

Following an electronic search of the databases for the literature published on the impact of COVID-19 and its treatment on the oral cavity and the oral manifestations that were reported in COVID-19 patients, a preliminary questionnaire was constructed under three sections:

- Demographic data
- Perceptions toward COVID-19-related oral manifestations
- Preparedness toward COVID-19-related oral manifestations.

The questionnaire was reviewed for clarity, face, and content validity by a panel of experts, followed by pilot testing on participants. It was further evaluated for internal consistency reliability (Cronbach's alpha – 0.82) and test-retest reliability among 40 participants (Intraclass correlation coefficients – 0.92). The questionnaire contained 5 knowledge-related questions with multiple correct responses and a score of 1 was given for each correct response, which accounted to a maximum possible knowledge score of 29.

Sample size

Sample size was calculated using G*Power 3.1.9.2 software (University of Dusseldorf, Germany). Based on the observations by Arora *et al.*^[5] on the knowledge and preparedness of Indian dentists during COVID-19 pandemic, a sample size of 400 was deemed sufficient at an alpha level of 5% and power of 80%. The formula used for sample size calculations was $Z\alpha 2pq/d^2$.

Data collection

The revised questionnaire prepared using Google forms was shared through E-mail and Whatsapp to the dental health care professionals through convenience and snowball sampling. Participants were informed that their participation in the study will be anonymous and will be considered their consent to participate and publish the data provided by them. In fulfillment of the key considerations proposed by Wicher and Wu^[6] for determining the need for an ethics oversight for survey research, the present study excluded participants who are vulnerable or with diminished autonomy, and there was no greater than minimal informational or psychological

harms present to the participants. Hence an ethical clearance was deemed not to be necessary from an Institutional Review Board. A total of 623 responses obtained during the study period were included in the study.

Statistical analysis

The data were compiled into Microsoft excel sheet and subjected to statistical analysis using the Statistical Package for the Social Sciences-20.0 (IBM SPSS, Armonk, NY, USA). The statistical tests used to analyze the data were Chi-square test, Kruskal–Wallis ANOVA, and Mann–Whitney *U*-test.

RESULTS

Background characteristics of the participants

Among the 623 dental professionals who had participated in this study, 342 (54.9%) were females. The mean age of the participants was 32.03 ± 10.83 years. Slightly more than half of the study participants were working in urban areas (51.7%) and only 18.6% had clinical experience of more than 15 years [Table 1].

Participants' perceptions toward coronavirus disease 2019-related oral manifestations

Nearly, half of the respondents (46.1%) had either screened or treated COVID-19 infected or recovered patients for oral health problems. However, only 17.3% of the study participants reported coming across COVID-19 diagnosed or recovered patients presenting with oral manifestations. Among the participants, males and those having a PhD with more than 15 years of clinical experience belonging to the specialties oral surgery and orthodontics reported significantly ($P < 0.05$) higher experience in treating or screening for oral health problems and noticing oral manifestations in COVID-19 patients [Table 1]. Osteomyelitis, ulcerations, and candidiasis were the most reported oral manifestations that were noticed by the study participants in COVID-19 patients [Table 2]. Nearly, 80% held the notion that oral tissues are one of the target organs in COVID-19 infection, and agreed that COVID-19-infected patients might present oral manifestations along with systemic features. Less than 40% of the study participants were aware of the term “*COVID tongue*.”

Participants' knowledge toward coronavirus disease 2019-related oral manifestations

Among the COVID-19-related oral manifestations reported in the published literature, alteration or loss of taste sensation (66%), ulcerations and blisters (59.6%), and opportunistic infections (58.4%) were the most known manifestations to the participants, whereas, melanin hyperpigmentation (4.8%), fixed drug eruptions (8%), and petechiae (8.2%) were the least known. About 80% of the participants believed that COVID-19-infected patients with underlying systemic diseases were most susceptible to develop oral manifestations. About 76.7% opined that the oral manifestations develop secondary to deterioration of systemic health in COVID affected patients and opted underlying



Table 1: Correlation of participants background characteristics with their experience of providing oral health care for and noticing oral manifestations among coronavirus disease 2019 infected/treated participants (n=623)

Variable	Category (n)	Question 1		Question 2	
		Yes, n (%)	P	Yes, n (%)	P
Gender	Male (281)	190 (67.6)	<0.001*	67 (23.8)	<0.001*
	Female (342)	97 (28.4)		41 (12)	
Specialty	None (BDS) (228)	51 (22.9)	<0.001*	32 (13.7)	<0.001*
	Oral surgery (47)	42 (89.3)		19 (40.4)	
	Endodontics (61)	41 (67.2)		11 (18)	
	Orthodontics (33)	26 (78.8)		11 (33.3)	
	Prosthodontics (37)	26 (70.3)		10 (27)	
	Periodontics (113)	49 (43.4)		15 (13.3)	
	Pedodontics (49)	19 (38.8)		5 (10.2)	
	Public health dentistry (30)	14 (46.7)		2 (6.7)	
	Oral medicine and radiology (16)	12 (75)		1 (6.2)	
	Oral pathology (9)	7 (77.8)		2 (22.2)	
	Clinical experience (years)	<5 (346)		96 (27.7)	
5-10 (86)		42 (48.8)	10 (11.6)		
11-15 (75)		55 (73.3)	28 (37.3)		
>15 (116)		94 (81)	21 (18.1)		
Location of workplace	Metropolitan (159)	93 (58.5)	0.092	21 (13.2)	0.001*
	Rural (322)	132 (41)		66 (20.5)	
	Urban (142)	62 (43.7)		21 (14.8)	

Chi-square test; $P \leq 0.05$ considered statistically significant; *Statistical significance. Question 1: Have you had the experience of treating or screening participants who are diagnosed/treated for COVID-19?, Question 2: Have you seen participants who are diagnosed/treated for COVID-19 presenting with oral manifestations?, COVID-19: Coronavirus disease 2019

Table 2: Oral manifestations observed and the respective treatments provided to the coronavirus disease 2019 diagnosed/recovered patients as reported by the study participants

Oral manifestations in COVID-19 diagnosed/recovered patients	Treatments provided
Osteomyelitis (n=27)	Surgical debridement; resection; medication
Ulcerations (n=24)	Multivitamin supplements; topical analgesic gels
Candidiasis (n=9)	Antifungals
Tooth mobility (n=6)	Scaling and root planing followed by splinting; extraction
Gingival bleeding (n=5)	Prescribed chlorhexidine mouthwash. Oral hygiene instructions given. Scaling and root planing performed after patient tested COVID negative
Mucormycosis (n=4)	Resection and obturator placement
Severe bone loss (n=2)	Flap surgery
Multiple abscesses/sinus openings (n=2)	Antibiotics
Bruxism (n=1)	Night guard
Xerostomia (n=7)	Not specified
Gingival enlargement (3)	Not specified
Loss of taste and smell (n=2)	Not specified
Others* (5)	Not specified

*Aphthous stomatitis with burning sensation, tonsillitis and redness of tongue, halitosis, periodontitis, dry socket (1 each). n: Number of cases reported for each oral manifestation noticed by the participants, COVID-19: Coronavirus disease 2019

systemic diseases (69.3%) and poor oral hygiene (61.2%) as the predisposing factors. About 80.3% chose steroids and 52.3% chose antivirals as the drugs used for COVID-19 treatment which are more likely to cause COVID-19-related oral manifestations when used for longer duration [Table 3].

Upon comparison of background characteristics, male oral health care professionals (13.5 ± 4.9), having a PhD (18.3 ± 5.8), belonging to the specialty of oral medicine and radiology (15.6 ± 4.7), with more than 15 years of clinical

experience (15.4 ± 4.7), and practicing in metropolitan areas (13.7 ± 5.03) demonstrated significantly higher mean knowledge scores ($P < 0.05$) [Table 4].

Specialists in oral and maxillofacial surgery, oral medicine, and radiology showed significantly higher mean scores compared to those with no specialization after adjusting for variables such as gender, highest level of education, years of clinical experience, and location of work. Similarly, study participants with more than 10 years of clinical experience had higher mean

Table 3: Participants' knowledge toward coronavirus disease 2019-related oral manifestations (n=623)

Knowledge associated questions concerning COVID-19-related oral manifestations:	n (%)
COVID-19 patients who are most susceptible to develop oral manifestations:	
Asymptomatic patients	92 (14.8)
Long haulers (✓)	213 (34.2)
COVID-19 infected patients with underlying systemic diseases (✓)	497 (79.8)
Patients with severe COVID-19 infection requiring hospitalization (✓)	307 (49.3)
Oral manifestations that can be seen in covid-19 patients: (all the options were correct responses)	
Opportunistic infections	364 (58.4)
Ulcerations and blisters	371 (59.6)
Desquamative gingivitis	103 (16.5)
Fixed drug eruptions	50 (8)
Xerostomia	191 (30.7)
Gingivitis	160 (25.7)
Melanin hyperpigmentation	30 (4.8)
Altered/loss of taste	411 (66)
Recurrent oral HSV infections	80 (12.8)
Petechiae	51 (8.2)
Tooth loss	64 (10.3)
Necrotizing disease	158 (25.4)
Burning sensation	168 (27)
Tongue depapillation	211 (33.9)
Osteomyelitis	216 (34.7)
Occurrence of COVID-19-related oral manifestations is: (all the options were correct responses)	
Due to COVID-19 infection	272 (43.7)
Secondary to the deterioration of systemic health	478 (76.7)
Due to treatments for COVID-19	356 (57.1)
Predisposing factors for COVID-19-related oral manifestations: (all the options were correct responses)	
Poor oral hygiene	381 (61.2)
Trauma secondary to intubation	100 (16.1)
Underlying systemic diseases	432 (69.3)
Stress	315 (50.6)
Old age	275 (44.1)
Hyperinflammatory response secondary to COVID-19 infection	360 (57.8)
Drugs used for treatment of COVID-19 which are more likely to cause COVID-19-related oral manifestations when used for longer duration:	
Anti-viral (✓)	326 (52.3)
Anticoagulants	50 (8)
Steroids (✓)	500 (80.3)
Antimalarial	52 (8.3)
Supplements	58 (9.3)

COVID-19: Coronavirus disease 2019, HSV: Herpes simplex virus

knowledge scores compared to those with <5 years of clinical experience [Table 5].

Preparedness of the participants toward coronavirus disease 2019-related oral manifestations

Teleconsultation and advising palliative care based on the oral manifestations present (65.5%) was found to be the most preferred way of managing oral manifestations in COVID-19 patients by the study participants. More than 92% of oral health care professionals participated in this study opined that dental check-ups are necessary for COVID-19 affected participants on long-term steroid therapy in light of their vulnerability for opportunistic infections. Eighty-seven percentage agreed that COVID-19 patients on

long-term steroid therapy with preexisting systemic diseases require prophylactic antibiotics and antifungals to prevent the emergence of secondary infections. Furthermore, 91% held the notion that inclusion of dentists in the intensive care unit (ICU) multiprofessional teams would contribute toward early diagnosis and management of oral manifestations in COVID-19 patients.

DISCUSSION

The novel coronavirus disease was found to impact oral health with about 45% of the COVID-19-infected patients presenting with oral manifestations.^[7] In the present study, almost 80% of the participants agreed that COVID-19 patients



Table 4: Mean knowledge scores and differences based on the background characteristics of the participants (n=623)

Variable	Category	Mean±SD	P
Gender	Male	13.55±4.97	0.001**
	Female	10.49±4.27	
Specialty	None (BDS)	10.5±4.39	<0.001*
	Oral surgery	14.19±5.09	
	Endodontics	12.7±4.84	
	Orthodontics	15.03±3.19	
	Prosthodontics	13.59±4.27	
	Periodontics	11.15±5.34	
	Pedodontics	11.67±3.87	
	Public health dentistry	12.27±5.14	
	Oral medicine and radiology	15.63±4.71	
	Oral pathology	12±4.61	
Years of clinical experience	<5	10.67±4.35	<0.001*
	5-10	10.24±4.22	
	11-15	13.71±4.43	
	>15	15.48±4.74	
Location of workplace	Metropolitan	13.77±5.03	<0.001*
	Urban	11.18±4.72	
	Rural	11.31±4.36	

Kruskal-Wallis ANOVA; ^aMann-Whitney U-test; P≤0.05 considered statistically significant; *Statistical significance. SD: Standard deviation

Table 5: Multiple linear regression model showing characteristics significantly related to knowledge scores regarding coronavirus disease 2019-related oral manifestations (n=623)

Characteristic	β	SE	t	P
Gender (Reference:Female)				
Gender (male)	0.887	0.465	1.908	0.057
Specialty (Reference:BDS)				
Oral and maxillofacial surgery	1.595	0.758	2.105	0.036*
Endodontics	-0.06	0.7	-0.094	0.925
Orthodontics	1.014	0.939	1.081	0.28
Prosthodontics	1.273	0.827	1.541	0.124
Periodontics	0.09	0.561	0.161	0.872
Pedodontics	-0.311	0.745	-0.417	0.676
Public health dentistry	0.167	0.89	0.188	0.851
Oral medicine and radiology	3.193	1.202	2.656	0.008*
Oral pathology	0.813	1.537	0.529	0.597
Clinical experience (Reference: <5 years)				
5-10	-1.11	0.601	-1.862	0.063
11-15	1.99	0.69	2.886	0.004*
>15	3.034	0.652	4.653	0.001*
Location (Reference: Metropolitan)				
Urban	-1.341	0.461	-2.91	0.004*
Rural	-1.697	0.531	-3.19	0.001*
Intercept	10.675	0.724	14.734	0.001*

R=0.429; R²=0.184; Adjusted R²=0.166; P≤0.05 considered statistically significant; *Statistical significance. SE: Standard error

might present oral manifestations and believed that oral tissues are one of the target organs in COVID-19 infection. Less

than 20% of the study participants reported coming across COVID-19 diagnosed or recovered participants presenting with oral manifestations. This could be due to missed diagnosis and reporting of the cases rather than less incidence of the cases alone. The mean knowledge scores concerning COVID-19-related oral manifestations was relatively high among participants having a PhD, with more than 10 years of clinical experience compared to BDS graduates with clinical experience of <5 years which could be attributed to their limited exposure to the current research data regarding the disease processes and manifestations.

Our study found that among the various COVID-19-related oral manifestations, alteration or loss of taste sensation (66%), ulcerations and blisters (59.6%), and opportunistic infections (58.4%) were known to the majority of the participants. This is in accordance with the occurrence of the findings reported by Santos *et al.*,^[8] where they stated gustatory disorders as most commonly noticed manifestations followed by oral mucosal lesions.

More than half of the participants believed that the occurrence of COVID-19-related oral manifestations is secondary to deterioration of systemic health and due to treatments used for COVID-19, mostly due to long-term usage of steroids and anti-virals. Brandini *et al.*^[9] suggested possible etiologies for the occurrence of these oral lesions:

- Direct or indirect interaction of SARS-CoV-2 with oral cells resulting in disruption of tissue integrity,
- Adverse reactions of the drugs used for COVID treatment triggering lesions of herpes simplex virus, candida, xerostomia, nonspecific ulcerations, and gingivitis and
- Hyperinflammatory, as well as immunosuppressive conditions induced due to COVID infection might cause viral reactivation and promote the growth of opportunistic organisms.

More than half of the study participants believed underlying systemic diseases, poor oral hygiene, hyperinflammatory response secondary to COVID-19 infection, and stress as the predisposing factors for the oral manifestations seen in COVID patients. On similar note, Fidan *et al.*^[10] supposed that these lesions were a consequence of various factors including poor oral hygiene, stress, and systemic infections. Viral invasion and dysbiosis induced by the use of therapeutic drugs might also be involved in the occurrence of oral lesions in COVID-19 patients.^[11] Among the various drugs used for the treatment of COVID-19, when used for longer duration, steroids were known to cause opportunistic infections and anti-virals can lead to dry mouth, taste disturbances, ulcerations, erythema multiforme, and parotid lipomatosis.^[12,13]

The infectious nature of this novel disease makes it challenging to adhere with the routine oral disease diagnosing and managing strategies. The study participants preferred the use of teleconsultation and advice palliative care based on the oral manifestations present. Guo *et al.*^[14] proposed that acute oral mucosal lesions treatments were based on the type of the lesion



seen and advised the use of systemic antibacterials, antivirals, antihistamines, immunosuppressants, and low-to-medium doses of glucocorticoids as required with topical adjuncts such as anti-inflammatory gargle and ointment, chlorhexidine and povidone-iodine solution, and topical glucocorticoid preparations, to ease the pain and promote healing of lesions. They also advised the use of web-based consultations and lesion photographs to aid in the diagnosis of oral-mucosal lesions, especially for patients with active COVID infection.

The majority of participants as preventive strategies, reckoned that dental check-ups are necessary for COVID-19 patients who were on long-term steroid therapy and believed that these patients might require prophylactic antibiotics and antifungals to prevent the emergence of secondary infections, especially those with systemic comorbidities. Santos *et al.*^[15] stated that dental follow-ups are necessary for the COVID patients following dismissal from the hospital. They also highlighted the role of dentists in maintaining the oral health of the ICU-admitted COVID-19 affected patients. In agreement with this, 91% of the participants deemed that inclusion of dentists in the ICU multiprofessional teams would contribute toward early diagnosis and management of oral manifestations in COVID-19 patients.

CONCLUSION

The myriad of oral manifestations occurring in COVID patients appears to be multifactorial in origin with varying severity. Although the majority of the reported cases are not fatal, certain conditions such as osteomyelitis and necrotic diseases can wreak havoc, if left untreated and needs to be diagnosed at the earliest. Dental professionals should scrutinize for the presence of oral lesions while treating patients who were infected and treated for COVID-19 to provide oral care and improve patient's quality of life. It is also imperative to develop guidelines for diagnosing and managing the COVID-19-related oral manifestations.

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Conflicts of interest

There are no conflicts of interest.

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Effect of nonsurgical periodontal therapy and smoking status on hematological variables related to anemia of chronic disease in chronic periodontitis patient: a case-control study

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Background: Chronic infectious, inflammatory, or neoplastic disorders are associated with anemia of chronic disease. Chronic inflammatory diseases such as periodontitis may contribute to masked anemia, especially in smokers. This study was aimed at verifying and comparing the efficacy of nonsurgical periodontal therapy (NSPT) for improving anemia among chronic periodontitis patients with and without the habit of smoking.

Methods: Thirty systemically healthy individuals with chronic periodontitis were divided into two groups of 15 each, smokers (group A) and nonsmokers (group B). The groups were compared based on hematological parameters such as serum erythropoietin (SE) and serum ferritin (SF) levels at baseline and 3 months after NSPT for anemia evaluation.

Results: The baseline SE levels in groups A and B were 11.84 and 15.19 mIU/mL ($p=0.031$), respectively; the corresponding levels at 3 months after NSPT were 13.00 and 17.74 mIU/mL ($p=0.022$). The baseline SF levels in groups A and B were 95.49 and 44.86 ng/mL ($p=0.018$), respectively; the corresponding levels at 3 months after NSPT were 77.06 and 39.05 ng/mL ($p=0.009$). Group B showed a significant increase and decrease in the SE and SF levels, respectively, at 3 months after NSPT ($p=0.035$ and $p=0.039$, respectively), whereas group A showed insignificant changes ($p=0.253$ and $p=0.618$, respectively).

Conclusion: NSPT led to an improvement in anemia among chronic periodontitis patients. However, the improvement is less in smokers compared to that in nonsmokers. Furthermore, SF and SE levels might serve as effective biomarkers for assessing anemia in smokers and nonsmokers with chronic periodontitis.

Keywords: Anemia; Chronic periodontitis; Erythropoietin; Ferritin; Smoking

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Introduction

Chronic infectious, inflammatory, or neoplastic disorders are associated with anemia of chronic disease (ACD) [1]. Pathophysiological factors such as limited iron availability to erythroid progenitor cells, a blunted response to erythropoietin, erythrophagocytosis, diminished erythropoiesis, and microbial/tumor cell infiltration of bone marrow contribute to the development of ACDs. Patients with ACD demonstrate diminished levels of serum iron, normal to elevated serum ferritin (SF) levels, and normocytic to microcytic anemia [2]. Periodontitis is a chronic inflammatory disease characterized by increased production of inflammatory cytokines that can contribute to the prevalence of anemia by directly inhibiting erythropoiesis and inducing changes in iron absorption and release [3].

Patients with chronic periodontitis (CP) who are regular smokers show lower gingival redness and bleeding on probing due to potential vasoconstriction caused by the nicotine content in tobacco. This may lead to an inaccurate assessment of periodontal status and failure to diagnose the underlying pathogenic state [4]. In addition, smoking causes an increase in hemoglobin (Hb) concentration mediated by carbon monoxide, which bonds with Hb and forms inactive carboxyhemoglobin (COHb) with a reduced ability to deliver oxygen to the tissues. Therefore, as a compensatory mechanism, smokers maintain a higher Hb level than nonsmokers, which is referred to as secondary polycythemia [5,6]. Despite the higher Hb levels found in smokers, this hypoxic state triggers erythropoietin production, thereby increasing erythropoiesis. Erythropoietin is a large glycoprotein hormone produced by the peritubular cells lining the kidneys and hepatocytes; it is the principal regulator of the erythrocyte lineage [7]. This clearly implies that the underlying anemic state in smokers is masked by high Hb values, which may lead to an underestimation of the prevalence of anemia among smokers [8]. In such a scenario, an estimation of serum erythropoietin (SE) levels may aid in assessing the anemia status of smokers.

In chronic inflammatory conditions such as CP, proinflammatory cytokines such as interleukin (IL)-1 α , IL-1 β , IL-6, tumor necrosis factor- α , and transforming growth factor- β not only increase hemolysis and impair erythropoiesis via direct bone marrow suppressive effects but also release reactive oxygen species that inhibit erythropoietin gene expression [9]. Singh et al. [10] and Hutter et al. [11] found lower erythropoietin levels in patients with CP, thereby strengthening the hypothesis that CP may lead to ACD.

Acute-phase proteins are biomarkers that show changes in plasma concentration that increase (positive acute-phase proteins), such as ferritin, or decrease (negative acute-phase proteins) by at

least 25% during inflammatory disorders, due to their altered production by hepatocytes [12]. Ferritin serves as the main iron storage protein in the body and contains 20% iron by weight. The serum iron concentration is directly proportional to its storage in the body, which increases under inflammatory conditions and iron overload. Chakraborty et al. [13] observed that SF levels were higher in patients with CP than in healthy controls, and a reduction was noted with remission of chronic inflammation following nonsurgical periodontal therapy (NSPT). Considering the prevalence of periodontal disease in the community at large, its deleterious effects on the systemic health of affected individuals, the increased use of tobacco in the form of cigarette smoking, and the resultant masking of anemia status, therapeutic measures such as scaling and root planning (both NSPTs) might significantly improve anemia status in such individuals.

The available literature suggests that NSPTs were not tested while evaluating erythropoietin levels in smokers and nonsmokers with CP. To date, only a few studies have evaluated NSPTs with a single hematological parameter to assess ACD in CP cases. Hence, multiple hematological parameters, such as SF and SE levels, collectively might serve as a better means to assess the anemia status of smokers with CP instead of any single parameter. The aim of the present study was to verify and compare the efficacy of NSPT in improving anemia status among patients with CP who do or do not smoke.

Methods

Ethical statements: This study was carried out following CONSORT (Consolidated Standards of Reporting Trials) guidelines and written informed consent was obtained from all participants who fulfilled the inclusion criteria and agreed to participate voluntarily. Details about the nature, risk, and benefits of the hematological investigations as well as the associated procedures were explained to all participants. The experimental protocol and consent form were approved by the Institutional Ethical Committee and Institutional Review Board (IRB) of Dr. R. Ahmed Dental College and Hospital (IRB No: DCH/07/18-19).

1. Study design

This clinico-biochemical study included 30 systemically healthy patients diagnosed with CP stage I/II (probing pocket depth of ≥ 4 mm but < 6 mm) requiring NSPT who were selected from the outpatient Department of Periodontics [14]. CP was confirmed clinically and radiographically according to the guidelines of the

2017 Periodontology Consensus Report [14]. Subjects were divided into two groups ($n = 15$ per group) based on their smoking history. Group A included current smokers (i.e., individuals with a history of smoking ≥ 100 cigarettes in their lifetime and currently smoking) and group B included never smokers (i.e., individuals who never smoked/history of smoking < 100 cigarettes in their lifetime) [15]. Pregnant and lactating mothers, former smokers, and those with any history of systemic illness, history of iron supplements or blood loss, any periodontal surgery in the last 6 months, and pocket depth ≥ 6 mm were excluded from the study.

After careful periodontal examination and diagnosis, venous blood samples were obtained in the early morning to avoid diurnal variations in SE levels. Peripheral venous blood (4 mL) was obtained by venipuncture (Mokshy Surgicals Ltd., Mumbai, India) in the antecubital fossa from each participant selected for hematological tests. The blood was transferred to non-vacuum clot activator (coated with micronized silica) blood collection tubes. The collected blood samples were kept at room temperature for approximately 2 hours to allow the blood to clot, and serum was obtained after centrifugation for 10 minutes at 2,500 revolutions per minute (Remi Elektrotechnik Ltd., Thane, India). The serum was then assayed for baseline periodontal and hematological parameters (SF and SE). The selected subjects in both groups then received NSPT, including ultrasonic scaling, root planing, and polishing, as required. Thorough oral hygiene instructions and demonstration of proper brushing technique were provided. The patients were asked to return at 1- and 3-month intervals for follow-up, and additional oral prophylaxis was administered at those times if required. All periodontal interventions were performed by an expert periodontist who was unaware of the specific grouping of the subjects. The final hematological data were recorded at the 3-month follow-up, and statistical analysis of the data was carried out. Oral hygiene was maintained at an optimal level during the study period.

SF levels were measured using an Access Immunoassay System and analyzed using an automated analyzer (Beckman Coulter Immunoassay System, Brea, CA, USA). SE levels were assessed using an enzyme-linked immunosorbent assay (ELISA) kit for erythropoietin and an Adonis ELISA plate reader system (Triveni Traders & Diagnostic Private Ltd., Thane, India).

2. Statistical analysis

The Shapiro-Wilk test was performed to assess the assumption of normality of the data. Data are presented as mean \pm standard error of the mean (SEM). An unpaired Student *t*-test was performed to compare the parameters of the two groups that showed normal distributions. Normally distributed paired data of each group were

compared using a paired Student *t*-test. Non-normally distributed unpaired data were evaluated using the Mann-Whitney *U*-test, and paired data were evaluated using the Wilcoxon matched-pairs signed-rank test. Correlations between two normally distributed parameters were evaluated using the Pearson correlation test. Sex distribution between the two groups was evaluated using Fisher exact test. The correlation between two non-normally distributed parameters was evaluated using Spearman nonparametric correlation. Direct and inverse correlations were indicated by positive and negative correlation coefficient (*r*) values, respectively. An absolute value of *r* of 1.0 to 0.5, 0.5 to 0.3, 0.3 to 0.1, and < 0.1 was considered strong, moderate, weak, and no correlation, respectively. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using Graph Pad Prism ver. 5, 2007 (Graph Pad Software Inc., San Diego, CA, USA).

Results

The Shapiro-Wilk test and visual inspection of the histograms and quantile-quantile plots suggested that the collected data were normally distributed. In the present study, the ages (mean \pm SEM) of the participants in groups A and B were 47.73 ± 2.33 years and 41.93 ± 2.79 years, respectively. An unpaired Student *t*-test showed that the mean age of the participants was not statistically different between the groups ($p = 0.122$) (Table 1). Regarding sex distribution between the groups, group A had nine male patients and six female patients, and group B had seven male patients and eight female patients. A chi-square test showed that there was no statistically significant difference in the sex distribution ($p = 0.457$). Hence, confounding variables, such as age and sex, did not affect the study results (Table 1). Group B showed higher SE levels than group A both at baseline (group A, 11.84 ± 1.24 mIU/mL and group B, 15.19 ± 0.79 mIU/mL; $p = 0.031$) as well as after 3 months of periodontal intervention (group A, 13.00 ± 1.40 mIU/mL and group B, 17.74 ± 1.36 mIU/mL; $p = 0.022$) (Table 2, Fig. 1). No significant alteration in the SE level was observed in group A

Table 1. Demographic details of the different study groups

Demographic variable	Group A	Group B	<i>p</i> -value
No. of patients	15	15	
Age (yr)	47.73 ± 2.33	41.93 ± 2.79	0.122 ^{a)}
Sex, male:female	9 (60.0):6 (40.0)	7 (46.7):8 (53.3)	0.457 ^{b)}

Values are presented as number only, mean \pm standard error of mean, or number (%).

Group A, smokers; group B, nonsmokers.

^{a)}Analyzed by independent samples *t*-test; ^{b)}analyzed by chi-square test. $p < 0.05$, statistically significant.

Table 2. Intergroup comparison of serum erythropoietin levels (mIU/mL) at baseline and at 3 months of treatment

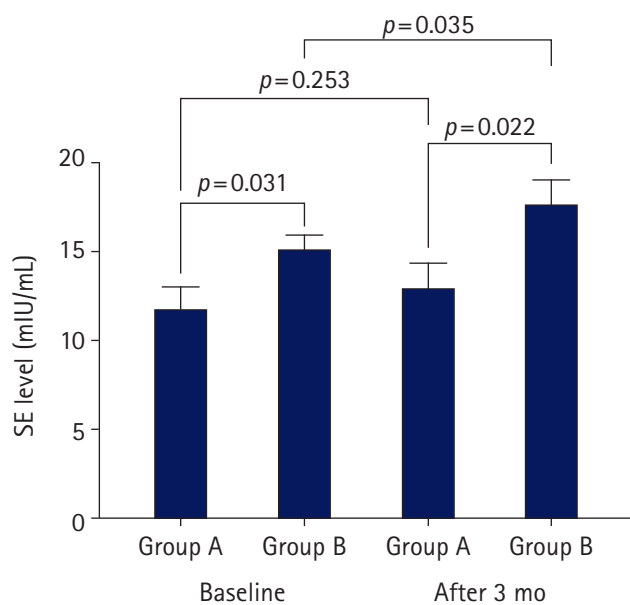
Time	Serum erythropoietin level (mIU/mL)		<i>p</i> -value ^{a)}
	Group A (n = 15)	Group B (n = 15)	
Baseline	11.84 ± 1.24	15.19 ± 0.79	0.031
At 3 mo	13.00 ± 1.40	17.74 ± 1.36	0.022
<i>p</i> -value ^{b)}	0.253	0.035	

Values are presented as mean ± standard error of mean.

Group A, smokers; group B, nonsmokers.

^{a)}Analyzed by independent samples *t*-test; ^{b)}analyzed by paired samples *t*-test.

p < 0.05, statistically significant.

**Fig. 1.** Evaluation of serum erythropoietin (SE) levels. Group A, smokers; group B, nonsmokers.

(*p* = 0.253) after 3 months of periodontal therapy (Table 2). However, the SE level in group B increased significantly (*p* = 0.035) after 3 months compared to the baseline level (Table 2, Fig. 1).

Considering the SF level data, group A showed statistically significant higher values of SF than group B at baseline (group A, 95.49 ± 21.53 ng/mL and group B, 44.86 ± 4.69 ng/mL; *p* = 0.018). Whereas group B showed statistically lower (group A, 77.06 ± 14.06 ng/mL and group B, 39.05 ± 5.46 ng/mL; *p* = 0.009) SF level compared to group A after 3 months of NSPT (Table 3, Fig. 2). However, no significant alteration (*p* = 0.618) in SF level was observed in group A after 3 months of treatment compared to the baseline level. However, the SF level in group B decreased significantly (*p* = 0.039) after 3 months of NSPT compared with the corresponding baseline level (Table 3, Fig. 2).

Table 3. Intergroup comparison of serum ferritin levels (ng/mL) at baseline and at 3 months of treatment

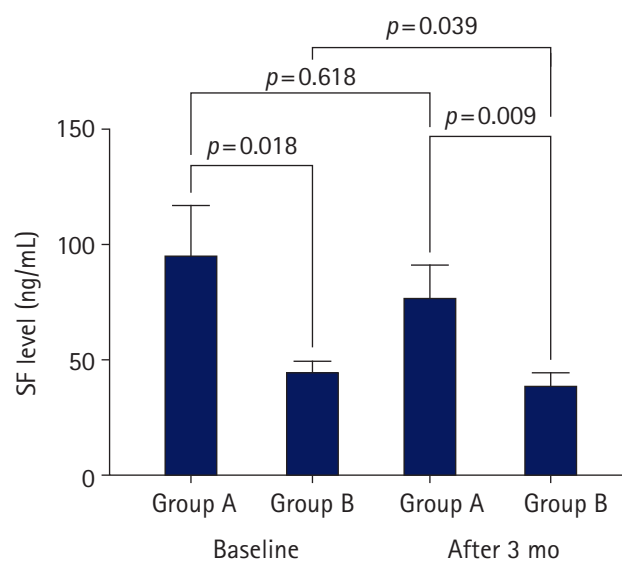
Time	Serum ferritin level (ng/mL)		<i>p</i> -value ^{a)}
	Group A (n = 15)	Group B (n = 15)	
Baseline	95.49 ± 21.53	44.86 ± 4.69	0.018
At 3 mo	77.06 ± 14.06	39.05 ± 5.46	0.009
<i>p</i> -value ^{b)}	0.618	0.039	

Values are presented as mean ± standard error of mean.

Group A, smokers; group B, nonsmokers.

^{a)}Analyzed by independent samples *t*-test; ^{b)}analyzed by paired samples *t*-test.

p < 0.05, statistically significant.

**Fig. 2.** Evaluation of serum ferritin (SF) levels. Group A, smokers; group B, nonsmokers.

Discussion

The underlying anemia status in smokers may be masked by relatively higher Hb values, which are usually tested to assess anemia. This may lead to an underestimation of the prevalence of anemia among smokers. The available literature suggests that only a few studies have analyzed the efficacy of periodontal interventions, such as NSPT, on the anemia status of such patients by assessing SE or SF.

In the present study, the baseline mean SE levels were significantly lower (*p* = 0.031) in smokers with CP than in nonsmokers with CP. In addition, SE levels were negatively correlated with smoking at baseline. These findings are consistent with those of Tanabe et al. [16] and Eisenga et al. [17]. During daytime smoking, higher SE levels lead to erythrocytosis, which in turn inhibits

further erythropoietin production through a negative feedback loop. Endogenous circulating erythropoietin with a half-life of 6 to 8 hours would result in low SE in the morning hours when blood samples are usually drawn [17]. This phenomenon is supported by the circadian rhythm of SE levels described by Miller et al. [18]. An alternative explanation may be derived from the study by Weinberg et al. [19] who observed the JAK2 V617F mutation in cigarette smokers and suggested that the erythrocytosis observed in smokers occurs via an erythroid cell-intrinsic erythropoietin-independent mechanism. They also stated that this may be an unidentified direct effect of smoking on erythropoiesis. Chronic smoking initially induces an increase in erythrocyte volume, plasma volume, and erythropoietin concentration, the latter of which is reduced when the erythrocyte volume increases. Hence, erythropoietin production represents a balance between stimulation by hypoxia and negative feedback by increasing erythrocyte volume [14]. The release of proinflammatory cytokines from peripheral neutrophils and various parameters of inflammation in plasma seem to be affected more by cigarette smoking than periodontal disease, which might contribute to the downregulation of erythropoietin production. Elevation of these inflammatory mediators leads to inhibition of the hormone erythropoietin and erythropoiesis, leading to the development of anemia [20].

In the present study, 3 months following the NSPT intervention, SE levels increased from baseline values in group A, although they were not statistically significant ($p = 0.253$). In contrast, the group B patients showed a statistically significant ($p = 0.035$) improvement in SE levels following periodontal intervention. This is in agreement with the results of Miller et al. [21], who failed to detect differences in SE levels when the COHb concentration changed following smoking cessation. As a possible explanation, they mentioned that small changes in COHb were not sufficient to trigger an erythropoietin response in persons with normal lung function. In the present study, the mean SF levels at baseline were significantly higher ($p = 0.018$) in group A than in group B. Ghio et al. [22] supported this finding of increased SF levels among smokers compared with those among nonsmokers. They correlated this finding with the systemic accumulation of iron after cigarette smoke exposure and concluded that cigarette smoke alters iron homeostasis both in the lung and systemically. However, that study did not include patients with CP. Contradictory findings were obtained in a study by Erdemir et al. [23], who noted similar SF levels among smokers and nonsmokers. These findings were in agreement with those of patients with ACD, who had normal to elevated SF levels. However, the possible cause of the similar SF values in both groups was not explained in that report. A cross-sectional study conducted by Prakash et al. [24] assessed the anemia status

of nonsmoking patients with CP by evaluating various hematological parameters. No significant changes in SF levels were observed between the study groups. In the present study, the mean SF levels in group B were significantly lower ($p = 0.0397$) at 3 months after NSPT than at baseline. This is in agreement with the study of Chakraborty et al. [13], who detected relatively higher SF levels in smokers with CP than in nonsmokers with CP, and these levels were restored to normal following NSPT intervention. The mean SF levels in the present study at 3 months after NSPT were reduced in group A compared to baseline; however, the difference was not statistically significant ($p = 0.618$). The available literature does not include any comparable studies.

One limitation of the present study is its relatively small sample size. Further studies involving larger sample sizes and other parameters may be conducted in the future if required.

Overall, NSPT leads to a relative increase in SE levels and a relative decrease in SF levels, thereby indicating an improvement in the anemia status of both smokers and nonsmokers. However, the magnitude of the changes was less in smokers. Hematological parameters such as SF and SE might serve as effective biomarkers for assessing anemia status in nonsmokers with CP. For smokers with CP, further studies with larger sample sizes may clearly demonstrate the effect of NSPT on SE and SF levels among these individuals.

Notes

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

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Author contributions

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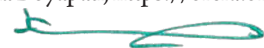
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Diplopia following posterior superior alveolar nerve block: a case report and review of literature

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Posterior superior alveolar nerve block (PSANB) is one of the most common and safe injection techniques in the field of dentistry. As with any other procedure, it also has inherent complications, of which ophthalmic complications are relatively rare. Transient diplopia following the administration of PSANB is rare and daunting for both the patient and the clinician. We present a case of transient diplopia in a 26-year-old female patient following administration of PSANB and review its probable pathophysiology and management and prevention.

Keywords: Dental Anesthesia; Diplopia; Nerve Block; Ocular Complications; Superior Alveolar Nerve.

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INTRODUCTION

Posterior superior alveolar nerve block is one of the most frequently administered injection techniques in dentistry. However, hematoma is among the most common complications of this technique, which includes mandibular nerve and V3 division anesthesia [1]. Other unusual complications attributed to drugs and procedural complications include pain on injection, edema, needle breakage, and post-anesthetic lesions, while toxicity and allergic reactions may be related to drug-body responses.

In addition to these local complications, ocular complications are also rare. To date, a very limited number of ophthalmologic complications secondary to intraoral anesthetic injections have been recorded.

Posterior superior alveolar nerve block anesthesia is, however, a common cause of ophthalmic complications after intraoral local anesthetic administration [2].

Motor/sensory complications, such as diplopia, occur due to the spread of the local anesthetic solution through the vascular, neurological, myofascial, or lymphatic networks [3]. These symptoms are generally temporary and regain normal function after the anesthetic effect is weaned off. However, such an event is distressing for both patients and clinicians. Hence, awareness of such situations is important for the management of unwarranted complications. Herein, the authors discuss one such case, in which the patient developed signs of diplopia after undergoing tooth extraction.

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<http://www.jdapm.org> 71



Fig. 1. No abduction in the left eye

CASE REPORT

A 26-year-old, otherwise healthy, woman was admitted to a private dental clinic for continuous pain in the upper left side of her jaw. Routine clinical and radiological assessments revealed chronic irreversible pulpitis with a grossly decayed maxillary left-third molar. After determining that there was no significant previous medical or dental history, extraction of the maxillary left third molar was planned. On the day of the extraction, routine preoperative vital signs were monitored and found to be normal. After obtaining consent for the tooth removal, a standard protocol was followed. Posterior superior alveolar and greater palatine nerve blocks were administered with 2% lignocaine HCl with 1:80,000 adrenaline in volumes of 1.5 ml and 0.5 ml, respectively. After confirmation of the signs and symptoms in the patient, the tooth was extracted with minimal trauma.

Just before discharge, she complained of double vision. Local measures were taken to control the hematoma. On examination, the pupils were equal, round, reactive to light, and accommodating. The patient complained of inadequate abduction in the left eye (Fig. 1). However, movement of the eye in all other directions was apparent. A clinical evaluation of the facial nerve was performed



Fig. 2. Restoration of the abductive movement in the left eye

using various methods and no abnormalities were observed.

A provisional diagnosis of transient diplopia, secondary to a posterior superior alveolar nerve block, was made based on the events leading to her clinical symptoms. The affected eyelid was taped in a closed position to provide corneal protection and to prevent further damage. Diplopia was checked every 15 min, and complete recovery was reported after 30 min. Ocular movements were reassessed to confirm restoration of the full range of movement in all gazes of the left eye (Fig. 2). The patient was kept under observation and no further complaints were noted at that time. The patient was referred to an ophthalmologist to rule out diplopia and ocular mobility. Appropriate tests were conducted to prove adequate mobility and to rule out diplopia. These were the forced duction test, Hess chart, and diplopia chart.

DISCUSSION

With myths related to loss of vision following removal of the upper teeth being very prevalent in the general public, extraction of maxillary teeth remains fearsome for a majority of patients. Despite a number of conversations

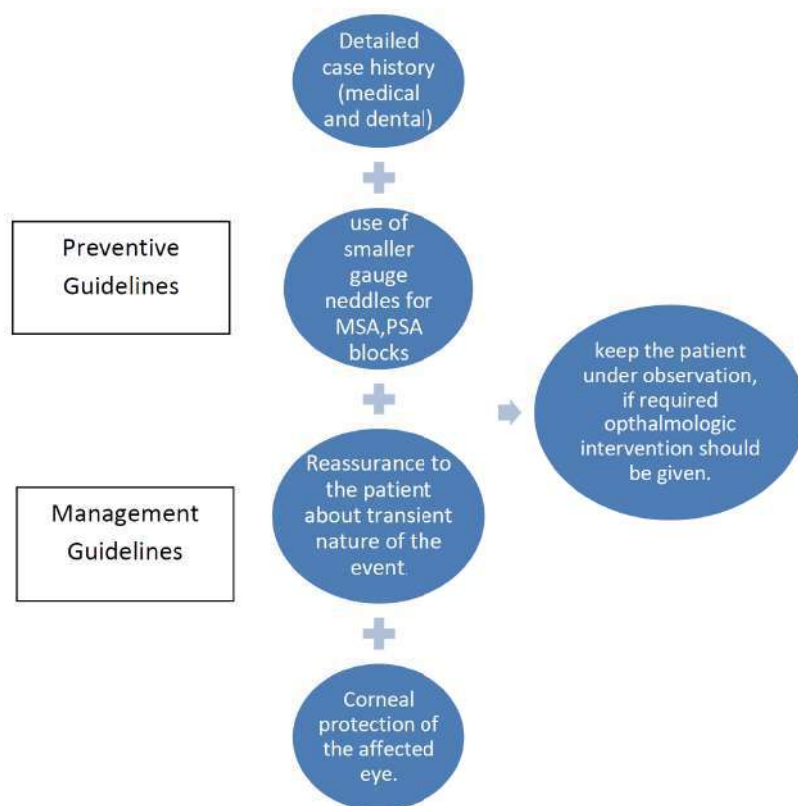


Fig. 3. Preventive and management guidelines

and solaces, patients tend to remain worried. Of all the intraoral procedures performed by a dental surgeon, local anesthetic injections are associated with the mainstream course of ophthalmologic complications. von Arx T et al. [2] reviewed typical incidences of ophthalmologic complications after intraoral local anesthesia and noted that conditions such as diplopia accounted for 39.8% of difficulties in their study. A case of permanent loss of vision in one eye after the administration of intraoral local anesthesia was also reported by Rishiraj et al. in 2005 [4]. Maxillary nerve block causes complications through the following pathways:

1) Direct diffusion of the anesthetic solution from the pterygomaxillary fossa to the orbit, through a defect in the bone.

2) Vascular causes in which the anesthetic solution is diffused through myofascial spaces or bony openings, such as through the inferior orbital fissure, to affect the extra ocular muscles [3].

Confluence of the orbital branch of the middle

meningeal artery and the recurrent meningeal division of the lacrimal branch of the ophthalmic artery leads to paralysis of the lateral rectus muscle and diplopia, in instances of intra-arterial deposition of local anesthesia [5]. Intraluminal injection into the inferior ophthalmic vein can also affect the extrinsic muscles of the eye through the inferior orbital foramen [6]. Diffusion of the anesthetic agent to the orbital cavity can also attain a pathway along the pterygomaxillary fossa through the sphenomaxillary cavity, indirectly impacting nerve endings [7]. As the authors discuss the occurrence of diplopia after posterior superior alveolar nerve block, the literature reveals that diplopia is more common in maxillary than in mandibular anesthesia techniques because of the difference in bone density due to increased dissipation. There are studies suggesting a higher prevalence of ophthalmic complications in female patients compared to men, and more, following usage of 2% articaine than any other local anesthetic agents [8,9]. In the present case, 2% lignocaine HCl was used

as a local anesthetic agent for a posterior superior alveolar nerve block in the recumbent position. Walker et al.[10] identified that the recumbent position of a patient during anesthesia delivery, along with the porous nature of the maxilla, may play a role in causing ophthalmologic complications.

Prevention and management

Awareness of the complications that can occur after a procedure will keep the practitioner prepared to handle the situation. Owing to the fact that ocular complications post-intra-oral local anesthesia are mostly transient, preventive measures taken can reduce anxiety in both patient and doctor. Some authors [5,11] have established preventive and management measures. We have summarized the same for the easier understanding of students and educators (Fig. 3).

In conclusion, complications of local and regional blocks may be less frequent in most cases, but certain conditions such as diplopia and ocular complications occur in rare situations. Correct knowledge and implementation of medical care is helpful in the management of such rare situations.

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Ramanarayana Boyapati: Project administration, Resources, Writing - review & editing

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CONSENT: Informed consent was obtained from the

patient in this case report.

The Ethical Committee constituted by MNR Dental College & Hospital, Sangareddy has approved the case for Publication. Reference Number – IEC/MNRD/2019/ 013

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COVID-19 and oral implications: An updated review

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Abstract

The emerging (COVID-19) pandemic is a global health disaster, caused by infection with severe acute respiratory syndrome coronavirus-2. The disease spreads at an alarming rate all over the world and presents a range of disease manifestations including asymptomatic, mild, moderate and severe symptoms irrespective of age groups. Most patients with severe symptoms exhibit underlying comorbidities such as diabetes, hypertension and obesity. Alternatively, there is an existing evidence for an association between oral health and nonoral systemic diseases. Since the oral cavity is a significant pool for many respiratory pathogens, patients with oral infections are more likely to develop pneumonia as a complication. Therefore, we emphasize that the oral hygiene status has a great impact on the recession and progression of oropharyngeal and respiratory diseases.

Keywords: Comorbidities, COVID-19, oral health, respiratory complications

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INTRODUCTION

The highly contagious viral disease COVID-19 or the severe acute respiratory syndrome is declared as a global pandemic by the World Health Organization (WHO) on March 11, 2020.^[1] A novel coronavirus was later recognized as the causative agent, provisionally named 2019 novel coronavirus (2019-nCoV). Coronaviruses (CoVs) are a group of viruses belonging to the family called Coronaviridae. The subfamily Coronavirinae consists of three genera, alpha coronavirus, beta coronavirus and gamma coronavirus. The spread of infection of severe acute respiratory syndrome CoV-2 (SARS-CoV-2) is either through direct transmission or inhalation of droplets or due to contact with the oral, nasal and eye mucus membranes.^[2] Genomic sequencing and phylogenetic research methods

have supported that the COVID-19-causing coronavirus is a betacoronavirus that belongs to the same subtypes as SARS virus but exhibits a different variant group. The receptor-binding gene site appears to coincide with that of the SARS-CoV and thus suggesting that the same receptor would be used for entry into the cell.^[3]

COVID-19 manifestations can range from asymptomatic state to acute respiratory distress syndrome and multiorgan dysfunction. Most commonly reported are flu-like symptoms of fever, dry cough, malaise, fatigue, myalgia and diarrhea.^[4] Certain risk factors for COVID-19 disease have been reported by the WHO and the Centers for Disease Control and Prevention (CDC) include diabetes, hypertension, aging, immunodeficiency and cardiovascular

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diseases.^[5] These risk factors are proposed to have a greater impact with the increased severity of COVID-19; however, there are various other comorbidities that could also be involved in affecting the prognosis. Persons with chronic lung disease, bronchial asthma, obesity, chronic kidney disease and liver disease are also at higher risk. A recent study lists hypertension, obesity and diabetes as the three major underlying conditions with the most unfavorable outcomes in COVID-19 patients requiring hospitalization.^[6] Recently, many studies have revealed the association between oral health status and systemic diseases including systemic infections, cardiovascular diseases and respiratory diseases.^[7] In addition, the impact of good oral hygiene on the reduction of risk in viral acute respiratory diseases has been reported in many studies.^[8] This article gives a bird's eye view about the impact of good oral hygiene status on the recession of oropharyngeal and respiratory diseases.

PATHOGENESIS

Recent studies have reported spike in the mortality rate of COVID-19 ARDS approaching 40%–50%.^[9] The rest of the cases have exhibited deterioration due to aggressive counteracting of the immune system, which is termed as “cytokine storm syndrome”. Certain cytokines such as tumor necrosis factor (TNF), interleukin-6 (IL-6) and IL-1 β when released at increased levels damage host cells to some extent and result in localized edema. During this phase, innate cytokine response could be a predictive factor for the disease progression.^[10] These responses may further stimulate the synthesis of inflammatory mediators and lead to multiorgan failures such as the kidneys and heart due to the hazardous complications of vascular permeability. In addition, a serum marker of hyperinflammation named as C-reactive protein (CRP) is generally elevated. Recent studies have revealed that the patients with elevated levels of CRP have been reported to show a poor prognosis with COVID-19 due to acute inflammatory pathogenesis.^[11] Moreover, the virus affects intestinal lymphocytes, hepatic cells, renal cells and T-lymphocytes inducing the apoptosis of T-cells ensuing in complete collapse of the immune system.^[12]

ORAL HYGIENE AND COVID-19 SEVERITIES

The oral cavity is well represented as “the window to general health.”^[13] Certain systemic conditions such as atherosclerosis, pulmonary infections, diabetes mellitus, osteoporosis and kidney diseases are influenced by oral hygiene status. Patients with poor oral hygiene and periodontal infections tend to suffer from recurrent

gingival inflammation and bacteremia, which activate the host inflammatory response.^[14] This chronic inflammatory condition elicits the release of multiple proinflammatory cytokines such as CRP, TNF- α , IL-1 β and IL-6, in association with bacteremia. These responses tend to increase the susceptibility of the vascular endothelium for injury, thus leading to atherosclerosis. The recent studies have established that the periodontal infection and poor oral hygiene have a strong connection with the risk of acute myocardial infarction and coronary heart disease.^[15] Researchers have suggested that periodontal disease ought to be considered as a major impediment of diabetes. There is better evidence to prove that prolonged hyperglycemia has negative effects on the heart, kidneys, eyes and peripheral nerves.^[16] This condition leads to the release of advanced glycation end products which exhibit a systemic impact that results in the prolonged excretion of cytokines causing inflammation and connective tissue loss.^[17]

Increased cytokine release is another important characteristic feature of severe COVID-19 cases, which exhibit an extreme elevation in inflammatory cytokines including IL-1 β , IL-2, IL-6, IL-7, IL-8, IL-10, granulocyte macrophage-colony stimulating factor (CSF), granulocyte-CSF, monocyte chemotactic protein 1, macrophage inflammation protein-1 α , IFN- γ and TNF- α , 2, 3, 12, and 15 establishing a “cytokine storm.”^[18] A spectacular increase in these cytokines levels over a short time period leads to a series of adverse reactions in the human body which initiate viral sepsis and inflammation-mediated lung injury, leading to respiratory distress, organ failure, shock and potentially death.^[19] Furthermore, in severe COVID-19 cases, elevated levels of inflammatory cytokines may result in shock and multiple organ failure. The constant release of increased levels of cytokines such as CXCL10, CCL7 and IL-1RA is related to lung dysfunction as well as poor prognosis, resulting in fatal outcome.^[20] Patients with poor oral hygiene status can elicit such immune responses which may result in the progression of thromboembolic complications. In other words, good oral hygiene and regular dental visits reduce the chances of incidence and progression of pulmonary diseases.

ORAL MANIFESTATIONS IN COVID-19

Literature reveals that the patients with COVID-19 disease have presented features such as ageusia, nonspecific anosmia and hyposalivation.^[21] Few reports have stated the presence of certain intraoral manifestations such as desquamative gingivitis, herpetic form ulcers on attached gingiva and tongue with multiple irregular ulcers on the dorsal surface in the oral cavity.^[22] Besides these,

the enlargement of submandibular glands and cervical lymph nodes have been noticed. The presence of ACE2 receptors on target cells could be the possible reason. These receptors help in the binding of SARS-CoV-2 virus spike proteins to the cells which pave way for consequent infections.^[23] The presence of ACE2 expressing cells in large numbers in the epithelial cells of the oral and nasal mucosa attributes to ageusia and anosmia, respectively.^[24] The oral conditions presented by some patients in few studies support the hypothesis that they are extremely indicative of secondary lesions ensuing due to the deterioration of systemic health or due to therapeutic measures for COVID-19. In COVID-19 patients, the presence of some oral manifestations including ageusia, traumatic ulcers, petechiae, candidiasis, HSV-1 infection and geographic tongue need to be considered. Various associated therapies in COVID-19 infection could perhaps contribute to adverse consequences regarding oral health such as unexplained oral ulcerations, recurrent oral herpes simplex virus (HSV-1) infection, opportunistic fungal infections, fixed drug eruptions, ageusia and xerostomia as a result of the altered immune system and its responses.^[25]

Among the opportunistic fungal infections, mucormycosis though rare yet is the rising fungal infection in COVID-19 cases. The causative fungi belong to Mucorales. Manifestations include cutaneous, mucosal, sinusitis, pulmonary, gastrointestinal or sometimes dissemination. These fungi affect the immunocompromised patients those that are on high-dose glucocorticoids, hemodialysis and uncontrolled diabetes mellitus.^[26] In a healthy individual, the spores and hyphae are attacked by immune cells and destroyed by monocytes, macrophages and polymorphonuclear phagocytes. When patients exhibit diminished immune status with low phagocyte count, altered phagocyte function or uncontrolled diabetes mellitus, they become progressively susceptible to the invasive type of mucormycosis.^[27]

OROFACIAL ADVERSE EFFECTS FOLLOWING COVID-19 VACCINES

Certain medication-related events such as adverse drug reactions (ADRs) are possibly life-threatening consequences due to the usage of medicines as well as vaccines. Hence, it is significant to recognize the adverse events on time to reduce the conceivable harm.^[28] Therefore, health professionals including dentists should be primed to diagnose orofacial ADRs probably being drug induced. The clinical trials and the development of effective vaccines against SARS-CoV-2 virus have been extremely progressing.^[29] At present, two RNA-based COVID-19 vaccines have

been permitted for emergency use and authorized for marketing by the regulatory agencies in Europe and America and are being used all over the world. Of late, another vaccine (AZD1222, Oxford–AstraZeneca) has been granted for use in the UK. Recent literatures have revealed the data on preliminary efficacy and safety for both BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) vaccines.^[30] As several billion doses of vaccination need to be administered, it is possible that an ample number of adverse events to be reported. The knowledge of the dentist regarding orofacial manifestations will enhance the diagnosis, management and reporting of vaccine-related adverse events. Recent study shows that COVID-19 vaccines have possible yet rare orofacial side effects including anaphylaxis associated with facial swelling, Bell's palsy and swelling of the face, lips or tongue. In addition to orofacial manifestations and systemic ADRs with anaphylaxis, both of the vaccines were seen associated with acute peripheral facial paralysis. It has been reported that there were two serious adverse events regarding facial swelling seen only with respect to mRNA-1273 vaccine recipients. The onset of the adverse event was reported 2 days after vaccine administration and was probably related to vaccination according to MHRA (UK).^[31] Finally, it has been concluded that both BNT162b2 and mRNA-1273 COVID-19 vaccines are associated with ADRs including orofacial manifestations with heterogeneity expression all over the world.

CONCLUSION

Patients with COVID-19 exhibit a range of disease manifestations including asymptomatic, mild, moderate and severe symptoms and high cytokine levels, which can be considered potential biomarkers for disease progression. The specific immune responses of COVID-19 tend to establish cytokine storm and thromboembolic complications which can further lead to multiple organ dysfunctions. In addition to this, compromised immune status leads to the rise of certain opportunistic infections such as mucormycosis which is rare yet extremely exhibit fatal outcome. On the other hand, poor oral hygiene can contribute to altered immune responses. Therefore, improving oral hygiene and reducing gingival and periodontal inflammation may reduce the risk of complications arising due to COVID-19 disease. Since knowledge about this concept and orofacial adverse effects due to vaccination is brainstorming, further researches are required to report cases of COVID-19 seen associated with oral diseases to add to the pool of knowledge on a comprehensive level.

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Marsupialization of odontogenic keratocyst using thermoform surgical splint in a pediatric patient, with 3-year follow-up

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Abstract

Odontogenic keratocyst (OKC), also known as keratocystic odontogenic tumor, is categorized as one of the developmental epithelial cysts, which accounts 10%–20% of all cystic lesions in jaws. Considering its high recurrence rate, combative treatment modalities such as enucleation and jaw resection are suggested. This case report aims to emphasize clinical, radiological and histological overview of OKC with conservative approach marsupialization using thermoform surgical splint in a 9-year-old female patient with no recurrence at 3-year follow-up.

Keywords: Marsupialization, odontogenic keratocyst, thermoform splint

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INTRODUCTION

Odontogenic keratocyst (OKC) that originates either from a dental lamina or primordial odontogenic epithelium^[1,2] is a benign tumor of the jaws, and the term was introduced by Philipsen in 1956.^[1,3] According to the World Health Organization's (WHO) 1992 classification, OKCs are typed under developmental (dysembryogenetic) cysts^[3] and were designated in 2005 as a keratocystic odontogenic tumor^[4,5] which is defined as "a benign uni or multicystic, intraosseous tumor of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium, and potential for aggressive, infiltrative behavior."^[5,6] In 2017, the fourth edition of the WHO/IARC Classification of Head and Neck Tumors were published where keratocystic odontogenic tumor is back into the cystic category as OKC.^[1,7] It is

frequently reported in the second to fourth decade of life and relatively less in the first decade.^[2] It has a multicentric growth pattern, infiltrating through cortical bone and extending into adjacent soft tissue.^[8] Conservative treatments are widely employed in children to avoid complications associated with radical operations.^[9] Marsupialization is one such conservative treatment, proved to be effective treating cysts among children and adolescents which secures adjacent vital anatomical structures such as inferior alveolar nerve, maxillary sinus and developing dentition.^[10]

CASE REPORT

A 9-year-old female patient reported to the Department of Paediatric and Preventive Dentistry with a complaint

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of pain and swelling in right lower back tooth region. On general examination, the patient was healthy. The patient had a history of pulp therapy in 84, 3 years back, and complained pain in the same region. Extraoral examination revealed facial asymmetry due to swelling noted on lower right side of face, with no visible pulsations or pus discharge [Figure 1a]. A single diffuse swelling, approximately 3 cm × 3 cm in size, extended anteriorly 1 cm behind the right corner of mouth to 3 cm posteriorly. Superiorly, extended 3 cm below the ala-tragal line and inferiorly to lower border of mandible. No paraesthesia was noted. Submandibular lymph nodes on right side were palpable, tender, mobile and firm in consistency. On intraoral examination, vestibular obliteration in right mandibular region from permanent lateral to the first molar with normal overlying mucosa was seen [Figure 1b]. Swelling was tender, nonfluctuant, bony hard in consistency. Other clinical findings included disto-proximal caries in 74 and deep occlusal caries in 36 [Figure 1b and c]. Panoramic radiography and cone beam computed tomography scan revealed a single homogenous well-defined radiolucency on the right body of mandible measuring approximately 2.5 cm × 3 cm in size, extending antero-posteriorly from distal aspect of 83 to distal side of 46 and expansion of the lesion resulted in displacement of permanent canine tooth bud downward and forward below incisors and premolars tooth buds downward and backward below permanent molar, 1 cm above the inferior border of mandible [Figure 1c]. The roots of deciduous first and second molar were resorbed. Inferior alveolar canal was not seen. Expansion of buccal and lingual cortical plates on the right side of mandible was observed [Figure 1d]. Based on clinical and radiological findings, the provisional diagnosis of radicular cyst was made. Histological examination was planned to differentiate from OKC, dentigerous cyst,

buccal bifurcation cyst and unicystic ameloblastoma. Enucleation was common treatment option. However, due to large size of the lesion, age, anatomical structures, and teeth involved, incisional biopsy was done followed by marsupialization after informed consent was obtained from the parent. Fine needle aspiration cytology was done using 5 ml syringe where a milky-yellow liquid content was obtained [Figure 2a]. Extraction of 84, 85 was performed and red wine-colored material appeared inside the cavity was collected [Figure 2b]. An impression was made followed by fabrication of thermoform surgical splint [Figure 2c], extending into surgical site with buccal and lingual flanges to support fragile walls from collapse [Figure 2d and e]. Parents were given oral hygiene instructions regarding maintenance of splint, and irrigation of socket with betadine, and saline thrice daily. The patient was explained regarding the treatment plan of 36, 74, but the patient was intended to get it done later as they were more worried about the existing lesion. Cytopathologic examination of the aspirate showed dense population of neutrophils, lymphocytes, foamy macrophages, few multinucleate giant cells and keratinocytes against hemorrhagic background suggestive of keratin filled lesion. Biopsy revealed parakeratinized stratified epithelial lining with prominent columnar basal cell layer, surface corrugation, and neutrophils, lymphocytes arranged against extravasated red blood cells [Figure 2f], which confirmed the diagnosis to be OKC. Follow-up was done every 3 days till 1st month which showed occlusal movement of developing tooth bud clinically, as well as reduction in radiolucency radiographically [Figure 2g]. Later, the patient was reviewed every 6 months till 3 years, in which there was a gradual reduction in radiolucency, occlusal movement of developing tooth buds and accordingly surgical splint was refabricated. During the 2nd-year follow-up, endodontic treatment was performed in 36 followed by composite inlay. By the end of 3 years, there was complete healing of the lesion with eruption of all permanent teeth [Figure 3a-f].

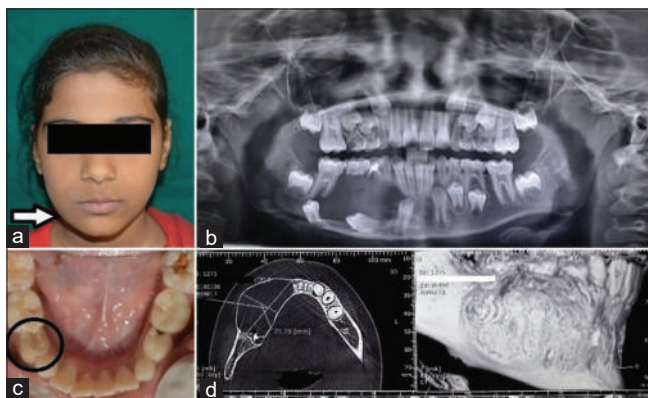


Figure 1: Preoperative pictures. (a) Extraoral swelling on right side of face (arrow) with facial asymmetry (b) Vestibular obliteration (circled) at 84, 85 (c) Orthopantomogram showing single homogenous radiolucency in the region of 83, 84, 85, 46 (d) Cone beam computed tomography showing expansion of buccal and lingual cortical plates

DISCUSSION

OKCs are common developmental odontogenic cysts occurring three times more often in the mandible than the maxilla and is most frequently seen in the region of canine to premolar, mandibular retromolar, ramus areas and maxillary second permanent molar area.^[3] It should be often differentiated from Dentigerous cyst, radicular cyst and lateral periodontal cyst which are other endodontic lesions.^[11] Radiographically OKCs display a radiolucency extending along the length of the mandible displacing developing teeth in children and adolescents. Histopathologically, OKCs are characterized by 5–8

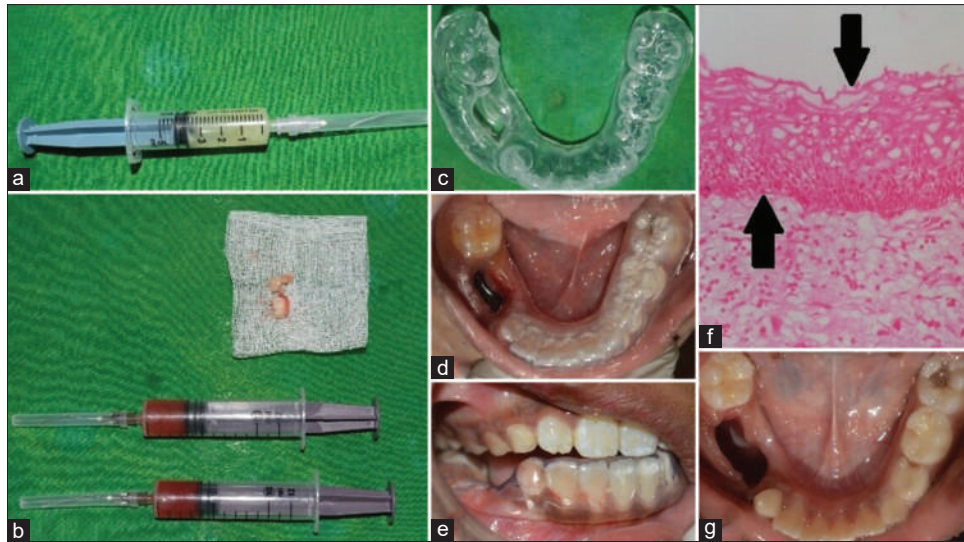


Figure 2: (a) Aspirated fluid (milky yellow in color) (b) Extracted 84, 85 and red wine-colored fluid collected (c) Fabricated thermoform surgical splint (d and e) Inserted marsupialization splint (f) Histologic picture showing parakeratinized stratified epithelial lining with prominent columnar basal cell layer (arrow), surface corrugation (arrow) (H&E stain, x40) (g) Follow-up after 1 month

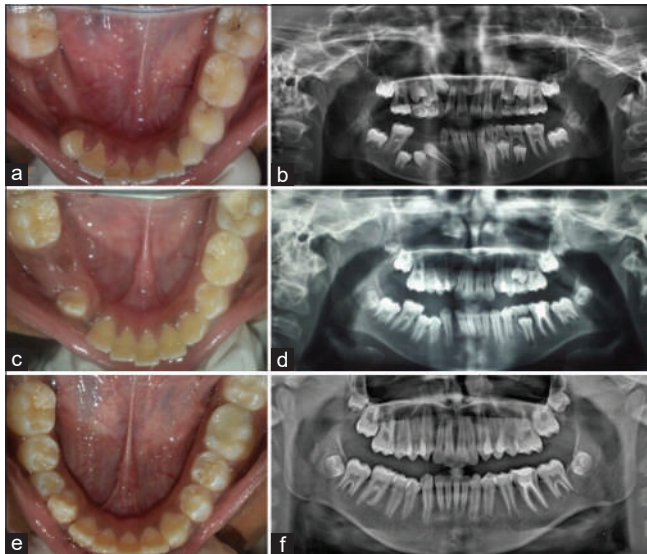


Figure 3: Postoperative clinical and radiographic follow-up showing remarkable reduction in lesion size and eruption of premolars (a and b) After 10 months (c and d) After 2 years (e and f) After 3 years

layered parakeratinized stratified squamous epithelium lining connective tissue capsule. Basal layer has palisade arrangement of hyperchromatic nuclei in columnar epithelial cells. The epithelial projections are absent in epithelial-connective tissue junction. The consistency of luminal content can be described as a “straw-colored fluid,” “thick pus-like” material or a caseous, cheesy, thick, milk-white mass which was similar in this case.^[5] Treatment of OKCs remains controversial because of their higher recurrence potential,^[12] and options include decompression, marginal resection, *en bloc* resection and adjuvant therapy, like cryotherapy, peripheral ostectomy and

Carnoy’s solution.^[2] However, marsupialization (Partsch I procedure)^[7] is reported to be the treatment of choice in large cysts involving multiple unerupted teeth and anatomic structures. It aims to reduce the size of the lesion thus preventing extensive surgery, preserving vital structures.^[9] In literature, the term marsupialization is often used interchangeably with decompression, but they are distinguished as decompression is any technique that reduces the pressure within a cyst, while marsupialization is a process of converting cyst into an open pouch that communicates with the oral cavity.^[7] However, both the procedures allow new bone to fill the defect. Studies have shown that more effective results were observed after marsupialization in lesions of the mandibular body than in ramus.^[13]

Despite having advantages, it has few limitations like prolonged healing period, discomfort to the patient, requirement of irrigation twice daily and weekly modification of surgical splint maintaining the cystic opening.^[7] Various devices such as tubes, gauzes, obturators and acrylic stent can be used for cystic window, but due to drawbacks like retention failure, increased porosity, irritating effect of residual monomer and difficulty in processing, usage of thermoformed appliances has gained high demand.^[14] In this case, thermoform splint was used which is a polyoxymethylene-based material made up of acetal resin. It is retained in an adequate position by negative pressure created intraorally. This obturator has advantages like easy removal to irrigate the cavity, nontoxic, nonallergic, flexible, resists wear and fracture, making it an ideal material of choice as surgical stent.^[14] Ziccardi *et al.*

used marsupialization stent along with space maintainer to preserve space for unerupted teeth.^[10] In the present case, thermoform marsupialization splint itself maintained space between existing dentition and allowed the displaced teeth to erupt in proper alignment without requirement of an additional space maintainer or orthodontic traction.

Literature has reported that recurrence rate of OKC varies from 35% to 50% and 21.4% among lesions treated by marsupialization.^[13] An immunohistochemistry study noted that after marsupialization, the epithelium thickness was increased, and the keratinized epithelium was changed to a hyperplastic and nonkeratinized stratified squamous epithelium.^[7] There is a histological transformation of 23 aggressive keratocysts to either less aggressive OKC or nonkeratocysts before and after marsupialization. Interleukin 1 and Ki-67 reduction was also observed in many cases, which is considered to be the factor for decrease in volume of the cysts^[15] allowing new bone to fill the defect.^[7] Thus, growth characteristics of OKC may become less aggressive during the course of marsupialization.^[13] implying that recurrence rate is not affected by it.^[7] According to Forssell *et al.*, an active follow-up performed annually during a 3-year period in pediatric patients might be sufficient, to ensure absence of recurrence and adequate eruption of the permanent teeth.^[13]

In conclusion, the diagnosis of OKC can be confirmed histologically along with radiological findings. In the selection of the most appropriate treatment, one must consider patient factors such as age, general medical condition and lesion characteristics. Considering the young age of the patient, subsequent growth of mandible and facial contour, treatment by marsupialization was performed in this case without any recurrence at 3-year follow-up which was an unique scenario. Although marsupialization is an unconventional method of treating OKC, it is a very effective method in children where there is proximity of lesion to the developing tooth buds and vital structures in the jaws. For the treatment success, regular follow-ups, along with patient awareness in maintaining oral hygiene, are important.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal.

The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Comparison of efficiency of different crowns in primary children an original research

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Abstract---Introduction: The restorations to anterior teeth in children is very significant. Hence in this study we compared efficiency of strip crowns versus primary anterior zirconium crowns in 3 to 6 year old children. Material and Methods: We selected 100 children of ages 3-6 years to receive the anterior crowns, attending the institution department of pedodontics. They were divided to 50 each of zirconia and strip crown groups. The various parameters were checked at 3,6 and 12 months. Values were compared keeping $p < 0.05$ as significant. Results: Zirconia crowns showed significantly lower plaque



accumulation, gingival bleeding restoration failure, at the 3- and 6-months no, but more wear to opposing teeth. With regards to recurrent caries no significant difference was found between the two crowns. Conclusion: Based on our data we conclude that overtime teeth covered with zirconia crowns show better gingival health and less bleeding, plaque accumulation as well as less loss of material. On the other hand, zirconia can cause more loss of opposing tooth structure.

Keywords---Primary anterior teeth, Strip crowns, Zirconia crowns.

Introduction

The children are more prone to caries than adults due to multiple reasons. Providing care to children who are considered to be at risk of caries, is to be done under specialist care. For the anterior primary teeth caries there are various restorations. Primary incisors with large or multi-surface caries can be restored with resin composite strip crowns if there is sufficient tooth structure after removing all carious tissues [1]. When remaining tooth structure is less or isn't enough for bonding, pre-veneered aesthetic crowns is a favorable solution. Stainless steel crowns with cosmetic facing have good aesthetics and good retention even with minimal remaining tooth structure. Moisture and hemorrhage control are not critical with these restorations which need minimal chair time and offer full coverage and protection.

Off late, zirconia aesthetic crowns for children appeared in the market. Zirconia has properties like metal and esthetics of the teeth, and the ready to use zirconia crowns are available for primary teeth. Although there is high acceptance of zirconia crowns, the literature lacks solid proof for their pediatric clinical performance [2-5]. There are limited clinical studies that are currently ongoing, however until the outcomes of adequate number of prospective researches with good long-term follow-up. Hence in this study we compared efficiency of strip crowns versus primary anterior zirconium crowns in 3 to 6 year old children.

Material and Methods

We selected 100 children of ages 3-6 years to receive the anterior crowns, attending the institution department of pedodontics. After obtaining the ethics clearance the consent was taken from the guardian of the children. We included both the genders for the study. Subjects with no major health issues, or bruxism were selected for the study. Any subjects with medical issues or problems were excluded.

The subjects were randomly divided to two groups to receive the zirconia and strip crown groups. The restorations were done according to the treatment protocols and the manufacturer's guidelines. The Gingival health, Plaque index, Secondary caries, Restoration failure, Tooth wear were evaluated. The subjects were followed up for a period of 3,6 and 12 months and at each visit the above parameters were



noted. These readings were compared using Chi square test keeping $p < 0.05$ as significant.

Results

We observed that significantly more teeth in the strip crown group were bleeding compared to the zirconia groups at the 3, 6-months follow-up. Table 1 We observed that that none of the teeth covered with zirconia crowns developed caries during the entire period of follow up. On the other hand, teeth that received strip crowns restorations had no recurrent lesions in the 3- and 6-months follow up but when it reached the 12-months follow up, 6.7% presented with recurrent caries. Table 2 We observed that at three months follow up, no failures was noticed in both groups. In the zirconia crown group, two crowns were lost completely in 6 and 12 months follow up due to trauma caused by falling of patients and hitting hard objects. On the other hand, failure was more in the strip crown group as 11.7% showed small but noticeable area of material marginal loss and 1.7% presented with large loss of crown during the 6 months follow up. Also, this loss increased with time to reach 30% small but noticeable area of loss of material and 8.3% large loss of restorations were clinically observed for crown by the 12 months follow up. Table 3 We observed that incisal and labial surfaces of the teeth opposing the full-coronal restorations were clinically observed for any sign of abrasion. 10 teeth opposing to zirconia crowns showed loss of enamel surface characteristics and minimal loss of contour. Table 4

Table 1: Comparison of the gingival status.

Chi-square test	Zirconia crowns	Strip crowns	p-value
<i>Gingival Health (assessed as bleeding on probing)</i>			
At 3 months	24 (40.0%)	40 (66.7%)	0.006 ^a
At 6 months	0 (100.0%)	28 (46.7%)	<0.001 ^a
At 12 months	0 (100%)	0 (100%)	N/A ^c
Friedman test	Mean rank		
	Total cases	Zirconia crown	Strip crown
Baseline	3.61	3.80	3.43
At 3 months	2.68	2.59	2.77
At 6 months	2.09	1.80	2.37
At 12 months	1.61	1.80	1.43
p-value	<0.001 ^b	* <0.001 ^b	<0.001 ^b

^a Significant using Chi-Square Test @ < 0.05 level

^b Significant using Friedman Test @ < 0.05 level

^c No statistics are computed because variable is a constant

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Table 2: Comparison of the secondary caries

Chi-square test		Zirconia crowns	Strip crowns	p-value
<i>Recurrent caries</i>				
At 3 months	No caries present	60 (100.0%)	60 (100.0%)	N/A ^b
At 6 months	No caries present	59 (100.0%)	60 (100.0%)	N/A ^b
At 12 months	No caries present	58 (100.0%)	56 (93.3%)	0.135
	Caries present	0 (0.0%)	4 (6.7%)	
Friedman test	Mean rank			
	Total cases	Zirconia crown	Strip crown	
Baseline	2.48	2.50	2.47	
At 3 months	2.48	2.50	2.47	
At 6 months	2.48	2.50	2.47	
At 12 months	2.55	2.50	2.60	
p-value	0.007 ^a	N/A ^b	0.007 ^a	

^a Significant using Chi-Square Test @ < 0.05 level

^b No statistics are computed because variable is a constant

Table 3: Comparison of the restoration failure

Chi-square test		Zirconia crowns	Strip crowns	p-value
<i>Restoration failure</i>				
At 3 months	Crowns appear normal, no cracks, chips, or fractures	60 (100.0%)	60 (100.0%)	N/A ^c
At 6 months	Crowns appears normal, no cracks, chips, or fractures	59 (98.3%)	52 (86.7%)	0.024 ^a
	Small but noticeable area of loss of material	0 (0.0%)	7 (11.7%)	
	Large loss of crown	0 (0.0%)	1 (1.7%)	
	Complete loss of crown	1 (1.7%)	0 (0.0%)	
At 12 months	Crowns appears normal no cracks, chips, or fractures	58 (98.3%)	37 (61.7%)	< 0.001 ^a
	Small but noticeable area of loss of material	0 (0.0%)	18 (30.0%)	
	Large loss of crown	0 (0.0%)	5 (8.3%)	
	Complete loss of crown	1 (1.7%)	0 (0.0%)	
Friedman test	Mean rank			
	Total cases	Zirconia crown	Strip crown	
Baseline	2.37	2.49	2.24	
At 3 months	2.37	2.49	2.24	
At 6 months	2.5	2.49	2.51	
At 12 months	2.77	2.53	3.01	
p-value	< 0.001 ^b	0.392	< 0.001 ^b	

^a Significant using Chi-Square Test @ < 0.05 level

^b Significant using Friedman Test @ < 0.05 level

^c No statistics are computed because variable is a constant



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Table 4: Comparison of the tooth wear

Chi-square test		Zirconia crowns	Strip crowns	p-value
<i>Tooth wear of opposing</i>				
At 3 months	No loss of enamel surface characteristics, no loss of contour	60 (100.0%)	60 (100.0%)	N/A ^c
At 6 months	No loss of enamel surface characteristics, no loss of contour	60 (100.0%)	60 (100.0%)	N/A ^c
At 12 months	No loss of enamel surface characteristics, no loss of contour	53 (88.3%)	60 (100.0%)	0.020 ^a
	Loss of enamel surface characteristics, minimal loss of contour	7 (11.7%)	0 (0.0%)	
Friedman test	Mean rank			
		Total cases	Zirconia crown	Strip crown
Baseline	2.47		2.44	2.50
At 3 months	2.47		2.44	2.50
At 6 months	2.47		2.44	2.50
At 12 months	2.59		2.68	2.50
p-value	<0.001 ^b		<0.001 ^b	N/A ^c

^a Significant using Chi-Square Test @ <0.05 level

^b Significant using Friedman Test @ <0.05 level

^c No statistics are computed because variable is a constant

Discussion

In assessment of gingival health, this study shows better gingival response in zirconia crowns which can be explained by the fact that zirconia is biocompatible and possesses a polished and smooth surface leading to less plaque accumulation and hence less gingival irritation [6–8]. This is similar to the study of Walia et al. [3] who evaluated anterior primary crowns for 129 patients. In agreement with our study, Walia et al. [3] also reported that zirconia crowns showed improved gingival health due to less plaque accumulation when compared to composite strip crowns and pre-veneered SSCs. Our data showed that none of the teeth covered with zirconia crowns showed recurrent caries during the entire follow up. In contrast, teeth restored with composite resin strip crowns showed that 6.7% developed recurrent caries in the 12-months follow up. Significant factors that influence development of caries are poor oral hygiene and high cariogenic diet.[9,10] A recent study by Holsinger et al. [4] reported results similar to our study's in their evaluation of 57 crowns treated with zirconia for primary anterior teeth in 18 patients. Their and other similar studies showed no recurrent caries after a follow-up period of 24 months. [6-10] The greater restoration failure of the composite strip crowns in this study may be explained by the fact that treatment was done under nitrous oxide sedation and physical restrains to manage the children behavior. Regarding the zirconia crowns, the success rate in this study was 98.3% by the end of the 12 months follow up. Only two crowns failed due to trauma. Current research on the clinical success of prefabricated primary zirconia crowns for primary incisors is still limited. Walia et al.[3] reported the retention rate of zirconia crowns as 100% after 6 months. These crowns have no facial upper structure, as they are made up of solid zirconia leading to no chance of facial veneer fracture. In our study seven teeth opposing to zirconia crowns



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showed loss of enamel surface, minimal loss of contour compared to 100% no loss of enamel surface characteristics in strip crown group. This results is in agreement with Walia et al. [3] who found four opposing primary teeth out of 38 zirconia crowns having loss of enamel surface characteristics and minimal loss of contour. Although one of the inclusion criteria was selecting patients with positive behaviour, some children occasionally became uncooperative due to prolonged procedure. Another limitation was the high cost of Zirconia crowns. A longer follow-up studies on zirconia crowns is suggested.

Conclusions

We can conclude that zirconia crowns showed better gingival health and less bleeding, plaque accumulation as well as less loss of material. On the other hand, zirconia can cause more loss of opposing tooth structure.

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Comparative evaluation of chelating efficacy of nano-chitosan, pomegranate extract, and ethylenediaminetetraacetic acid on primary radicular dentin: An *in vitro* study

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ABSTRACT

Background: Irrigation is a crucial aspect of root canal treatment, and it is imperative to employ chelating agents to eliminate the smear layer during biomechanical preparation. They in turn react with mineral content of dentin, leading to decreased strength and increased susceptibility to fracture. **Aims:** This study aimed to assess and compare mineral loss and microhardness from primary root canal dentin following the usage of different irrigating solutions and determine the least detrimental irrigant among the tested solutions. **Materials and Methods:** Sixty-six primary anterior teeth were divided into three groups with 22 in each- Group I: 17% ethylenediaminetetraacetic acid (EDTA) Group II: 0.2% Nano chitosan Group III: Pomegranate extract. The decoronated teeth were split longitudinally. Half of it was directly subjected to Vickers test, and the other half was immersed in a magnetic stirrer bath containing test solution to record the mineral loss from solution. Postmicrohardness values were recorded on the specimen and compared with initial values. **Statistical Analysis Used:** Statistical analysis was done using SPSS software (Version 20, SPSS, IBM, Armonk, NY, U. S. A). **Results:** Descriptive statistics were calculated, and the groups were compared using analysis of variance test and *post hoc* Tukey test. Pomegranate extract showed least effect on mineral content and microhardness compared to 17% EDTA and 0.2% nano chitosan. $P \leq 0.05$ was considered statistically significant. **Conclusion:** Pomegranate aril extract showed better results with selected parameters.

KEYWORDS: Chelating agents, chitosan nanoparticles, microhardness, mineral loss, pomegranate extract, primary root dentin

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Introduction

A clean root canal system along with a three-dimensional seal is the clinician's path to success.^[1] Biomechanical preparation is of paramount importance where endodontic instruments are used in association with auxiliary chemical substances like irrigants, which help clean the root canal system areas

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that cannot be reached directly by instrumentation. Regardless of the instrument and technique employed, the maneuver of endodontic instruments against dentinal walls consequently promotes the formation of smear layer.^[2,3] The complete removal of smear layer requires chelating agents, followed by tissue solvents because no single solution can furnish both effects alone. Therefore, ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite solutions have been jointly advocated as an effective irrigation regimen.^[4]

Chelating agents were introduced to endodontics by Nygaard-Ostby^[5] as an adjunct to prepare narrow and calcified root canals and smear layer removal. They increase the penetration of irrigants into dentinal tubules, thus allowing adequate disinfection and a reduction in dentin microhardness that facilitates the access and action of endodontic instruments in the root canals.^[6,7] However, usage of endodontic irrigants like EDTA causes dentinal cracks and alterations in the chemical composition of dentin particularly, the calcium and phosphate ratio which in turn changes the microhardness, permeability, and solubility characteristics of dentin, thereby adversely affecting sealing ability and adhesion of dental materials to dentin.^[8]

Recently, the global scenario is inclining towards natural products like Chitosan which is a polysaccharide biopolymer produced by the alkaline deacetylation of chitin, the principal component of crustacean and shrimp exoskeletons.^[9] Chitosan contains ideal properties including biocompatibility, biodegradability, bioadhesion, high chelating ability for various metal ions in acidic conditions, lack of toxicity, and even antibacterial activity. Owing to these properties, proposed uses of chitosan in dentistry are diverse. It was used as an irrigant in several studies^[10,11] and Bastawy *et al.*^[9] reported that even at the lowest concentration, chitosan removed the smear layer as effective as or more than EDTA. Ikono *et al.*^[12] found that chitosan nanoparticles (Cs-NPs) have smaller size and better penetration than chitosan, and this property makes it unique with superior qualities.

Among the myriad of herbal alternatives available, pomegranate (botanical name: *Punica granatum*) finds a noteworthy mention of its several antioxidant activities, including radical scavenging ability, ferrous ion chelating, and ferric ion reducing power.^[13] Pomegranate is now finding prime uses in the dental arena as well. Prior studies^[14,15] employing the pomegranate rind or extract of pomegranate suggest that pomegranate has effective antimicrobial activity, but the chelating potency of pomegranate extract in endodontics has been left unexplored. Hence, an attempt is made to study pomegranate aril extract's effect on the primary radicular dentin.

Even though plentiful studies regarding the effect of root canal irrigants on permanent root dentin are available in the literature,^[3,8,11] their impact on primary root canals is quite limited. Hence, the results obtained cannot be pertinent to primary teeth due to morphological differences such as dentinal tubules, accessory canals, root tip bifurcation, and compositional variations.^[16] Henceforth, the present study was aimed to evaluate the effect of 17% EDTA, 0.2% Nano-chitosan and pomegranate aril extract as irrigating agents on the microhardness and mineral content of primary radicular dentin.

Materials and Methods

The present laboratory study investigated the effect of various root canal irrigants on the calcium and phosphate content and microhardness of primary root dentin using the Atomic Absorption Spectrophotometer (AA-6300, Shimadzu, Kyoto, Japan); Vandomolybdophosphoric acid method, and Vickers Hardness Test, respectively. After obtaining clearance from the institutional ethical committee, the present *in vitro* study was planned with a sample size of 70 teeth which was calculated using G * POWER 3.1.9.2 software with a power of 80%, effect size of 0.4, and an alpha error of 5%.

The sample included human primary anterior teeth which were un-restorable due to caries or trauma or over-retained. These teeth were collected from the tooth bank in our institute and stored in distilled water at room temperature (37°C) until use. The soft and hard debris on the root surfaces was cleaned using hand scaler, decoronated at the cemento-enamel junction, and at the root apex to obtain 10 mm standard-sized specimens using a low-speed diamond disc. Roots were sectioned longitudinally into two halves, and pulp tissue was removed using a brush. Four samples were discarded due to improper sectioning, and the remaining 66 samples were randomly divided into three groups of 22 specimens in each using computer randomization method depending on the irrigating solution used. Group I: 17% EDTA, Group II: 0.2% nano-chitosan and Group III: Pomegranate aril extract.

Before commencing the experiment, 17% EDTA solution (Prevest Denpro Ltd, Jammu) was procured. 0.2% nano chitosan solution was made by dissolving 0.2 g of chitosan powder (size 395 ± 98.5 nm) (Aura Biotechnologies, Chennai) in 1% acetic acid with the volume of 100 ml. To obtain a homogenous solution, the mixture was stirred with a magnetic agitator for 2 h. Chitosan was synthesized from shrimp shells (degree of deacetylation >75%) using ionic gelation method and polyanion tripolyphosphate (TPP) as crosslinker.^[11]

Fresh pomegranate fruits were brought to the laboratory, cleaned in running tap water; surface sterilized using 70% alcohol, and then rinsed with

sterile distilled water. Pomegranate aril juice was freshly prepared after removing the rind and separating the arils. The arils were blended in a domestic mixer without dilution. The blend was first filtered through a sieve followed by centrifugation of the obtained extract to precipitate the residual waste, and the supernatant clear solution obtained was again passed through a filtration assembly using a series of membrane filter papers of 0.45 microns.^[17] The final extract thus obtained was placed in the ultraviolet (UV) sterilizer and used in the study. Freshly prepared undiluted and filtered pomegranate aril extract has been used to know its indisputable effect on human radicular dentin and to avoid any bias that might occur with the use of preservatives. The pH of the test solutions of different groups I, II, and III were 7.2; 3.5; and 3.4, respectively, which was determined using a pHmeter with an accuracy of ± 0.002 .

In each group, one-half of the specimen was immersed in a magnetic stirrer bath containing 10 ml of test solution for 5 min. Accordingly, 1 ml of solution was aspirated from the bath after 5 min for Calcium (Ca) analysis using Atomic Absorption Spectroscopy technique.

The phosphate level of the solution was calculated using the vanadate-molybdate method which is based on the APHA Standard Method 4500-P C. 0.25 ml of vanadate-molybdate reagent was added to 1 ml of sample irrigating solution from the magnetic stirrer bath and the solution was mixed by pipetting up and down for several times till yellow color is developed in the cuvette. This sample was placed in the UV-visible spectrophotometer (Shimadzu UV-1700 Pharma Spec) to get the absorbance value of phosphate converted to phosphate concentration in parts per million.^[18]

The other half of the specimen was mounted horizontally on an acrylic block and the dentin surface was polished with silicon carbide sandpaper with three progressively increasing grit sizes (400, 600, and 1200, respectively) to obtain a smooth surface for analyzing surface microhardness using digital microVickers hardness tester under an indentation load of 150 g with an indentation time of 15 s. The mean average of the three indentations taken on the middle third of the root dentin was considered as baseline hardness value.

After immersing in the respective irrigating solutions, the part of the specimen used for mineral loss analysis was flushed with saline, air dried, mounted on an acrylic block, dentin surface was polished using silicon carbide sand papers (400, 600, and 1200) and subjected to Vickers test. The difference between initial and final microhardness was noted.

A single operator performed the entire procedure. However, to avoid bias, a second operator who was unaware of the prior results evaluated the samples

randomly. Interexaminer reliability was assessed by the kappa test. As an interexaminer reliability statistic of 0.93 was achieved indicating excellent agreement (Intraclass correlation coefficient = 0.93), the scores given by the first investigator were only considered for the analysis. Statistical analysis was done using SPSS Version 20 (SPSS, IBM, Armonk, NY, U. S. A), and the groups were compared using analysis of variance test and *post hoc* Tukey test as necessary. The $P \leq 0.05$ was considered statistically significant.

Results

The mean calcium and phosphate loss in the three groups after placing in the respective irrigants ranged from 1.9 ppm to 24.26 ppm and 2.6 ppm to 29.3 ppm respectively, with highest loss seen in group II followed by I and III [Table 1].

The preimmersion microhardness values of the samples ranged between 56.44 and 56.97 Vickers hardness numbers (VHN) which were not statistically significant ($P > 0.05$). However, when the pre and post-immersion microhardness values were compared, groups I and II showed statistically significant change from their baseline values ranging from 19.74 to 20.26 VHN. Whereas in group III samples, not much difference (1.23) was noticed between pre and postmicrohardness values, although the change was statistically significant ($P < 0.05$) [Table 2].

On intergroup comparison, the mean difference in calcium mineral loss varied between 17.75 and 22.36 with least variation recorded between groups I, II and the highest change observed between groups II, III. The amount of loss noticed was statistically significant ($P < 0.05$) among all the three groups [Table 3]. The mean difference in phosphate loss ranged between -23.73 and 26.67 with least change recorded between groups I, III and highest change observed between groups II, III. A statistically significant difference in phosphate loss was noticed on an intergroup comparison between I, II and II, III with no significant difference in phosphate loss between I and III, the value was 2.93 [Table 3]. The microhardness values span from -17.78 seen between groups I and III to 0.34 observed between groups I and II. As shown in Table 4, it was evident that intergroup comparison of

Table 1: Mean calcium and phosphate loss following the usage of different irrigating solutions

Variable	Group	Mean (ppm) \pm SD
Calcium loss	17% EDTA	6.5 \pm 1.58
	0.2% nanochitosan	24.26 \pm 3.86
	Pomegranate aril extract	1.9 \pm 0.82
Phosphate loss	17% EDTA	5.59 \pm 2.08
	0.2% nanochitosan	29.32 \pm 7.11
	Pomegranate aril extract	2.65 \pm 1.24

EDTA=Ethylenediaminetetraacetic acid; SD=Standard deviation

Table 2: Pre-and post-immersion microhardness values

Groups	Premicrohardness	Postmicrohardness	Mean difference between pre-and post-immersion microhardness values	P
17% EDTA	56.44	36.7	19.74	0.000*
0.2% nanochitosan	56.87	36.6	20.26	0.000*
Pomegranate aril extract	56.97	55.73	1.23	0.007*

*P<0.05=Statistically significant. EDTA=Ethylenediaminetetraacetic acid

Table 3: Inter group comparison of calcium and phosphate mineral loss

Variable	Inter group comparison		Mean difference	P
Calcium loss	17% EDTA	0.2% nanochitosan	-17.75	0.000*
		Pomegranate aril extract	4.6	0.003*
	0.2% nanochitosan	Pomegranate aril extract	22.36	0.000*
Phosphate loss	17% EDTA	0.2% nanochitosan	-23.73	0.000*
		Pomegranate aril extract	2.93	0.248
	0.2% nanochitosan	Pomegranate aril extract	26.67	0.000*

*P<0.05=Statistically significant. EDTA=Ethylenediaminetetraacetic acid

Table 4: Inter group comparison of post immersion microhardness values

Inter group comparison	Mean difference in microhardness values	P
17% EDTA		
0.2% nanochitosan	0.34	0.997
Pomegranate aril extract	-17.78	0.000*
0.2% nanochitosan		
Pomegranate aril extract	-18.13	0.000*

*P<0.05=Statistically significant. EDTA=Ethylenediaminetetraacetic acid

microhardness postimmersion showed no significant difference between groups I, II but these solutions have significant difference with group III [Table 4].

Thus, it can be hypothesized that pomegranate aril extract demonstrated minimal effect on calcium, phosphate loss and microhardness values. Although 17% EDTA showed less mineral loss than 0.2% nano chitosan, the variation in microhardness was analogous with it. Novel irrigating solution nano chitosan had the most significant effect on calcium and phosphate loss and microhardness values.

Discussion

The root canal system is remarkably complex and variable, limiting the ability to clean and disinfect it predictably. Peters *et al.*^[19] stated that irrespective of the instrumentation technique used, 35% or more of the root canal surfaces, including canal fins, cul-de-sacs, and isthmi remain un-instrumented with the evidence derived from their study on permanent teeth using micro-computed tomography scans before and after mechanical instrumentation. Thus, the use of chemical adjuncts is considered a *sine qua non* in root canal treatment.^[20,21] Concerning primary teeth, in particular, more ictus is on chemical means with limited mechanical debridement.^[22] Therefore, irrigation is an indispensable part of root canal debridement.

An ideal irrigant should have many prerequisites.^[23,24] In addition, the choice of a cleanser in the pulpal therapy of primary teeth should have auxiliary requirements such as: They must not irritate the periapical tissues to avoid harming the germ of the permanent successor tooth, should facilitate easy instrumentation in the ribbon shaped canals, increase root dentin permeability without altering the microhardness of primary teeth.^[25]

The most common chelating solutions are based on EDTA, which reacts with the calcium ions in dentin and forms soluble calcium chelates.^[10] Chelating solutions remove dentin calcium ions, favoring smear layer removal and altering the microhardness. Baldasso *et al.*^[26] have hypothesized that these changes could increase the tooth's susceptibility to fracture. Biomaterials like Cs-NPs reduce the possibility of bacterial penetration, prevents dentinal micro-fractures, and improves the mechanical properties of the root dentin.^[27] Its high chelating capacity for different metallic ions and low cost, made it a preferable irrigant for the study. Natural phytochemical pomegranate has active components such as organic acids and bioactive compounds like phenolics and flavonoids, principally anthocyanins that inhibits the plaque-forming microorganisms and effectively removes smear layer in the root canals.^[28]

This study quantified the calcium ions chelated by different solutions from the root canal dentin by atomic absorption spectroscopy, a susceptible procedure popular for its elemental selectivity.^[29] Among the various phosphate determination methods, spectrophotometry involving molybdovanadate and ammonium molybdate is most widely used for biological applications as it is more sensitive, and the preparation of reducing agent is easy.^[30]

Microhardness determination is sensitive to composition and surface changes of the tooth structure; hence, it can provide indirect evidence of mineral changes, either loss or gain in dental hard

tissues.^[9] Previous investigations have demonstrated that Vickers microhardness test is an appropriate and practical tool for evaluating dentin surface changes treated with chemical agents.^[9,31] The fact that there were no statistically significant differences between the pretreatment microhardness values of all the tested groups proved that the specimens were standardized. The resulted difference in microhardness values was because of the different irrigating solutions used. In the current study, the longitudinal sectioning of the roots was preferred as it could show an accurate representation of clinical situation,^[6] and as the irrigants first contact the most superficial layer of root canal dentin, its microhardness was measured.^[32] In the present study, the root canals were not prepared before analysis; thus, no smear was present on the dentin surface. This step was omitted so that calcium and phosphate loss from intact root dentin could be measured, avoiding any possible contamination of readings that could result from calcium and phosphate being incorporated into loosely bound dentin.^[8]

The rationale behind using 17% EDTA was to test a low yet commonly used EDTA concentration on calcium and phosphate removal. 0.2% chitosan application for 5 min is the most viable combination for its use on root dentin and smear layer removal.^[33,34] Hence, a similar concentration of nano-chitosan has been used for equitable comparison. In the present study, a 5-min exposure time was advocated for each chelating solution as it may simulate the clinical application time of the irrigant solution.^[35]

Atomic absorption spectrophotometric analysis of calcium and vanadomolybdophosphoric acid method for phosphate evaluation exposed that 0.2% nano-chitosan produced the highest release of calcium and phosphate followed by 17% EDTA and pomegranate aril extract. Almost similar results were obtained in the studies done on permanent teeth by Pedro *et al.*^[2] However, contrast findings were seen in studies by Spanó *et al.*^[36] and Bastawy *et al.*^[9] in which 17% EDTA and 0.2% chitosan groups contained the highest concentration of calcium ions extracted from root canals, without significant difference between them. Nonetheless, study by Berastegui *et al.*^[37] exhibited the highest calcium loss from root dentin with EDTA than chitosan. The discrepancy in the results may be attributed to factors influencing the agents' demineralization capacity such as contact time, pH, concentration and the amount of available solution, assessment of calcium and phosphate ion concentration in the specimen rather than in solution, irrigation protocol followed coupled with difference in methodology.^[7] Furthermore, permanent dentin is richly mineralized, and concentration of calcium and phosphorus in both peritubular and intertubular dentin is also high. Even dentinal micromorphology studies showed potential differences between primary and permanent teeth.^[38]

It is believed that adsorption, ionic exchange, and chelation properties of chitosan are responsible for eliminating dentin calcium ions.^[39] There was a significant decrease in calcium and phosphate levels in dentin following EDTA treatment which was less than nano-chitosan. This might be attributed to the fact that EDTA forms a stable complex with dentin's calcium ions. Correspondingly, EDTA molecule's carboxyl groups ionize, releasing hydrogen atoms that compete with calcium ions. After all available ions are bound, equilibrium is formed, and no further dissolution occurs. It is also possible that dentin's organic matrix may act as a barrier in the dissolution of the inorganic component. Thus decalcifying action of EDTA stops.^[9] Pomegranate aril extract displayed least mineral loss, and possible reasons accredited to this may be weak organic acids such as ascorbic acid, citric acid, and malic acid in the arils, which have a weak chelating ability.^[28,40]

Notable variation in VHN values following irrigation protocol indicates the potential effect of these chemical solutions on radicular dentin components. The postimmersion VHN values in the present study suggest that dentin microhardness was significantly reduced by irrigating solutions compared to baseline values. As compared to pomegranate aril extract group, both 0.2% nano chitosan and 17% EDTA showed an identical reduction in root canal dentin's microhardness. The chelating activity of EDTA produces softening of calcified components of dentin, and subsequently, a reduction in the microhardness of the root canal dentin is noticed.^[29] The chelating property of chitosan responsible for calcium and phosphate extraction from radicular dentin might also be accountable for reduction in microhardness of dentin which was analogous with that of 17% EDTA. The results are in accordance with Pimenta *et al.*^[33] and Silva *et al.*^[34] who found that 0.2% chitosan is equally effective as 15% EDTA in microhardness reduction. On the other hand, Bastawy *et al.*^[9] reported that 0.2% chitosan caused less reduction in dentin microhardness than 17% EDTA. In case of pomegranate aril extract, it may be hypothesized that the presence of weak acids could have altered dentin's microstructure that is liable for a minimal reduction in root dentin VHN values. Nevertheless, it may be theorized that the time-dependent demineralization effect of the irrigation regimens tested herein may be reduced in the clinical settings by the presence of smear layer, volume of irrigant used, contact period, and irrigation protocol followed.

Conclusion

All the irrigants used in the study demonstrated mineral loss from the root canal dentin with maximum loss observed in 0.2% nanochitosan and least with pomegranate aril juice. A significant difference in the VHN values was recorded in all the tested groups,

suggesting that microhardness is directly proportional to mineral loss. Thus, the present study's observations demonstrate the need to incorporate herbal agents such as pomegranate extract as a valuable adjunct during endodontic procedures; however, further research to enhance its clinical applicability and establish its capability as a root canal irrigant needs to be evaluated.

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Conflicts of interest

There are no conflicts of interest.

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ORIGINAL RESEARCH

Remineralizing Potential of Various Commercially Available Dentifrices on Artificial Enamel Lesions

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ABSTRACT

Aim: The aim of the present study was to determine the remineralizing potentiality of artificial carious lesions in primary teeth after application of three commercially available dentifrices using pH cycling.

Materials and methods: A total of 24 human primary teeth were randomly divided into three groups. Group I: Clinpro Tooth Creme, Group II: Enafix, and Group III: Amflor. All the teeth in each group were demineralized for 96 hours, sectioned longitudinally using hard tissue microtome and subjected to pH cycling for 7 days. The specimens were viewed under polarized light microscope, depth of demineralization and remineralization was recorded using Micap Microview analyzer software.

Results: The results were analyzed using one way-ANOVA test. All the test groups showed statistically significant decrease in mean depth of lesion $21.62 \pm 9.41 \mu\text{m}$, $20.75 \pm 14.61 \mu\text{m}$, $26.13 \pm 17.84 \mu\text{m}$ for group I, II, and III, respectively. However, no statistical difference was noticed on intergroup comparison.

Conclusion: All the tested dentifrices demonstrated remineralizing potential which was elicited by decrease in the lesion depth. Amflor showed better results than other remineralizing agents.

Clinical significance: To emphasize the importance of minimal invasive treatment of incipient carious lesion by remineralizing agents. Opting for remineralizing agents at the early stage can save the time, money and manpower. Usage of dentifrices containing remineralizing agents shown long-term beneficial effects on early caries lesions. The present study demonstrated that all the tested agents can be used to induce remineralization of early enamel lesions, so that complex invasive treatments can be prevented in near future.



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Keywords: Demineralization, Dentifrices, Fluorides, Polarized light microscope, Remineralization

INTRODUCTION

Dental caries is the most prevalent oral disease and is considered as the major worldwide health concern. “White spot lesion” is the earliest clinical sign of dental caries.¹ In the recent decade, white spot lesions can be prevented through various noninvasive preventive modes, as the routine operative treatment is hard to achieve and may also need special behavior management for treating children.

In children, the demineralization potential at low oral pH is high while the remineralization potential at normal pH is low.² And also low mineral and high organic content of enamel made the primary teeth more susceptible to caries development. Hence, the progression of caries will be faster and reversal will be slower in children, as they depend upon the balance between demineralization and remineralization.³ Thus, in children while managing the initial caries lesions the most preferred noninvasive intervention is the usage of dentifrices containing remineralizing agents.

The ions liberated upon the application of remineralizing agents will act as diffusion barrier by the formation of thick surface coatings and also deposition of minerals within the enamel crystallites in turn decrease the solubility of enamel.⁴ Orientation of the remineralized enamel crystals is similar to that of the original enamel crystals and are generally more resistant to decalcification.

Fluoride’s role in remineralization is well established and effective method of remineralization. Fluoride containing dentifrices usually have a dose-response relationship; therefore dentifrices containing 250 to 500 ppm fluoride are used for children to reduce fluorosis risks. In accordance with IAPD Recommendations (2020), using fluoridated tooth paste containing 1000 ppm of fluoride twice a day with age-appropriate amounts of tooth paste on the toothbrush are effective in preventing dental caries in children.⁵

As science and research advances, newer fluoridated and nonfluoride remineralizing agents have been introduced in the market. The Clinpro Tooth Cremem contains functionalized tri-calcium phosphate ingredient (fTCP) with fluoride range of about 950 ppm. Each gram of it contains 0.95 mg of fluoride ion in a neutral pH base consisting of

water, hydrated silica, sorbitol, glycerin, polypropylene glycol, polyethylene-polypropylene glycol, flavor, sodium lauryl sulfate, titanium dioxide, carboxymethyl cellulose, and sodium saccharin.⁶ This organically modified TCP technology prevents undesirable reactions with fluoride, but may dissolve away when particles contact saliva. This system is stable in aqueous environment and does not affect the fluoride activity.⁷

Enafix contains anticay 5% which is a complex mix of calcium sucrose phosphate inorganic amorphous calcium phosphate, sorbitol, purified water, silica, glycerin, sodium lauryl sulphate, flavors, sodium methyl cocodyl taurate, sodium carboxy methyl cellulose, titanium dioxide, potassium acesulfame, sodium methyl hydroxyl benzoate, and sodium propyl hydroxyl benzoate. During brushing, calcium sucrose phosphate (anticay) quickly breaks down and releases calcium, phosphate, and sucrose phosphate ions into the saliva. Calcium and phosphate ions rapidly adsorb onto the enamel, decrease the rate of enamel solubility under acidic conditions and at neutral pH and increase the rate of remineralization.⁸

Amflor contains 1000 ppm amine fluoride, sorbitol, purified water, propylene glycol, silica, cocamidopropyl betaine, titanium dioxide, Hydroxyl ethyl cellulose and sodium saccharin. Amflor toothpaste contains the active ingredient in the form of amine fluoride (organic fluoride) which has caries inhibitory properties due to its antiglycolytic and surface-active properties. It also involves the accumulation of fluoride onto the surface of the tooth, which readily forms calcium fluoride and acts as a labile fluoride reservoir.^{8, 9}

These three remineralizing agents were chosen based on their difference in composition and also studies comparing the above forementioned dentifrices on the primary teeth are sparse. Therefore present in vitro study was undertaken to compare remineralization potential of three commercially available dentifrices (Clinpro Tooth Creme, Enafix, and Amflor) on artificial carious lesions.

MATERIALS AND METHODS

The present in vitro study was performed after obtaining institutional ethical committee approval (pr.171/IEC/SIBAR/2018). The sample size determination was done using G* 3.1.9.2; the effect size is 0.795 and sample size derived is 24. Teeth with preshedding mobility, natural exfoliation, extracted over-retained or orthodontic purpose were included. Whereas the teeth with cracks, white spots and dental caries, or any discolored teeth and hypoplastic teeth were excluded from the study.

Out of total 40 sound human caries-free primary teeth collected, 24 teeth, which fulfilled all the inclusion and exclusion criteria were selected for the study and stored in a 0.1% thymol solution until use.

Sample Preparation and Lesion Formation

The selected teeth were rinsed, hand scaled and thoroughly cleaned and dried. A rectangular window of 2 mm × 4 mm was labeled in the center of the buccal surface and the remaining surface of the tooth was coated with nail varnish. The teeth were randomly divided into three groups with 8 in each using computer randomization technique. The groups are as follows: Group-I: Clinpro Tooth Creme (3M ESPE Dental Products, St.Paul, MN, USA), Group-II: Enafix (Group pharmaceuticals pvt Ltd, Banglore, India), and Group-III: Amflor (Group pharmaceuticals pvt Ltd, Malar, India).

To create artificial caries lesions, all the specimens were immersed for 96 hours in a glass beaker containing 500 mL of demineralizing solution. With a diamond disk mounted on a slow-speed handpiece, the teeth are sectioned longitudinally in buccolingual direction. Each specimen shows a portion of the normal enamel and demineralized area. Out of two sections obtained from each tooth, one section was used to record depth of demineralization and other to record depth of remineralization.

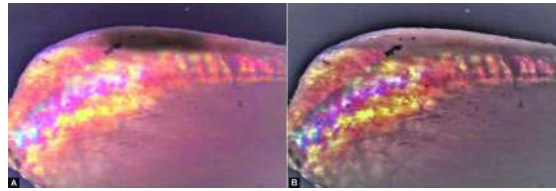
The specimens used to record depth of demineralization in three groups were embedded in self cure acrylic (DPI- PR Cold Cure) resin blocks using a preformed mould. Later these specimens were sectioned using hard tissue microtome (Leica SP 1600 Saw Microtome, Germany) longitudinally through the lesions to produce sections of approximately 100 to 150 μm thickness to examine under polarized light microscope.

Recording the Demineralization Depth

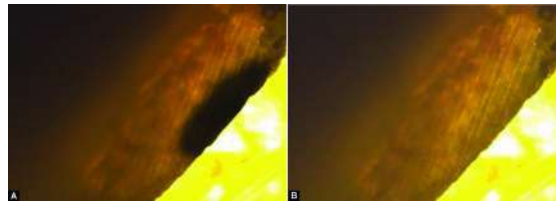
The sectioned samples of each group were immersed in water for a clear demarcation between sound and carious enamel and then viewed under polarized light microscope (Olympus BX51 penta head microscope, Japan) at 40x magnification. After attaining the microscopic images (Fig. 1A, 2A, 3A), the depth of demineralization was noted at three different points from the surface of a tooth to the maximum depth of demineralization and the average of the three was considered as mean value (μm).



Figs 1A and B: (A) Polarized light microscopic image after demineralization in group I; (B) Polarized light microscopic image after remineralization in group I



Figs 2A and B: (A) Polarized light microscopic image after demineralization in group II; (B) Polarized light microscopic image after remineralization in group II



Figs 3A and B: (A) Polarized light microscopic image after demineralization in group III; (B) Polarized light microscopic image after remineralization in group III

pH Cycling

Ten Cate and Duijsters pH cycling model was used for all the samples after demineralization.¹⁰ It involved 3 hours of demineralization twice daily, with 2 hours of remineralization between periods of demineralization for 7 days. Each section was then treated with dentifrice supernatant for 60 seconds (5 mL/section).

The demineralizing, remineralizing solutions and dentifrice slurries were freshly prepared for each cycle and stored in separate containers for each group throughout the experimental period.¹¹ These solutions were changed daily to prevent depletion or saturation of the solutions and accumulation of enamel dissolution products.³

Recording Depth of Remineralization

Following pH cycling the specimens were embedded in acrylic blocks and sectioned using hard tissue microtome. Each section was visualized under polarized light microscope at 40X magnification to determine the amount of remineralization on microscopic images (1B, 2B, 3B) using Micaps-micro view 3.7 version software.

The entire procedure was performed by a single operator, however to avoid bias, a second operator who is unaware of prior results evaluated the procedural aspect and

recorded the values. intraclass correlation coefficient (ICC) values were calculated to examine the reliability. An intraclass correlation value of 0.93 was achieved which indicates good agreement among the investigators, hence the earlier observations were only taken into account.

RESULTS

The de- and remineralization values obtained were tabulated using SPSS and subjected to statistical analysis. Paired t-test was used to calculate mean lesion depth and standard deviations and one-way ANOVA for inter group comparison. The p -value of < 0.05 was considered statistically significant.

The mean lesion depth among the three test groups after demineralization ranged from 167.38 μm to 177 μm which was not statistically significant and after remineralization the values varied between 141.25 μm to 155.38 μm , which were also statistically insignificant. However, a decrease in the mean lesion depth was observed in the all test groups after remineralization.

Coming to reduction in mean lesion depth after remineralization Group III exhibited highest decrease in mean lesion depth (26.13 \pm 17.84 μm) followed by Group I (21.62 \pm 9.41 μm) and Group II (20.75 \pm 14.61 μm) samples. There was statistical significant decrease in mean lesion depth values in all the three groups after de- and remineralization for Group I, II, and III with a p -value of 0.001*, 0.005*, and 0.004*, respectively (Table 1).

Table 1: Comparison of mean lesion depth and difference in mean lesion depth before and after remineralization

Group	N	Mean \pm SD before remineralization	Mean \pm SD after remineralization	Mean difference	p value
I	8	177.00 \pm 11.88	155.38 \pm 16.37	21.62 \pm 9.41	0.001*
II	8	170.13 \pm 14.04	149.38 \pm 19.89	20.75 \pm 14.61	0.005*
III	8	167.38 \pm 16.02	141.25 \pm 24.4	26.13 \pm 17.84	0.004*

Paired t test; $p \leq 0.5$ considered statistically significant

DISCUSSION



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Remineralization is a noninvasive treatment modality for the initial caries lesions that bridge the gap between preventive and invasive dentistry.⁸ Caries process is said to be dynamic when demineralization periods are far more than the periods of remineralization.¹ Saliva's inherent buffering capacity and the presence of calcium ions, phosphate ions, fluoride, and other components prevent the early tooth demineralization.¹² If this natural system of saliva fails, then remineralizing agents are needed to compensate the oral defensive mechanisms at that site and protect the tooth from cavitation. Thus, if remineralizing agents are used at this stage, caries can be arrested or reversed.¹³

In the present study, artificial carious lesions were produced as they are more homogeneously reproducible than natural lesions and showed all the principal histological features of natural caries of enamel *in vitro*.³ Extracted or naturally exfoliated primary teeth (molars, canines, and incisors) were used for lesion formation. Though there are variations in the morphology of individual teeth, it was hypothesized that these variations among the teeth do not have a significant role in caries formation.⁷

Single section model proposed by Wefel et al.¹⁴ was used for this study as it provides the advantage that the same tissue can be evaluated before and after the exposure period. Thus, any change could only be due to the exposure of the experimental solutions. Similarly, Kiranmayi et al.³ and Malekafzali et al.¹⁵ used single section pH cycling models for evaluating the efficacy of fluoride dentifrices on primary teeth.

Ten Cate and Duijsters pH cycling model can mimic the introral pH changes and results in more representative findings in evaluating remineralization efficacy of an agent.¹⁶ A 10-day pH-cycling model can be used on the enamel of permanent teeth whereas a 7-day pH cycling or 10-day cycling with added 0.25 ppm fluoride can be used for primary teeth.^{3,7} In the present study, 7 days pH cycling model without the addition of fluoride was used as incorporation of the same could interfere with the hypothesis being tested.

Polarized light microscope was used in the present study to assess the lesion depth of the artificial caries lesion for all specimens because the characteristic features of enamel can be better visualized due to its birefringence property, which is not well appreciated in a transmitted light microscope.¹⁷

The results of the present study quantified that all the tested groups showed better remineralization potential by decreasing the mean lesion depth. However, Group III elicited higher remineralization potential; which could be due to high fluoride content (1000 ppm) and the fluoride compound used (amine fluoride).

The results were similar to the studies conducted by Galuscan et al.¹⁸ and Madlena et al.¹⁹ who reported that amine (organic) component in amine fluoride (AmF) exhibited an antiplaque effect that resulted in the inhibition of bacterial adhesion. Also, the tensioactive

property of the amine component favors the accumulation of fluoride close to the tooth surface, providing a sustained fluoride release. Shetty et al.²⁰ also evaluated the enamel surface microhardness after the application of organic fluoride (AmF) and inorganic fluoride (NaF) dentifrices on permanent teeth and found AmF released a larger amount of fluoride than NaF, which caused the fluoride to be more bioavailable for a longer period of time. Likewise, Prasad et al.²¹ compared the remineralizing effect of organic fluoride (AmF) and inorganic fluoride (NaF) and found AmF showed better remineralization on the enamel surface of permanent teeth. This might be due to formation of a thick calcium fluoride layer between NaF (inorganic fluoride) and hydroxyapatite of enamel which inhibited the further diffusion of fluoride, thus providing a relatively lower bioavailability of fluoride ions. Arnold HW et al.²² Naumova EA et al.²³ also found that more amount of fluoride was deposited on enamel by treating with AmF than sodium fluoride which resulted in enhanced remineralization.

In this study group I (Clinpro Tooth Creme) showed a significant reduction in mean lesion depth thereby promoting remineralization. The reason may be due to the presence of a combination of sodium fluoride with functionalized tricalcium phosphate (f-TCP) which provides a continuous reservoir of ions. As it not only ensures a controlled supply of calcium and phosphate ions, but also enhances the action of fluoride on enamel surfaces. TCP can provide maximum advantages even when administered in a neutral pH environment, whereas other calcium phosphate additions may require an acidic pH.

In accordance to the present study, Rao R et al.²⁴ also found that ClinPro tooth Creme showed better remineralization potential and this could be due to beta-TCP is present in Clinpro tooth creme, which is similar in structure to apatite and has unique calcium environments capable of reacting with fluoride and enamel. While phosphate floats free, the exposed calcium environments are protected, preventing calcium from prematurely interacting with fluoride by providing catalytic amounts of calcium to boost fluoride absorption and promote remineralization.

Similarly, Krishnan et al.²⁵ found Clinpro as a promising agent for remineralizing primary teeth. Likewise, Sreekumar et al.,²⁶ Patil et al.¹ found that a higher concentration of calcium ion in f-TCP and addition of fluoride was responsible for its superior performance.

On Contrary, study done by Premanth et al.²⁷ found better remineralization with clinpro than Amflor in permanent teeth. This might be due to the synergistic effect of both fluoride compound (NaF) and functionalized tricalcium phosphate (f-TCP). The aqueous-stable functionalized calcium phosphate system has no effect on the fluoride activity added to dentifrices, which increases enamel surface strength. The available evidence, therefore indicated that TCP-containing dentifrices could remineralize teeth in a significant manner.



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The significant reduction in mean lesion depth in group II samples could be due to rapid absorption of ions onto enamel surface, make the availability of the sucrose phosphate ion which decreases the rate of acid dissolution of hydroxyapatite that inhibits demineralization and presence of calcium and phosphate ions increase the rate of remineralization by common ion effect. Similarly, Gade²⁸ and George et al.²⁹ in their studies found Enafix as a favorable remineralizing agent for mineralization of artificially demineralized human enamel.

Although remineralizing ability of CaSP with inorganic ACP (Enafix) has shown promising results. However, in comparison to the dentifrices containing fTCP+NaF (Clinpro) and amine fluoride (Amflor), Enafix has shown less effectiveness in promoting remineralization in this study. This might be due to the nonavailability of fluoride ions, as soluble calcium and phosphates were unable to substantially localize at the tooth surface to produce effective concentration gradients for better remineralization. This concept was further supported by studies done by Titty et al.⁹ Kakkar et al.¹² in which Enafix formed a thick layer of calcium fluoride by reaction with hydroxyapatite of enamel that in turn reduced the bioavailability of fluoride ions.

Even though this *in vitro* experiment provides qualitative research, certain limitations do exist while performing remineralization in *in vitro* designs such as inadequate simulation of complex and diverse intraoral conditions, lower level of salivary proteins, lack of bacteria in the artificial saliva, control over the salivary flow rate, and lack of pellicle and biofilm formation on the tooth surface. Moreover the specimens were also exposed to multiple cycles of de- and remineralization, which may be severe than actual acid attack in the oral cavity. Hence, further studies need to be conducted with the forementioned points taking into consideration to obtain optimal clinical results.

CONCLUSION

All the three dentifrices used in the study demonstrated remineralization of artificial carious lesions. Among the three, amine fluoride containing dentifrice exhibited a superior remineralizing potential which might be due to the presence of high fluoride content and organic fluoride component (amine component). Also properties like antiglycolytic, tensioactive property, antiplaque, and anticariostatic effect are further considered for the beneficial effects of remineralization.

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Orthodontic pain control following arch wire placement; a comparison between pre-emptive tenoxicam and chewing gum: a randomized clinical trial

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Background: Pain during fixed orthodontic treatment can have a detrimental effect on patient treatment compliance. To overcome this, there is a definite need to establish the best pain-relieving methods suitable for orthodontic patients in terms of efficacy and use. The objective of this study was to compare the effect of chewing gum and pre-emptive tenoxicam on pain after initial archwire placement and to evaluate the pain perceptions of orthodontic patients in the two groups while performing various functions at specific time intervals.

Methods: Forty-two patients were selected and randomly divided into two groups: group A (chewing gum) and group B (pre-emptive tenoxicam). Pain perception was documented by patients immediately; at 4 h; at bedtime on the day of archwire placement; the next morning; at 24 h; and at bedtime on the 2nd, 3rd, and 7th day after the initial archwire placement. Pain scores were noted during fitting of the posterior teeth, biting, and chewing using a visual analog scale. The data obtained were subjected to statistical analysis.

Results: Group A showed a significant increase in pain until the next morning while fitting the posterior teeth, biting, and chewing [36.2, 52.0, 33.4, respectively], followed by a gradual decrease by the 7th day. Group B showed a significant increase in pain at bedtime on biting, with a peak value of 47.5. Pain on chewing, fitting posterior teeth, peaked the morning of the next day (100.0, 45.0). The Friedman test showed a statistically significant difference with a p-value of < 0.01. Higher pain scores were observed while chewing and biting compared with that while fitting the posterior teeth in both groups. The overall comparison of pain control between the two groups was not statistically significant [P > 0.05] between the two groups.

Conclusions: Chewing gum was not inferior to pre-emptive tenoxicam. Thus, chewing gum is a non-pharmacological alternative to analgesics for orthodontic pain control that eliminates the chance of adverse reactions and can be used in the absence of adult observation.

Keywords: Chewing Gum; Orthodontic Treatment; Pain Control; Tenoxicam.

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INTRODUCTION

Anxiety regarding pain and uneasiness during the

treatment phase is a concern for orthodontic patients. It is a crucial limiting factor for fixed-appliance therapy and may have a negative impact on patient compliance [1]. Soon after placement of the initial archwire, biological

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variations occur in the periodontal ligament (PDL), resulting in the release of inflammatory mediators such as prostaglandins and bradykinin etc., [2] which mediate orthodontic pain [3,4]. Pulpal inflammation during fixed appliance therapy may also induce the pain [5,6]. The pain typically intensifies progressively after 2 h of applied orthodontic force, reaches a peak by 24 h, and gradually reduces by the seventh day [7-9].

To relieve pain and discomfort, clinicians employ either conventional pharmacological analgesic administration of nonsteroidal anti-inflammatory drugs (NSAIDs) or nonpharmacological methods. The latter includes the use of a plastic wafer [10], chewing gum [11,12], transcutaneous electrical nerve stimulation [13], low-level laser therapy [14,15], and vibratory stimulation [16].

Cheng et al. [17] revealed that preemptive analgesia is effective for orthodontic pain control. The mechanism involves the generation of analgesia prior to the induction of a pain stimulus, which may reduce pain intensity [18]. The inhibition of prostaglandin synthesis by NSAIDs also have adverse effects on tooth movement [19]. Tenoxicam (20 mg) was convenient for pain control without any impediments related to upper canine tooth movement [20]; hence, tenoxicam was used in the present study. Tenoxicam belongs to the oxycam group of non-selective COX inhibitors (NSAIDs) with a half-life of 67 h. It is beneficial in terms of usage to administer only a single dose of tenoxicam per day for orthodontic pain relief. In contrast, other non-selective COX inhibitors are prescribed three times a day, which increases drug intake.

The overuse and adverse reactions of NSAID challenge their use in contemporary analgesic management in adolescent patients [21,22]. Owing to these limitations, non-analgesic pain control methods have been approved [10]. Few studies have reported the efficacy of chewing gum for orthodontic pain control [23-25].

However, in a recent systematic review by Fleming et al. [26], very low-quality evidence was observed regarding pain relief with the use of chewing adjuncts. In this context, the present study aimed to compare the effectiveness of chewing gum and tenoxicam for pain

relief following initial archwire placement in patients receiving orthodontic treatment. To date, few randomized controlled trials (RCTs) have been included in systematic reviews on orthodontic pain management methods. To our knowledge, no RCTs has compared chewing gum and tenoxicam for pain relief in orthodontic treatment. Therefore, the present study aimed to compare the pain-relieving effect of chewing gum with that of pre-emptive tenoxicam.

METHODS

The present study was a uni-centered, two-arm parallel investigation approved by the Institutional Research Ethics Committee (University registration number: D168408007). One hundred twenty-one subjects requiring fixed orthodontic therapy were assessed for eligibility. Only female patients were included in the investigation to eliminate the influence of sex on the outcome assessment, as sex-dependent differences in pain perception have been reported [27].

Inclusion criteria: physically and mentally healthy women in the age group of 18–25 years, patients undergoing fixed orthodontic treatment for the first time, full arch bonding in both arches, extraction in both arches, and crowding with a minimal index of 4–9 mm.

Exclusion criteria: patients with systemic diseases, pregnant patients, patients in whom tenoxicam is contraindicated, patients under medication for chronic pain, and patients undergoing functional appliance therapy and orthognathic surgery.

A comprehensive medical history and informed consent were obtained.

Sample size determination: This was designed to preserve the power of the study at 0.8 (80%) and the level of significance at 0.05 (5%). [28] A total of 34 subjects were needed for the trial to detect a minimum difference of 10 mm on pain scale on visual analog scales (VAS) between any two subjects for any given function or time point. Considering a possible dropout rate of 20%,

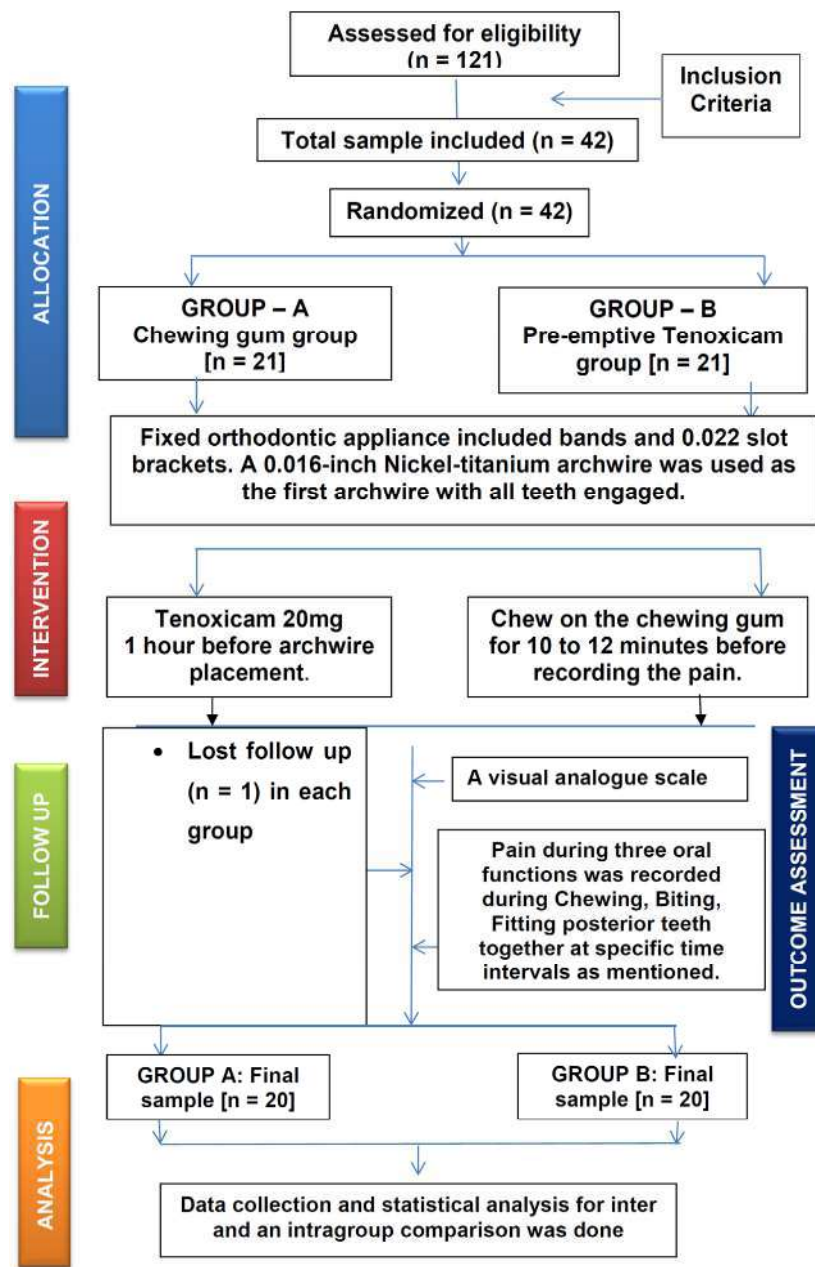


Fig. 1. Consolidated Standards of Reporting Trials flow diagram showing the study design.

a total of 42 female participants were selected for the trial and equally divided into two groups of 21 each.

Randomization: The patients were coded with a numerical value, and the data were entered into an Excel sheet and fed to a computer application. Simple randomization was performed using a 1:1 allocation. The sequences of the subjects assigned to the chewing gum group (group A) and the tenoxicam group (group B) were computer-generated with random numbers using a

research randomizer. Allocation was performed using opaque sealed envelopes. On the day of archwire placement, which was also the first day of the intervention, each subject chose one envelope to detect her randomized allocation.

Treatment: In both groups, orthodontic appliances included bands and 0.022 slot M.B.T brackets (3M Unitek™ Gemini Metal Brackets, USA). As an initial archwire, 0.016-inch nickel-titanium was placed. Patients

Table 1. Depiction of the differences in the demographic data between the 2 groups (Chewing gum and Tenoxicam group) (P-value > 0.05) is statistically insignificant

Demographic characteristic	Group	Number of participants	Mean \pm SD	P-value
Age	Group A	20	19.6 \pm 1.6	0.64
	Group B	20	20 \pm 3.4	
Height	Group A	20	165.05 \pm 7.1	1.00
	Group B	20	165.05 \pm 5.4	
Weight	Group A	20	53.5 \pm 8.2	0.76
	Group B	20	53.15 \pm 6.8	

SD, standard deviation.

in the chewing gum group were asked to chew on chewing gum (Trident, sugar-free gum with xylitol, U.S.A.) whenever they experienced discomfort. However, patients were advised to chew it for 10 to 12 min prior to pain recording at specific time intervals. In the tenoxicam group, the subjects took Tenoxicam B.P. (Tilkotil, 20 mg, Radiant Pharmaceuticals, Bangladesh.) 1 h before archwire placement. The work strategy was illustrated using a Consolidated Standards of Reporting Trials flowchart (Fig. 1).

Data collection: Subjects were given regular post-treatment guidelines and were suggested to fill out a feedback form at suitable intervals in the week after the bonding procedure. The questionnaire was given in a 7-page booklet containing a succession of 10 cm VAS, and each centimeter was divided into 10 mm. The patients were instructed to note the grade of pain experienced, from 0 to 100 (with 0 representing no pain and 100 representing severe pain), at the indicated time intervals, immediately after archwire placement (T_0), 4 h after archwire placement (T_1), at bedtime of the day of archwire placement (T_2), the next morning (T_3), at 24 h (T_4), and bedtime of the 2nd (T_5), 3rd (T_6), and 7th day after the engagement of the initial archwire (T_7).

The subjects were advised to record pain during three oral functions: fitting the posterior teeth together, chewing, and biting. To fit the posterior teeth, the subjects were instructed to fit the posterior teeth using a slight force. The patients used an almond for biting and chewing. They bit and chewed almonds and recorded their perceived pain.

Table 2. The Descriptive Data of the VAS scores for the 2 groups under study

Function	Time interval	Chewing gum	Tenoxicam
		Mean \pm SD	Mean \pm SD
Fitting posterior teeth	T_0	11.1 \pm 7.5	4.4 \pm 6.4
	T_1	16.7 \pm 5.35	16.2 \pm 6.01
	T_2	30.6 \pm 9.32	27.7 \pm 5.93
	T_3	36.2 \pm 10.04	31.3 \pm 5.25
	T_4	31.5 \pm 9.19	18.7 \pm 5.51
	T_5	29.4 \pm 9.69	13.2 \pm 2.78
	T_6	23.6 \pm 8.19	10.07 \pm 2.32
	T_7	16.8 \pm 5.44	5.35 \pm 1.49
Biting	T_0	17.5 \pm 4.27	14.0 \pm 4.46
	T_1	29.1 \pm 4.90	28.2 \pm 6.48
	T_2	39.9 \pm 9.44	47.5 \pm 11.5
	T_3	52.0 \pm 11.1	47.3 \pm 9.41
	T_4	41.6 \pm 8.59	39.6 \pm 6.98
	T_5	41.6 \pm 8.86	32.9 \pm 7.81
	T_6	28.4 \pm 4.53	28.0 \pm 4.11
	T_7	11.6 \pm 3.24	12.7 \pm 6.03
Chewing	T_0	12.2 \pm 3.71	24.9 \pm 4.95
	T_1	33.1 \pm 5.79	39.0 \pm 8.60
	T_2	33.7 \pm 5.34	62.6 \pm 9.62
	T_3	42.9 \pm 4.35	68.1 \pm 8.76
	T_4	34.1 \pm 6.62	45.5 \pm 6.80
	T_5	33.7 \pm 6.13	33.7 \pm 5.52
	T_6	18.8 \pm 3.62	29.5 \pm 5.13
	T_7	13.3 \pm 3.95	11.4 \pm 2.90

SD, Standard deviation.

All patients were stringently communicated to prevent the intake of supplementary analgesics. If rescue medicine was needed, the patient was advised to contact the investigator immediately and to document the

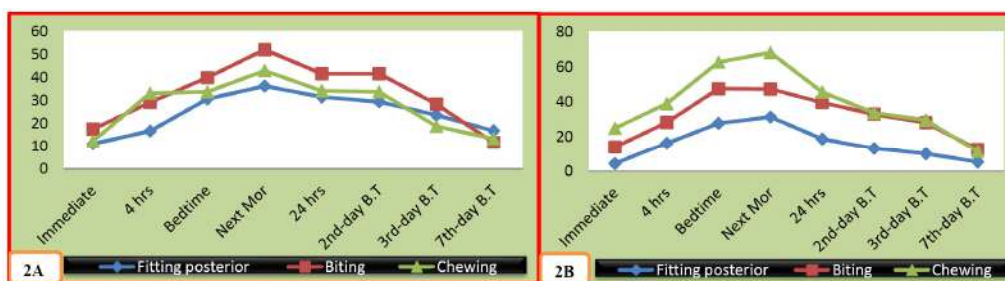


Fig. 2. Comparison of VAS scores while performing various functional activities at different points of time in the two groups (A) chewing gum group, (B) tenoxicam group

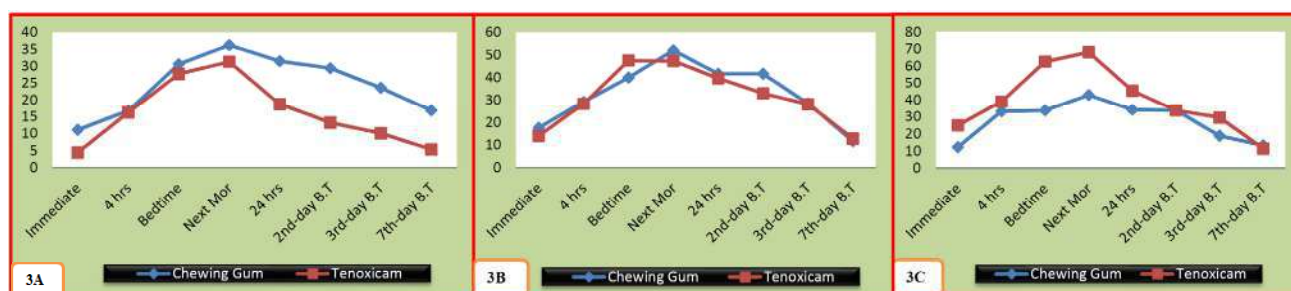


Fig. 3. Comparison of VAS scores among the two groups while performing various functions at different points of time. (A) fitting posterior teeth, (B) biting, (C) chewing. VAS, visual analog scale.

analgesic used. Patients were requested to return to the completed pain diary at the next appointment.

Blinding: The clinician and patients were not blinded to the intervention because the usage instructions needed to be explained based on the intervention. However, the operator dealing with the feedback forms and the statistician evaluating the records were blinded to the intervention.

Outcome: Valuation of pain by patients using VAS at specific time intervals after initial archwire placement.

Statistical analysis: All statistical analyses were performed using SPSS Statistics for Windows software package, Version 21.0. (Armonk, NY: 2012). The histogram assessed the normal distribution of the sample variables and the results showed that the parameters were normally distributed. An unpaired t-test was used to demonstrate the differences in demographics between the two groups. The Friedman test was used to compare pain experienced at different points in time during the different functions within the group in both clusters. The Mann-Whitney U test was performed to determine pairwise comparisons between the two groups at different time

points for different functions and to assess the overall pain perception between the two groups.

RESULTS

Two patients, one in each group, discontinued participating in the study for personal reasons. Finally, the final sample size for statistical analysis was 20 participants in each study group. The demographic details of the study participants are presented in Table 1. The unpaired t-test revealed no significant differences in the mean age, height, and weight between the groups.

1. The course of post intervention pain

The descriptive data for the two groups displayed in Table 2 shows that the peak pain occurred at T3 in chewing gum group during biting and in the tenoxicam group during chewing. There was a slow decrease in T₇ pain in both groups. The pain experienced during biting or chewing was greater than that experienced while fitting posteriors in both groups.

Table 3. Comparison of Pain experienced at different points of time during the different functions in Chewing gum and Tenoxicam group- Friedman test (P-value = < .001)** is statistically significant

Function	Time intervals	Chewing gum group		Tenoxicam group	
		Friedman test value	P value	Friedman test value	P value
Fitting Posterior	T ₀ -T ₇	129.42	< 0.001**	129.956	< .001**
Biting	T ₀ -T ₇	133.66	< 0.001**	133.581	< .001**
Chewing	T ₀ -T ₇	123.10	< 0.001**	139.180	< .001**

Table 4. Pairwise comparisons of pain perceptions between the two groups at different points of time for different functions - Mann-Whitney test (P-value < 0.05)*, ** is statistically significant (P-value > 0.05) is statistically insignificant

Time intervals	Different functional activities		
	Mann-Whitney test P-value		
	Fitting posterior	Biting	Chewing
T ₀	< .001**	0.002*	< .001**
T ₁	0.577	0.532	< .001**
T ₂	0.227	0.002*	< .001**
T ₃	0.043*	0.023*	< .001**
T ₄	< .001**	0.174	< .001**
T ₅	< .001**	< .001**	0.849
T ₆	< .001**	0.633	< .001**
T ₇	< .001**	0.630	0.102

1) Chewing gum group

A significant increase in pain was observed until T₃ during fitting of the posterior teeth, biting, and chewing, followed by a gradual decrease by T₇ (Fig. 2A). The p-value was highly significant (P < 0.001) at all time points while performing various functional activities (Table 3).

2) Tenoxicam group

There was a significant increase in pain during biting at T₂. Pain on fitting the posterior teeth and chewing showed a peak value at T₃ (Fig. 2B). P-values indicated high significance (P < 0.001) at all the mentioned points of time while performing various functional activities (Table 3).

2. Comparison of VAS scores between two groups while performing various functions at different points of time

- The tenoxicam group showed less pain than the

chewing gum group during fitting of the posterior teeth at all times (Fig. 3A). In both groups, the pain increased until T₃ and gradually decreased by T₇.

- During biting, the tenoxicam group reported less pain, followed by the chewing gum group at all time points except T₂ (Fig. 3B). The tenoxicam group showed peak pain at T₂, whereas the chewing gum group showed peak pain at T₃.
- During chewing, patients in the chewing gum group reported less pain, followed by those in the tenoxicam group at all time points (Fig. 3C). Both groups showed an increase in pain until T₃ and a gradual decrease in pain by T₇.

3. Comparisons of pain perceptions between the two groups at different points of time for various functions (Table 4)

T₀, T₃: There was a statistically significant difference (P < 0.05) between the two groups in all functional activities.

T₁, T₂: There was a statistically significant difference (P < 0.05) between the two groups during chewing. However, statistical significance was not observed when fitting posterior teeth and biting.

T₄, T₆: There was a statistically significant difference (P < 0.05) between the two groups while performing all functional activities, except while biting, where there was no statistical significance.

T₅: There was a statistically significant difference (P < 0.05) between the two groups while performing all functional activities except chewing, which showed no statistical significance.

T₇: There was a statistically significant difference (P < 0.05) between the two groups while fitting posterior

teeth, whereas during chewing and biting, the difference was not statistically significant.

The Friedman test values of the two groups were similar during fitting posterior teeth and biting, whereas during chewing, the chewing group showed a lower value of pain (123.10) than the tenoxicam group (139.180), indicating that chewing gum is not inferior to tenoxicam in controlling orthodontic pain (Table 3). The overall comparison of tenoxicam and chewing gum revealed no significant difference ($P = 0.305$) between the groups.

DISCUSSION

Tenoxicam is a long-acting nonsteroidal anti-inflammatory agent that is effective in pain control. Its long-acting effect may be correlated with its high serum binding properties [29]. Previous investigations have focused on the efficacy of chewing gum for pain management [12]. Few studies have compared two analgesics [30], chewing gum, and short-acting NSAIDs [31] in regulating orthodontic pain. No study has compared the efficacy of chewing gum with that of long-acting NSAIDs for pain reduction during orthodontic therapy.

Therefore, this work aimed to compare the effects of chewing gum and pre-emptive tenoxicam on pain perception once initial archwire engagement was done. In this study, the present pain levels for both groups followed a similar curve. In both groups, the intensity of pain during various functions gradually increased from T_0 , reached its extreme intensity at T_2 and T_3 and gradually decreased by T_7 . This outcome was supported by the observations of Law et al. [32], Polat et al. [33], and Jones and Chan [34]. They established that the pain was maximum in the morning after initiating treatment, with uneasiness progressively declining by day 6.

Fixed appliance therapy results in variations in the vascular supply. This occurs because of the sensitization of different proprioception centers by prostaglandins, glutamate, and gamma-aminobutyric acid [35,36]. The

prostaglandin production reaches a maximum level by 24 h and then decreases in the next 7 to 14 days [37]. Similarly, the increased production of neuropeptides resulting in antidromic inducement of afferent nerve terminals leads to pain. The increased levels of inflammatory mediators could be the reason for the pain experienced by patients after archwire insertion in the present study.

Factors such as the amount of orthodontic force applied to dentoalveolar structures and the severity of crowding do not play a key role in the patient's distress. Evidence regarding the impact of patients' age on pain perception after treatment is unpredictable [38]. To avoid the impact of age on outcome assessment, the current study was limited to 18–25-year age clusters.

To date, investigations related to the use of NSAIDs as pre-emptive analgesic for orthodontic pain control have been successful [30]. According to Davidovitch and Shanfield [37], NSAIDs are the gold standard for pain regulation during orthodontic treatment. According to Law et al. [32], usage of ibuprofen prior to orthodontic therapy shows positive discrimination towards orthodontic pain control. Pre-emptive analgesia works by blocking afferent nerve impulses in advance; therefore, there is no central sensitization of the nervous system. Administration of NSAIDs prior to the initiation of treatment facilitates biotransformation of the drug and further helps minimize inflammation and tissue trauma [33].

The use of certain agents that might momentarily move the teeth and adjacent tissues during orthodontic treatment will aid in the resolution of inflammation, thereby decreasing pain. Based on this conviction, Proffit and Fields [38] suggested chewing gum for orthodontic pain management. Patients often request a substitute pharmacological approach for pain management. Chewing gum is the best alternative because it is economical and safe to use. Therefore, in this study, chewing gum was compared with tenoxicam.

The recorded pain scores were higher while biting and chewing than while fitting posterior teeth in both groups. This could be attributed to the fact that the teeth are in

maximum intercuspation position during biting and chewing. This concurs with the findings of Polat [33]. Both tenoxicam and chewing gum were effective in fitting posterior teeth and biting. Chewing gum helps loosen the firmly arranged PDL fibers around the neurovascular bundles, repairing the regular flow of the lymphoid and circulatory system of the PDL, consequently eliminating inflammation and edema, and finally reducing pain and distress. This might have contributed to the effective pain control in the chewing gum group during chewing. Alshammari et al. [39] also reported the effectiveness of chewing gum as equivalent to paracetamol after initial archwire placement. Silva et al. [40] advocated chewing gum as a non-pharmacological alternative to acetaminophen and ibuprofen. The overall pain perception among the chewing gum and tenoxicam clusters was similar and not statistically significant. Hence, chewing gum might be an alternative to preemptive tenoxicam. The findings of our study were similar to those of Ireland AJ [31].

The limitations of the current study are that it was a unicentric study, and the sample size was small. Another limitation is the possibility of bias during the study, because the previous VAS scores noted by the participants may have influenced the subsequent pain scores. To minimize this, each VAS score was recorded on a new page in the booklet. There is a definite need to perform evidence-based research, including multicenter randomized clinical trials with a larger sample size to formulate a standard protocol for pain management by comparing various non-pharmacological options with long-acting NSAIDs during fixed orthodontic treatment.

In conclusion, the intensity of pain during various functions gradually increased after initial archwire placement, reached its extreme by the next morning or within 24 h, and then gradually decreased by the seventh day in both groups. Pain experienced during biting or chewing was significantly greater than that experienced while fitting posterior teeth. Chewing gum was not inferior to pre-emptive tenoxicam. Thus, chewing gum is a non-pharmacological alternative to analgesics for

orthodontic pain control that eliminates the chance of adverse reactions and can be used in the absence of adult observation.

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Sobitha Obili: Data curation, Investigation, Writing - review & editing

Thejasree Keerthipati: Investigation, Validation, Writing - review & editing

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Mandava Prasad: Conceptualization, Supervision, Writing - review & editing

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Case Series

Driftodontics – A case series

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ABSTRACT

In spite of the tremendous improvements in technology that allow for fewer patients to require extractions, there are always patients who have significant enough crowding that ultimately necessitate the removal of premolars.

Alleviating the lower anterior crowding prior to placement of the fixed appliances shortens the time in braces, and allows self correction by natural drift. One such treatment strategy which allows all this is Driftodontics.

This paper is intended to bring forth the clinical advantages of driftodontics by showing some of our cases which achieved efficient results.

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1. Introduction

Space gaining methods in orthodontic treatment of crowded teeth include expansion, distalization, proximal slicing or extraction of teeth. Cases which necessitate extraction of teeth should be critically evaluated, for selection of teeth to be extracted and time of extraction to avoid undesirable tooth movement into extraction space.¹ As physiological drift of the teeth is most often in the mesial direction because of mesial component of force, greater mesial movement of molars is seen with second premolar extraction than first premolar extraction.² However, teeth mesial to the extraction space move in distal direction.

Potential benefits from a period of physiologic dental drift post-extraction, was proposed by Bourdet.³ “Driftodontics” is a term that can be attributed to Dr. R.G. “Wick” Alexander in his textbook.⁴ The benefits include shorter period of fixed appliance therapy because of unprompted alignment of the teeth, oral hygiene problems due to appliance therapy decreases because of less time

period of appliance in the oral cavity and the dentoalveolar support also increases.^{5,6}

2. Case Reports

2.1. Case 1

An 18-years old female patient presented with a chief complaint of forwardly placed upper front teeth. Clinical examination revealed convex profile with competent lips, Angles class I malocclusion with crowding in the lower anterior region, skeletal class I with normodivergent growth pattern, proclined upper and lower anteriors (Figure 1). Based on clinical and cephalometric findings we have planned for all 4's extractions and driftodontics in the lower arch. Immediately after the extractions, banding and bonding of upper arch was done with 0.022 MBT bracket prescription. Initial alignment of upper arch was done with 0.016 inch, 16x22inch and 19x25inch NiTi wires. After 4 months of upper arch strap up, lower arch was bonded and aligned (Figure 2). Space closure was done using 19x25SS wire.

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2.2. Case 2

A 13 year old male patient reported with a chief complaint of irregularly placed teeth. Initial examination revealed convex profile with incompetent lips, Angles class I malocclusion with crowding in the upper and lower anterior region, cross bite in relation to 26, lower midline shifted to right by 3mm. Skeletal class I with normodivergent growth pattern and proclined upper incisors (Figure 3). Due to excessive crowding in the upper and lower arches, we decided to treat the case by extracting upper and lower first premolars. After the extractions, banding and bonding of upper arch was done with 0.022 in MBT bracket prescription. Expanded transpalatal arch was placed between 16, 26. Initial alignment of upper arch was done with NiTi wires sequentially starting from 0.014 inch wires. Lower arch was left for driftodontics for 5 months in this case, later on bonding was done and aligned (Figure 4).



Fig. 1: Pretreatment intraoral and extraoral photographs for case 1



Fig. 2: Series of lower occlusal photographs showing driftodontics over a period of 4 months (A-D) for case 1

2.3. Case 3

A 17-year-old female patient came with chief complaint of irregularly placed teeth. The patient was mesoprosopic and had convex profile with competent lips. Skeletal class I with normodivergent growth pattern and proclined upper incisors. Intraoral examination revealed Angles class I malocclusion with crowding in the upper and lower anterior region, cross bite in relation to 22,25, lower midline shifted to right by 2mm. (Figure 5). Extraction of all 4's done followed by banding and bonding of upper arch with 0.022 in MBT bracket prescription. Anterior bite plate was inserted to correct the crossbite. Initial alignment of upper



Fig. 3: Pretreatment intraoral and extraoral photographs for case 2



Fig. 4: Series of lower occlusal photographs showing driftodontics over a period of 5 months (A-D) for case 2

arch was done with 0.014 inch followed by 0.016 inch, 16x22inch and 19x25inch NiTi wires. In a period of 3 months significant amount of crowding was relieved in the lower arch (Figure 6). After 3 months of driftodontics in the lower arch, strap up was done and aligned.



Fig. 5: Pretreatment intraoral and extraoral photographs for case 3

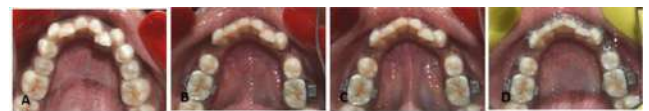


Fig. 6: Series of lower occlusal photographs showing driftodontics over a period of 3 months (A-D) for case 3

3. Discussion

In orthodontics, treatment plan for cases with severe crowding usually include extraction of premolars and immediately banding and bonding of upper and lower arches. In contrast, driftodontics encourage delayed lower arch bonding to allow physiological drift of canines distally into first premolar extraction spaces which will create space for the anterior teeth to relieve crowding.

In his study^{2,4} Papandreas showed that when the maxillary arch is treated and the mandibular arch is allowed to drift for 6 months, predictable movements will take place:

1. Canines will drift distally
2. Incisors will upright
3. Anterior crowding will diminish
4. Molars will drift mesially, but only slightly

Weber⁷ showed that approximately one third of the space closure following first premolar extraction is taken up by mesial molar movement, two thirds is accounted for distal canine movement. Berg and Gebauer⁸ attributed 80% of the space reduction to distal drifting of the canines.

Mandibular molars will be in relatively in more stable position.² In critical anchorage cases lower lingual arch can be placed to prevent lower molar mesial movement which accounts for use of total extraction space for decrowding of anteriors.

Driftodontics will be most successful, if there is minimal skeletal discrepancy and an average overbite. Drifting of crowded canines distally will be more influenced by the lip and tongue muscle action.⁹ So we had chosen cases with skeletal class I, Class I molar relation with crowding in the lower anterior region, matching soft tissue with competent to potentially competent lips.

This physiological drift will be more only in first six months after extractions,² so we have observed changes in the present cases in a period of 4, 5 and 3 months respectively.

Bonding of crowded teeth will be much difficult. Aligning of severely rotated teeth eventually needs change in the bracket position after derotation which will consume much time because the orthodontist again has to go for undersized wires. Complicated mechanics or multilooped arch wires are needed to correct severe crowding which will be difficult for the patient to maintain oral hygiene and which leads to fewer emergency appointments and most importantly happier patients. Hence, extracting and allowing teeth to drift makes bonding easier, will simplify treatment mechanics and reduce the chair side time and overall treatment time.

The other advantage of driftodontics is in case of instanding lower incisors. Placement of continuous archwire in these cases will lead to movement of roots further linguallly. Driftodontics will help in this situation to achieve much better result in lesser time by natural drift into the

extraction spaces.

Generally in driftodontics cases, upper arch aligning and levelling, retraction will be carried simultaneously so that any kind of occlusal interferences for lower canines to move distally will be reduced.

As second and third cases had Class I molar relation with single tooth crossbites, anterior bite plate was used to avoid occlusal interferences effect on the changes in position of lower canines. Most of the lower anterior crowding was relieved by distalization of anteriors into extraction space within 3-5 months.

4. Summary

Alleviating the lower anterior crowding prior to placement of the fixed appliances will have advantages like

1. Shortens the time in braces, which leads to fewer emergency appointments
2. Avoid movement of roots of blocked out incisors further linguallly
3. Avoid complicated mechanics and multilooped archwires
4. Placement of bracket in correct position
5. Better anchorage control
6. Better oral hygiene,

5. Conclusion

As said by Creekmore, "Sometimes doing nothing is doing something".¹⁰ Sometimes letting teeth move in a physiologic way is better than moving them by orthodontic force. Proper case selection and less traumatic extraction of premolars play an important role in the success of driftodontics cases.

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None.

7. Conflict of Interest

The authors declare no conflict of interest.

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Comparative Evaluation of Microleakage Under APC Plus, APC Flash-Free and Conventional Stainless Steel Brackets: An In Vitro Study

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Abstract

Aim: The aim of the study was to compare and evaluate the microleakage under the Adhesive Precoated Plus (APCP), APC Flash-Free (APCF), and conventional stainless steel (SLS) brackets on the occlusal and gingival sides of the bracket base.

Materials and Methods: 66 extracted premolar teeth were collected and divided equally into 3 groups. Bonding was done with APCP, APCF, and conventional SLS brackets using Transbond XT. After bonding, the samples were stored in a distilled water and thermocycled for 3000 cycles, followed by immersion in a 2% methylene blue for 24 h. After cleaning, the teeth were mounted in acrylic and sectioned longitudinally at about the center of the bracket in a buccolingual direction. Microleakage was recorded using a stereomicroscope.

Results: Kruskal–Wallis H test showed a significant difference ($P < .005$) in the microleakage scores between the 3 groups. Mann–Whitney U test showed a significant difference between group 1 to group 2 and group 1 to group 3 ($P < .005$) and an insignificant difference between group 2 and group 3 on the occlusal side ($P = .116$) and on the gingival side ($P = .063$).

Conclusion: Conventional SLS bracket group exhibited greater microleakage scores at both occlusal and gingival sides, when compared with APCP and APCF groups.

Keywords

APC plus, APC Flash-Free, microleakage, stainless steel brackets

Introduction

The introduction of the direct bonding technique in orthodontics by Buonocore¹ in 1955 revolutionized orthodontic therapy. Bracket bonding with light-cured composite has become increasingly popular in orthodontics because of longer material working time and flexibility in initiating the polymerization process, which means “on-demand polymerization.”² In 1992, a new-generation bracket system called adhesive precoated (APC) brackets were introduced. Each bracket is individually packaged, and the bracket bases are prepaste with the optimal amount of adhesives.^{3,4} According to Cooper et al,⁵ APC offers some advantages over the conventional system, such as consistent quality and quantity of adhesive, easy debonding, reduced adhesive wastage, improved asepsis, and better inventory control. The Adhesive Precoated Plus (APCP) system has provided better tolerance to humidity and has a fluoride-releasing effect. The adhesive's color change property helps the

practitioner clean up the flash by providing a visual marker of adhesive placement. Conventionally, the flash needs to be removed before polymerizing the adhesive to avoid gingival irritation and plaque accumulation.⁶

Studies aimed to reduce the excessive flash adhesive led to the development of different systems. The APC Flash-Free adhesive coated (APCF) appliance system developed by 3M Unitek (Monrovia, California, USA) uses individually packaged brackets with a low viscosity resin, applied to a

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nonwoven polypropylene mesh, which eliminates the need for flash removal and creates a seal to reduce microleakage.⁷ Microleakage is an essential drawback of orthodontic adhesives because of photopolymerization shrinkage of the adhesive during curing. The shrinkage creates a gap between adhesive and enamel surface, allowing the infiltration of bacteria, fluids, and ions from the oral cavity. In addition to polymerization shrinkage, thermal stresses to the adhesive in the oral cavity would cause the adhesive joint's fatigue, causing microleakage. Microleakage can lead to white spot lesions under and surrounding the brackets and reduce the bond strength at the enamel-adhesive-bracket interface.^{8,9}

Previous studies^{10,11} have shown increased microleakage at the enamel-adhesive interface rather than at the bracket-adhesive interface. Arhun et al⁹ showed increased microleakage with metal brackets compared to ceramic brackets and on the brackets gingival side compared to the occlusal side. Kim et al⁷ compared microleakage under APCP and APCF ceramic brackets and reported no significant difference between the systems in the extent of microleakage.

However, comparative studies reflecting the extent of microleakage under conventional metal brackets, APCP metal brackets, and APCF metal brackets are scanty. Hence, the present study has been taken up to assess the microleakage under APCF metal orthodontic brackets from gingival and occlusal surfaces at the enamel-composite interface and compare that with conventional metal brackets bonded with conventional composite adhesive and with APCP metal brackets.

Materials and Methodology

66 freshly extracted premolar teeth were collected. The sample size was determined using G*power version 3.1.9.2 (SPSS VERSION 14(SPSS Inc., Chicago, Illinois, USA)) with a confidence interval of 95%, power of 80%, $\alpha = 0.05$, and effect size of 0.40. The sample included in this study was teeth with intact buccal enamel, teeth that were not subject to any pretreatment chemical agents (such as hydrogen peroxide), teeth having no cracks, no caries, and no previous orthodontic bonding. Teeth were stored in 0.1% thymol to inhibit bacterial growth. All samples were cleaned and polished with pumice and rubber prophylactic cups for 10 s and consequently etched with 37% phosphoric acid (D-tech dental technologies, Wagoli, Pune, India) for 15 s and then rinsed with water and air-dried to see the white frosty appearance of etched enamel. Teeth were divided into 3 groups: group 1 bonded with conventional metal brackets with conventional composite, group 2 with APCP metal brackets, and group 3 with APCF metal brackets. Each group consisted of 22 samples.

Group 1: Prepared samples from group 1 (22 premolars) were taken, and a thin layer of Transbond XT primer was applied onto the enamel surfaces, and a gentle burst of air was applied to uniformly spread the primer. The Transbond XT (3M Unitek, Monrovia, California, USA) adhesive composite

was placed on the conventional stainless steel (SLS) bracket (3M Unitek) bonding surface, and then it was placed in the middle of the crown on the labial surface. The gentle force was applied to squeeze out the excess resin, which was removed using an explorer without disturbing the bracket.

Group 2: Prepared samples from group 2 (22 premolars) were taken, and a thin layer of Transbond XT primer was applied to the enamel surfaces, and a gentle burst of air was applied to uniformly spread the primer. APCP adhesive system brackets were placed on the middle of the crown on the labial surface. The gentle force was applied to squeeze out the excess resin, which was removed using an explorer without disturbing the bracket.

Group 3: Prepared samples from group 3 (22 premolars) were taken, and a thin layer of Transbond XT primer was applied to the enamel surfaces, and a gentle burst of air was applied to uniformly spread the primer. APCF adhesive system brackets were placed in the middle of the crown on the labial surface. All the adhesives were cured with light emitting diode (LED) with a wavelength between 450 nm and 470 nm and at an intensity of 1100 mW/cm² for a total of 20 s (10 s each on the mesial and distal sides).

To ensure complete polymerization of the bonding materials, all the teeth were stored in distilled water bath at 37°C for 24 h. All teeth were thermocycled for 3000 cycles between 5°C and 50°C. During the thermocycling procedure, all the teeth were exposed to 8°C for 30 s, followed by normal room temperature for 30 s and later to 45°C for 30 s. After the thermocycling procedure, roots of all teeth were sectioned 5 mm from the cervical margins, and exposed root canals were blocked with sticky wax. All teeth surface except 1 mm around bracket bases were coated with 3 different color varnishes to differentiate various groups (group 1 with red, group 2 with green, and group 3 with brown).

Microleakage Evaluation

All the teeth were immersed in 2% methylene blue for 24 h. The teeth were then thoroughly cleaned and brushed around the brackets to remove any excess dye and stains. All the teeth were embedded in cold cure acrylic blocks taking care to keep the brackets' center in line with the long axis of the

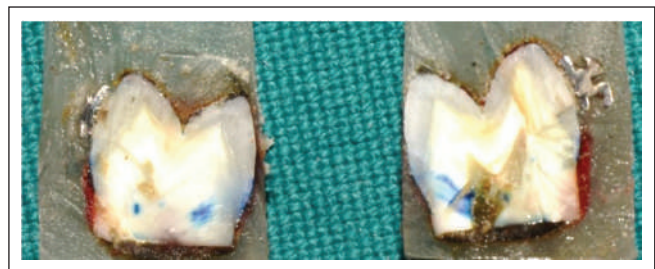


Figure 1. Teeth Sectioned in a Buccolingual Direction Through the Center of the Bracket.

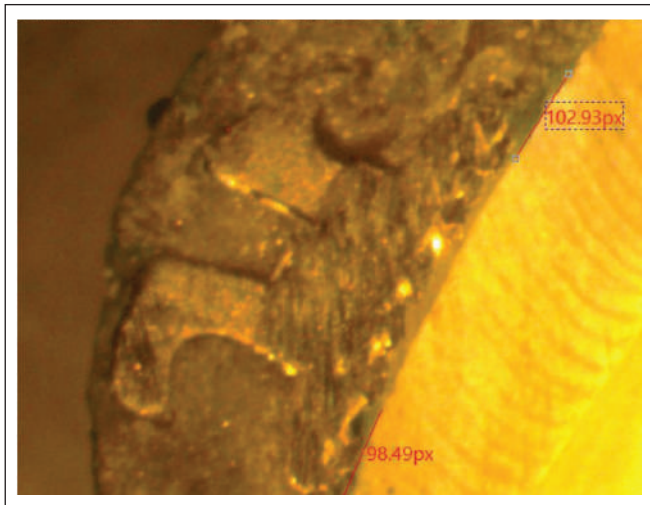


Figure 2. Sample Showing Microleakage at the Occlusal and Gingival Border of the Bracket Base.

acrylic blocks. Teeth were sectioned longitudinally through the center of the bracket, using diamond cutting equipment, and cut surfaces were thoroughly polished (Figure 1). Microleakage was recorded using a stereomicroscope with MICAPS-microview software on the occlusal and gingival regions of the brackets at the tooth adhesive interface (Figure 2). Half of the samples were randomly selected and examined again by the same observer after 2 weeks to measure the intraobserver error of measurements.

Statistical Analysis

Statistical analysis was performed using Kruskal–Wallis H tests to test the difference between the groups and Mann–Whitney U test to compare the differences between 2 independent groups. The level of statistical significance was set at $P < .005$. Intraobserver error was evaluated with the Kappa statistics.

Results

The mean intraobserver agreement was acceptable ($\kappa = 0.64$). Mean microleakage scores measured at the occlusal side of the

bracket in the conventional SLS group are 0.106 ± 0.005 mm and on the gingival side are 0.349 ± 0.015 mm. In the APCP group, the measured mean scores on the occlusal side is 0.057 ± 0.004 mm, and on the gingival side, it is 0.104 ± 0.006 mm. In the APCF group, the measured mean scores on the occlusal side are 0.052 ± 0.004 mm and 0.088 ± 0.007 mm on the gingival side (Table 1). The conventional SLS bracket group exhibited more microleakage than the APCP group and APCF group, both on the gingival and occlusal side of the brackets.

Higher microleakage scores were observed on the gingival side of the bracket than on the occlusal side in all the 3 groups and the difference was statistically significant ($P < .005$; Table 2; Figure 3).

Kruskal–Wallis H test showed a significant difference ($P < .005$) in the microleakage scores on the occlusal side and

Table 2. Inferential Statistics Showing Comparison of Microleakage Between Occlusal and Gingival Borders of the Bracket Base in 3 Different Experimental Groups

Type of Bracket	Median Difference Between Occlusal and Gingival Side	Mann–Whitney U Test P-Value
Conventional SLS	0.242	.0001*
APC Plus	0.047	.0001*
APC Flash-Free	0.037	.0001*

Abbreviations. SLS, stainless steel; APC, adhesive precoated.

Note. Mann–Whitney U test. Results are statistically significant if * $P < .005$.

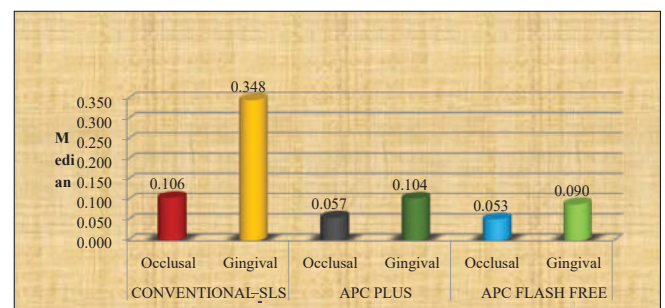


Figure 3. Depiction of Microleakage (mm) on Occlusal and Gingival Side of the Bracket in 3 Experimental Groups.

Table 1. Descriptive Statistics Showing Median, IQR, Mean, and SD Values of Microleakage (mm) at the Enamel–Adhesive Interface Measured from the Occlusal and Gingival Borders of the Bracket Base

Statistics	Conventional SLS		APC Plus		APC Flash-Free	
	Occlusal	Gingival	Occlusal	Gingival	Occlusal	Gingival
Median	0.106	0.348	0.057	0.104	0.053	0.09
IQR	0.008	0.021	0.009	0.012	0.009	0.012
Mean	0.106	0.349	0.057	0.104	0.052	0.088
SD	0.005	0.015	0.004	0.006	0.004	0.007

Abbreviations. SLS, stainless steel; APC, adhesive precoated; SD, standard deviation; IQR, interquartile range.

Table 3. Comparison of Microleakage (mm) Among Conventional SLS, APC Plus, and APC Flash-Free in Occlusal and Gingival Borders of the Bracket Base Using Kruskal–Wallis H Test.

	Conventional	APC Plus	APC Flash-Free
	Kruskal–Wallis H test		
	P-Value		
Occlusal	.0001*		
Gingival	.0001*		

Abbreviation. APC, adhesive precoated.

Note. Kruskal–Wallis H test. Results are statistically significant if * $P < .005$.

Table 4. Intergroup Comparison of Microleakage (mm) Among Conventional SLS, APC Plus, and APC Flash-Free in Occlusal Side and Gingival Borders of the Bracket Base Using Mann–Whitney U Test.

Comparing Groups		Occlusal	Gingival
		Mann–Whitney U Test	
		P-Value	
Conventional SLS	APC Plus	.0001*	.0001*
Conventional SLS	APC Flash-Free	.0001*	.0001*
APC Plus	APC Flash-Free	.116	.063

Abbreviations. SLS, stainless steel; APC, adhesive precoated.

Note. Mann–Whitney U test. Results are statistically significant if * $P < .005$.

gingival side between the 3 groups (Table 3). While the Mann–Whitney U test comparison between independent groups showed a significant difference between conventional SLS–APCP and conventional SLS–APCF groups ($P < .005$) and an insignificant difference between APCP and APCF groups on the occlusal side ($P = .116$) and on the gingival side ($P = .063$; Table 4).

Discussion

Microleakage from an orthodontic point of view is the seeping and leaking of fluids and bacteria between the enamel–adhesive or adhesive–bracket interface. Polymerization shrinkage of the adhesive and differences in the rates of thermal expansion and contraction between the adhesive, bracket, and the enamel during exposure to hot and cold foods can cause gaps between the adhesive and enamel or between the adhesive and the bracket thus contributing to microleakage.

Microleakage presents the likelihood of formation of white spot lesions on the enamel at the adhesive–enamel interface and a reduction in the shear bond strength of the adhesive leading to bracket failure. It is reported that an average of 2 of the 3 teeth bonded with an orthodontic adhesive were affected

by some form of enamel opacity after orthodontic treatment; the most common type identified as diffuse opacity. O'Reilly and Featherstone¹² and Øgaard¹³ have shown that visible white lesions can develop within 4 weeks. Although microleakage-oriented caries is a well-documented entity in the restorative dentistry literature, the potential of caries adjacent to and beneath orthodontic brackets as a result of microleakage remains an underestimated threat to the permanent tooth, especially concerning long-term fixed appliance therapy. Several techniques have been introduced to assess microleakage around dental restorations. Microleakage is determined by a variety of in vitro techniques such as air pressure, fluid filtration, dye penetration, and microcomputed tomography^{14–16} and the most commonly used evaluation method is that of dye penetration.^{14,17,18} Researchers use colored dye agents or chemical tracers, which can penetrate and stain. The specimens are then sectioned longitudinally through the research area and assessed with stereo optical microscopy or scanning electron microscopy. To evaluate the relevance of a leakage test, the effective size of oral bacteria must be considered. Because of the range of bacteria sizes, dyes such as methylene blue and fuchsin are realistic agents to identify the presence of a clinically relevant gap.^{19,20} 2% methylene blue dye penetration was chosen for this study for the assessment of microleakage at the enamel–adhesive interface on the occlusal and gingival sides of the bracket. Thermal cycles were used in this study to mimic thermal changes in the mouth for the production of thermal stress at the tooth–adhesive interface.

The procedure of bonding orthodontic brackets at the enamel surface with composite resin consists a series of preparatory steps. New orthodontic materials are getting evolved in an effort to reduce the procedure time without reducing the bond strength of the bracket. APC brackets, a new generation bracket system introduced in 1992, had bracket bases prepaste with the optimal amount of adhesives. The APCP system provided better tolerance to humidity, has a fluoride-releasing adhesive, and the color change property of the adhesive helps to practitioner clean up the flash by providing a visual marker of adhesive placement. Excess flash is removed before curing an orthodontic bracket to avoid gingival irritation and plaque accumulation. Efforts aimed at reducing the excessive flash led to the development of APCF bracket systems. When APCF brackets are pressed on the enamel surface, the adhesive resin spreads out and conforms to the surface and creates a channeling border at the edges of the brackets, thus creating a seal to reduce microleakage.

This study evaluated and compared the microleakage scores at the enamel–adhesive interface on the occlusal and gingival side in conventional SLS, APCP, and APCF brackets. The mean of the microleakage scores both at the gingival and occlusal sides of the bracket was significantly lesser in APCP and APCF compared to conventional metal brackets. However, although the scores were higher on the gingival and occlusal sides of the bracket in APCP over APCF, the difference was insignificant.

The mean microleakage scores with metal brackets in the current study were in the ranges reported by Ulker et al²¹ and James et al,²² but were lesser than the mean values reported in the study by Abdelnaby and Al-Wakeel,⁸ In the present study LED light was used to bond the metal brackets, while James et al in their study used halogen light, argon laser, and plasma arc curing light. The results of the present study support the results of Ulker et al²¹ who concluded that the type of light-curing unit has an insignificant influence on the microleakage scores. Microleakage scores were measured at the gingival and occlusal sides of the bracket in the present study, while James et al in their study measured at the bracket margins after debonding the bracket. Abdelnaby and Al-Wakeel⁸ measured microleakage at the underneath the bracket base after debonding.

Significantly lesser microleakage scores with APCP and APCF brackets are consistent with studies that show that the filler content of the adhesive has an inverse relation to the polymerization shrinkage. Decreased filler content causes greater polymerization shrinkage.²³ Transbond XT has approximately 3% lesser filler content than the APCP, and this could have contributed to lesser microleakage with APCP brackets. The APCF system has a nonwoven mat at the bracket base, saturated with resin adhesive. When pressed on the enamel surface, the transparent resin forms a channeling border at the edges of the brackets, thus sealing the bracket on the margins. The superior performance of APCF brackets over APCP brackets is consistent with the findings of Foersch et al,²⁴ which reports dye penetration in 35% of interfaces with APCF brackets relative to 97.5% of interfaces in APCP brackets. The results of the present study are consistent with the findings of Lee and Kanavakis⁴ that proved the APCF system showed increased shear bond strength compared with the APCP system and shear bond strength is an absolute indicator of gaps and microleakage in the adhesive. However, microleakage scores with APCP metal brackets in the present study were significantly lesser than the values reported by James et al²² in those brackets cured by argon laser and halogen light but were comparable to the values obtained with plasma arc curing.

Mean microleakage scores in all the 3 systems of stainless-steel brackets were higher at both occlusal and gingival side of the bracket than the scores with conventional, APCP, and APCF ceramic bracket systems reported in the previous studies.^{7,25} A possible explanation for this finding is the “incomplete polymerization” phenomenon. In restorative dentistry, researchers have documented several factors that affect the depth of photoactivated cures, including duration and intensity of light exposure, filler type and shade of adhesive resin, and the reflective characteristics of the adhesive resin bulk.²⁶⁻²⁸ As light passes through the bulk of the restorative resin material, its intensity decreases greatly, thus decreasing the potential for cure. This decrease results in a gradation of the cure such that it decreases from the top surface inwards.²⁹ In orthodontics, brackets may act as the

bulk of restorative material. Because metal brackets do not conduct light, the underlying adhesive resin may remain incompletely polymerized. Several studies have shown that ceramic brackets produce significantly stronger bond strength compared with conventional metal brackets.³⁰⁻³² Increased bond strength with ceramic brackets resulted in bond failure at the enamel surface rather than at the bracket–adhesive interface, resulting in more enamel fractures after debonding.^{23,32-34} This increased strength and difficulty in debonding for ceramic brackets may be attributed to the close adhesion of the ceramic bracket to the adhesive in the absence of microleakage. Similarly, the weaker bond strength of metal brackets may be attributed to relatively more microleakage between the bracket and the adhesive.

In the present study, microleakage was significantly greater on the gingival side than on the occlusal side. In the present study, the curing device was used from both sides for equal time duration. However, the difference in the microleakage scores could be explained by the fact that a greater surface curvature anatomy at the gingival side may result in a thicker adhesive at the gingival margin. The results of the present study are consistent with the previous studies^{9,13,35} which report increased microleakage at the gingival margin than on the occlusal margin.

In restorative dentistry, the shrinkage of the resin caused by the rapid curing with high-intensity lights has been considered a disadvantage because of the large amount of resin placed in the cavity. Fast curing may generate excess shrinkage by permitting little opportunity for the flow of cured resin; also, it may result in gap formation along the resin–tooth interface, which most likely increases the potential for microleakage. However, from an orthodontic perspective, this condition is different. Adhesives at the edges of the bracket can absorb some shrinkage,⁹ and this shrinkage can pull the bracket closer to the enamel by the bracket's free floating. In contrast to the thick composite resin put in the prepared cavity in restorative dentistry, polymerization shrinkage and the subsequent microleakage is less of a concern in orthodontic adhesives because only a thin layer is used.

In the present study, APCP and APCF groups were cured with the LED light-curing unit, etched with 37% phosphoric acid, and microleakage was observed at enamel–adhesive interface only. However, the compatibility of APCP, APCF bracket systems with various curing methods such as plasma arc, argon laser, and halogen light curing and with various methods of enamel surface preparation such as self-etching primers, Er:YAG laser, and Er, Cr:YAG laser could significantly influence the level of microleakage between enamel–adhesive–bracket interface.

Future studies are recommended to test in vivo properties of the APC and APCF systems as well as the compatibility of the above systems to various curing and enamel preparation methods at both enamel–adhesive and adhesive–bracket surfaces.

Conclusion

1. Microleakage was observed in all the investigated groups.
2. The conventional SLS bracket group exhibited higher microleakage scores compared with APCP and APCF bracket systems at both occlusal and gingival sides.
3. Gingival sides in all groups exhibited higher microleakage scores compared with those observed in occlusal sides for the enamel–adhesive interface.
4. APCP and APCF bracket systems do not have a significant difference in the microleakage levels at the enamel–adhesive interface.

Declaration of Conflicting Interests

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Statement of Informed Consent and Ethical Approval

Necessary ethical clearances and informed consent was received and obtained respectively before initiating the study from all participants.

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Evaluation of Dimensionality and Reliability of the Autonomy over Smoking Scale among South Indian Smokers

Abstract

Introduction: In spite of the efforts being directed at reducing the tobacco use among public, it remains a significant concern facing India today. In the quest of providing tobacco cessation counseling, documentation of the tobacco dependence of the individuals is quintessential. This study aims to assess the psychometric properties of the autonomy over smoking scale (AUTOS) among patients seeking oral health care at a teaching dental institution in southern India. **Materials and Methods:** The study sample constituted 199 subjects who satisfied the eligibility criteria of self-reported current smoking and were willing to participate in the study. Participants' age, gender, and years of smoking were documented along with their nicotine dependence by administration of Fagerstrom test for nicotine dependence (FTND) and AUTOS by a trained interviewer. The dimensionality of AUTOS was verified by confirmatory factor analysis using the Classical and Bayesian Instrument Development software program. Correlation analysis between FTND and AUTOS subscale scores was performed along with multiple linear regression analyses to identify the predictors for AUTOS subscale scores. **Results:** The symptom type-wise subscales of AUTOS and the overall scale demonstrated good internal consistency reliability (Cronbach alpha ≥ 0.758). Significant positive correlation was observed between age, years of smoking, FTND score, and all the AUTOS subscale scores. Linear regression analyses showed that the number of years for which the subject had been smoking was a significant predictor of all the three AUTOS subscale scores. **Conclusion:** AUTOS was observed to be a very useful tool with good internal consistency reliability that measures tobacco dependence in consistency with FTND among South Indian population, and while doing so, it captures the various forms of tobacco dependence in an independent manner.

Keywords: Confirmatory factor analysis, tobacco dependence, withdrawal symptoms

Introduction

The ever-increasing tobacco use is one of the significant problems world is facing today. The habit of tobacco consumption is responsible for over 8 million deaths across the globe.^[1] The years of life lost due to tobacco use have been on a consistent rise worldwide, with developing countries contributing around 70% to these premature deaths.^[2] In India, it is reported by a study from the World Health Organization that 1% of the nation's GDP is lost to the disease burden posed by tobacco consumption and the associated mortality.^[3] It has been estimated that the revenue obtained from tobacco accounts for only 12% of the costs associated with tobacco use, implying that the nation's

economy loses Rs 8.16 for every rupee of tax obtained from tobacco products.^[3]

It is noteworthy that in spite of the huge efforts being directed toward reducing the use of tobacco products in India in the form of awareness campaigns and increased taxation on tobacco products, etc., the achievement of desired outcomes remains a distant dream with nearly 29% of the adult Indian population using tobacco in some form according to the Global Adult Tobacco Survey, India (2016–2017).^[4] The Government of India in association with the Dental Council of India has taken an initiative and made it mandatory to establish tobacco cessation clinics in teaching dental institutions across the country.^[5] Similar efforts have been

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underway with regard to full integration of tobacco cessation counseling in undergraduate medical curriculum.^[6] Though the attitudes of students and faculty at teaching health-care institutions with regard to provision of tobacco cessation counseling at these settings are equivocal, international experiences suggest positive influence of tobacco cessation counseling on bringing down the use of tobacco among subjects seeking care at these facilities.^[7-10] Nevertheless, it has been a common observation that health-care providers belonging to diverse disciplines who take part in tobacco cessation counseling must be equipped with the knowledge on multitude of reasons for nicotine dependence and in various pharmacologic means of tobacco cessation counseling besides the motivational persuasion of potential quitters.^[11]

It is to be discerned that nicotine dependence is a complex disorder. People with nicotine addiction consume tobacco products at differing doses and frequencies depending on the demands of the body.^[12] Nicotine traverses through the blood-brain barrier and quickly gets distributed into the cerebral tissue. Tobacco users experience a sense of arousal, relief from stress, and improvement in concentration after the intake of the tobacco products.^[13] However, cessation of tobacco use among these subjects result in manifestation of withdrawal symptoms such as irritability, anxiety, depression, etc., which need attention from health-care professionals. Understanding the nicotine dependence of an individual is therefore quintessential in the course of provision of tobacco cessation counseling as it also determines the likelihood of quitting the habit of tobacco consumption. Autonomy over smoking scale (AUTOS) is a 12-item psychometric tool developed and previously validated among other populations outside India.^[14-16] With this background, the objective of this study was to assess the psychometric properties of AUTOS among subjects seeking care at a teaching dental institution in the South Indian state of Andhra Pradesh.

Materials and Methods

This study was conducted among patients seeking oral health care at a teaching dental institution in the South Indian state of Andhra Pradesh. The study was conducted between January and December 2017. AUTOS is a 12-item psychometric tool, to assess the autonomy over smoking, originally developed and validated at the University of Massachusetts Medical School.^[14] The scale consisted of items relating to three symptom types as follows: withdrawal symptoms, psychological dependence, and cue-induced craving. All the items were administered on a 4-point Likert scale ranging from “not at all” to “very well.” Thus, the symptom type-wise scores in AUTOS range from 4 to 16, whereas the overall scale score ranges from 12 to 48. The scale demonstrated excellent internal consistency reliability in previous studies (Cronbach alpha ≤ 0.91) and showed concurrent validity with the Fagerstrom test for cigarette dependence.^[15] The 12-item

scale was translated to the local language Telugu by a bilingual expert, which was then back-translated to English by another person proficient in both the languages. Minor modifications in the local language version were made to achieve semantic equivalence. A trained interviewer administered the scale to the participants in a quiet room. Details pertaining to participants’ age, gender, and years of smoking were collected. Fagerstrom test for nicotine dependence (FTND) was also administered along with AUTOS. The average time taken for the conduct of each interview was 8 minutes. All the participants signed a written informed consent before the administration of the scales. Ethical approval for this study [SJDC/CEC/2015-2016] was obtained from the Institutional Ethical Committee of St Joseph Dental College, Eluru, on December 22, 2015. A total of 199 subjects satisfying the eligibility criteria of self-reported current smoking who were willing to participate in the study constituted the final sample. This study aimed to verify the factor structure of AUTOS by performing confirmatory factor analysis (CFA). CFA was performed using Classical and Bayesian Instrument Development software program.^[17] The sample size of 199 considered in this study was in accordance with the sample size requirements for structural equation models suggested by Wolf *et al.*^[18] IBM SPSS version 20 software was used to check the internal consistency reliability of the AUTOS subscales. Further, correlation analyses between AUTOS subscale scores, age, years of smoking, and FTND scores were evaluated using Spearman correlation coefficient. Linear regression was performed to check the amount of variance in each of the symptom-wise subscale scores and the overall scale score that could be explained by age of the participant and the number of years for which the participant has been smoking.

Results

The mean age of the study participants was 43.92 ± 16.48 years and the mean duration for which the participants have been smokers was 19.16 ± 14.77 years. All the study subjects were males. The symptom type-wise subscales of AUTOS demonstrated good internal consistency reliability (Cronbach alpha ≥ 0.758). Table 1 presents the corrected item total correlations, Cronbach alpha values for the AUTOS subscales along with Cronbach alpha values with deletion of individual items. Both the unidimensional factor structure and the symptom type-wise tridimensional factor structure of AUTOS were verified in CFA. Table 2 presents the factor loadings of items in both the unidimensional and tridimensional models obtained using CFA. Table 3 summarizes the model fit indices of both the CFA models evaluated. It was observed that the symptom type-wise tridimensional model showed good model fit indices compared to the unidimensional model. Significant positive correlation was observed between age, years of smoking, FTND score, and all the symptom type-wise

Table 1: Internal consistency reliability statistics and the corrected item total correlations of the symptom type wise subscales of autonomy over smoking scale

Measure	Item	Corrected item total correlation	Cronbach alpha if item deleted	Cronbach alpha
Withdrawal symptoms subscale	When I go too long without a cigarette, I get impatient. (Item 1)	0.661	0.814	0.846
	When I go too long without a cigarette, I get strong urges that are hard to get rid of. (Item 2)	0.764	0.768	
	When I go too long without a cigarette, I lose my temper more easily. (Item 3)	0.588	0.845	
	When I go too long without a cigarette, I get nervous or anxious. (Item 4)	0.724	0.787	
Psychologic dependence subscale	I rely on smoking to focus my attention. (Item 5)	0.667	0.732	0.805
	I rely on smoking to keep from feeling bored. (Item 6)	0.618	0.756	
	I rely on smoking to deal with stress. (Item 7)	0.618	0.758	
	I would go crazy if I couldn't smoke. (Item 8)	0.581	0.774	
Cue-induced craving subscale	When I feel stressed, I want a cigarette. (Item 9)	0.466	0.746	0.758
	When I see other people smoking, I want a cigarette. (Item 10)	0.605	0.672	
	When I smell cigarette smoke, I want a cigarette. (Item 11)	0.538	0.711	
	After eating, I want a cigarette. (Item 12)	0.621	0.663	

Table 2: Standardized estimates from the unidimensional and tridimensional models of AUTOS using confirmatory factor analysis

Model	Factor	Item	Standardized estimate	Standard error	Z-value
Unidimensional	AUTOS	Item 1	0.71	0.04	17.66
		Item 2	0.82	0.02	29.74
		Item 3	0.61	0.04	14.24
		Item 4	0.82	0.03	27.49
		Item 5	0.58	0.05	11.76
		Item 6	0.8	0.02	28.11
		Item 7	0.54	0.05	10.17
		Item 8	0.75	0.03	20.54
		Item 9	0.76	0.03	22.08
		Item 10	0.68	0.03	17.72
		Item 11	0.67	0.04	15.68
		Item 12	0.7	0.04	16.35
Tridimensional	Withdrawal symptoms	Item 1	0.794	0.039	20.31
		Item 2	0.883	0.027	33.12
		Item 3	0.693	0.045	15.5
		Item 4	0.882	0.03	29.69
	Psychologic dependence	Item 5	0.606	0.05	11.9
		Item 6	0.852	0.031	27.18
		Item 7	0.567	0.054	10.41
		Item 8	0.79	0.04	19.92
	Cue-induced craving	Item 9	0.852	0.032	26.23
		Item 10	0.755	0.037	20.38
		Item 11	0.738	0.041	18.2
		Item 12	0.789	0.043	18.49

AUTOS, autonomy over smoking scale.

subscale scores of AUTOS [Table 4]. Linear regression analyses showed that the number of years for which the subject had been smoking was a significant predictor of all the three AUTOS subscale scores [Table 5].

Discussion

This study tested the dimensionality of AUTOS among subjects seeking oral health care at a teaching dental institution in southern India by the conduct of confirmatory factor analysis. The study results support the symptom type-wise factor structure of AUTOS unlike the unidimensional factor structure observed in previous studies.^[14,19] It is important at this juncture to underscore the fact that AUTOS was originally developed with a notion that the manifestation of tobacco dependence can be manifold and three domains of withdrawal, psychologic dependence, and cue-induced craving were identified as different forms of tobacco dependence.^[14] Such explicit distinction of items in

the scale to be belonging to different domains holds an assumption that the factors responsible for each of these forms of tobacco dependence, the underlying mechanism of development of dependence, and the influence of each of these forms of dependence on the ability of the subject to quit tobacco are different.^[19] Thus AUTOS possess an intrinsic tridimensional factor structure which was also established among the validation studies performed among adolescent smokers. Nevertheless, confirmatory factor analysis demonstrated a unidimensional factor structure of AUTOS among adult smokers of the United States America, the reason for which was identified to be the diminishing boundaries between the AUTOS subscales with increasing age.^[19] However, in the present study performed among a

Table 3: Comparison of model fit indices of both the unidimensional and tridimensional models tested in CFA

Model fit index	Unidimensional model	Tridimensional model
Model fit test statistic (df, P-value)	261.87 (54, 0.0001)	98.45 (51, 0.0001)
RMSEA (90% CI)	0.14 (0.12–0.15)	0.069 (0.048–0.089)
Tucker–Lewis index	0.953	0.989
Comparative fit index	0.961	0.991
SRMR	0.1	0.062
WRMR	1.602	0.982

df, degrees of freedom; RMSEA, root mean square error for approximation; CI, confidence interval; SRMR, standardized root mean square residual; WRMR, weighted root mean square residual.

Table 5: Multiple linear regression models with autonomy over smoking scale score and its subscale scores as outcome variables

Outcome variable	Model	Unstandardized coefficient (β)	P-value	R ² value
Withdrawal symptoms score	Constant	26.51	<0.001*	0.172
	Age (years)	-0.102	0.005*	
	Number of years of smoking	0.315	<0.001*	
Psychologic dependence score	Constant	10.04	<0.001*	0.156
	Age (years)	-0.07	0.384	
	Number of years of smoking	0.14	<0.001*	
Cue-induced craving score	Constant	8.53	<0.001*	0.205
	Age (years)	-0.021	0.654	
	Number of years of smoking	0.116	<0.001*	

R², coefficient of determination. *Statistically significant.

Table 4: Correlation between age, years of smoking, autonomy over smoking scale, and its symptom-wise subscale scores

	Age	Years of smoking	Withdrawal symptoms score	Psychologic dependence score	Cue-induced craving score	FTND score
Age		0.867*	0.217*	0.365*	0.189*	0.325*
Years of smoking	0.867*		0.379*	0.47*	0.245*	0.421*
Withdrawal symptoms score	0.217*	0.379*		0.611*	0.469*	0.809*
Psychologic dependence score	0.365*	0.47*	0.611*		0.649*	0.789*
Cue-induced craving score	0.189*	0.245*	0.469*	0.649*		0.688*
FTND score	0.325*	0.421*	0.809*	0.789*	0.688*	

Spearman correlation coefficient test; P ≤ 0.05 considered statistically significant. FTND, Fagerstrom test for nicotine dependence.

* Statistically significant.

sample of South Indian adult population, the mean age of whom was 43.92 ± 16.48 years, the tridimensional factor structure of AUTOS showed better model fit indices compared to the unidimensional factor structure. Each of the three subscales of AUTOS demonstrated good internal consistency reliability (Cronbach alpha ≥ 0.758). Similar observations were reported by Wellman *et al.*^[16] The reasons cited for diminishing boundaries between AUTOS subscales with increasing age were the evolution of physical dependence early in the process of habituating tobacco consumption and the increasing association between physical dependence and psychologic dependence, cue-induced cravings with increasing durations of smoking.^[20,21] Though higher AUTOS subscale scores were observed in this study with increasing age, the explanations surrounding the fading distinction between subscales with age did not hold well among the population considered in the present study. Prospective studies that follow subjects from adolescence to late into their adulthoods may be necessary to acquire concrete insights into the differential inter item relations of AUTOS for participants from different age groups. It was observed in this study that the subscales of psychologic dependence and cue-induced cravings were the most correlated of the three subscales. All the three subscales of AUTOS showed good concurrent validity with the FTND score. Of the three subscales, withdrawal symptoms demonstrated strongest positive correlation with FTND score which could be understood in light of the nature of FTND items that attempt to document the compelling feelings of smokers to smoke. These findings were consistent with the results reported by DiFranza *et al.*^[22] The AUTOS subscale scores showed weak to moderate positive correlations with the years for which the subjects have been smoking. Contrary to these findings, strong positive correlation between AUTOS scores and duration, frequency of smoking were found in the studies conducted among the US and German populations.^[14,15] One of the limitations of this study is its reliance on a convenience sample of subjects seeking care at an oral health-care facility which limits the ability of the investigators to define the population from which the sample was drawn. Another potential limitation in this study is the social desirability bias inherent in all self-reported data, particularly with regard to a deleterious habit which is known to be harmful both for the individual and the society in a broader context.

Conclusion

Within the limitations of this study, AUTOS was observed to be a very useful tool that measures tobacco dependence in consistence with FTND among South Indian population, and while doing so, it captures the various forms of tobacco dependence in an independent manner. Future directions for research include the conduct of longitudinal studies that document the transformations in the factor structures of

AUTOS with time and the reasons for such dynamic nature of association between items belonging to different symptom domains.

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Conflicts of interest

There are no conflicts of interest.

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Peritraumatic distress in Southern Indian inhabitants during second wave COVID-19 pandemic: A community-level survey

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ABSTRACT

Introduction: As coronavirus disease 2019 (COVID-19) is a new disease which has ruining and dismantling the harmony of people internationally, its development and spread, creates turmoil, nervousness and dread among worldwide. **Aim:** To evaluate seriousness of peritraumatic problems among southern Indian inhabitants during the COVID-19 pandemic. **Methodology:** A referral sampling technique was continued until a sufficient sample size was reached, while this self-administered survey catches insights about depression, fears, psychological change, evasion cognitive change, and collectively quantifies the stress on a scale of 0–60. Expressive factual examinations were utilized to sum up clear cut information and inferential measurable investigations included Chi-square tests and Pearson's correlations were done. **Results:** The study included only those participants who understood English and had access to the internet. Inconsequential outcomes were found among gender in which both male and female members frequently showed apprehension and nervousness about COVID-19. 44.8% tried not to watch the news on COVID-19 as they were excessively frightened and make frenzy to family members. 90.9% of participants with lower education levels were more stressed over the thought of getting COVID-19 when showing manifestations related with the novel corona virus, which was statistically significant. **Conclusion:** The current assessment shows that almost all the study participants have felt restless, uncomfortable and terrified of watching the news whilst stressed over appearances related with COVID-19 due to their greater access to information.

Keywords: Coronavirus, mental stress, pandemic, peritraumatic distress

Introduction

Since the outbreak of affirmed case arose in Wuhan, China, in December 2019, the novel coronavirus disease 2019 (COVID-19) has spread around practically the entirety of the world at an alarmingly quick rate influencing more than 7 million individuals and causing more than four lakh's deaths.^[1,2]

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The Government of India forced a cross-country lockdown beginning from March 24, 2020, results in complete limitation of movement outside the house except for basic supplies or other crisis reasons, which has unhinged the lives of people across the country.^[3]

As COVID-19 is a new disease and are having the most devastating effects globally, its emergence and spread causes confusion, anxiety, and fear among the general public.^[4,5] Nations have been burdened with COVID-19 for more than one year and presently going through the another possibility of third flood of COVID-19. Starting on February 3, 2021, there have been

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more than 100 million affirmed cases throughout the planet.^[2] A second wave beginning in March 2021 was much devastating than the first, with shortages of vaccines, hospital beds, oxygen cylinders, and other medicines in parts of the country. On April 30, 2021, it became the first country to report over 400,000 new cases in 24 hrs.^[6]

Outbreaks such as the COVID-19 pandemic have a negative psychological impact which increases demand for mental health care needs. Factors like lockdown, social isolation, disruptions in life routines may cause peritraumatic distress.^[7] During the outbreak of these infections, several psychiatric co-morbidities such as depression, panic attack, anxiety, psychomotor excitement, suicide, and stress symptoms were reported.^[8]

The most noteworthy quantities of new deaths were accounted for from India (7875 new deaths; 0.6 new deaths per 100,000; a 69% increment), Indonesia (885 new deaths; 0.3 new deaths per 100,000; a 26% lessening), and Bangladesh (622 new deaths; 0.4 new deaths per 100,000; a 39% increment). Because of the increasing COVID cases in India, during the second wave pandemic, the fundamental motivation behind the current examination is to quantify mental experiencing like pressure, nervousness, and dread among the overall population.^[9] A second wave of coronavirus swept across the country causing widespread destruction and death. While we're still unclear, there are countless reports of a third wave of COVID arriving, which could be just as intense and put a strain on healthcare resources.^[10] Despite the fact that anxiety and depression are the most common mental health problems encountered in primary care. The difficulty in recognizing and diagnosing mental health problems is a multifaceted problem influenced by the knowledge and attitudes of primary care physicians. Diagnosing and treating a mental health problem in primary care begins with the clinician's response to a patient disclosure of emotional problems. So, it is important for primary care physicians to perceive, evaluate, and react to this distressing pandemic situation.^[11]

Health crises such as the COVID-19 pandemic lead to psychological changes, not only in the medical workers but also in the citizens, and such psychological changes are initiated by fear, anxiety, depression, or insecurity.^[12] Thus, this study aimed to assess the severity of peritraumatic distress among the general population during the COVID-19 pandemic.

Methodology

A cross-sectional investigation was conducted among general population of Andhra Pradesh, India in April 2021. With a certainty level of 95%, the assessed test size was 967 utilizing an edge mistake of 5%. A cold-calling sampling method was adopted in the study, and the questionnaire was sent to the contacts of the surveyors. All the participants were further requested to forward the tool in their respective contacts. Data were gathered utilizing Google forms in English language comprising demographic details like age, gender, education,

work, and recently approved peri-traumatic distress scale which was adjusted by the current investigation region, and the link was shared among various platforms. Participants were requested to forward the questionnaire further to their contacts. Obscurity was guaranteed as there is no close-to-home contact, as only the responses were recorded in the study. This self-administered survey catches insights about depression, fears, psychological change, evasion cognitive change, and collectively quantifies the stress on a scale of 0–60. A modified peri-traumatic index scale score under 20 shows '**low or no stress**', 21–40 demonstrates '**gentle to moderate stress**' and more than 41 shows '**extreme stress**'. Study approval was taken from the institutional review board (36/IRB/SIBAR/2021). Before the start of the study, an informed consent was obtained from all the individuals willing to participate and ensured their participation was voluntary and anonymous. The investigators assured the participants that the data shall be kept confidential and shall only be used for research purposes. The information was pooled, arranged, and examined utilizing SPSS version 23 (IBM Corp., Armonk, NY, USA). Descriptive statistical analyses were used to summarize categorical data and Inferential statistical analyses included Chi-square tests and Pearson's correlation were done. Straight relapse examination was finished utilizing stepwise factor determination to decide critical indicators of mental misery while P value ≤ 0.05 was considered as statistically significant.

Results

All the participants were above 18 years of age and of Indian origin, while study included only those participants who understood English and had access to the internet. The majority of respondents had a degree and above qualification, 901 (93.2%), and 912 (94.3%) were employed. There were 967 participants from both genders (636 males, 65.8%, and 331 females, 34.2%). In the present study, 1.3% are health care workers and the remaining 98.7% are general public [Table 1].

About 46.4% of study subjects felt more nervous and anxious while only 9.7% of the participants were normal, irrespective of situation. The majority of the participants felt sympathetic toward COVID-19 patients and their families. Almost all the age groups are worried and felt nervous and scared of watching the news about COVID-19. At the same time majority of the participants worried about symptoms associated with COVID-19. 38.2% of the participants said that they don't believe fake news without confirmation and would not like to share it with others. 44.8% sometimes avoid watching the news on COVID-19 as they were too scared as it could make to family members panic. Similarly, 65.7% of respondents felt helpless, and angry about people around them, while 34.9% were occasionally irritable and have frequent conflicts with family members than in normal times [Table 2].

Participants with lower education level are more scared to watch the news, which was statistically significant ($P \leq 0.01$). 90.9% of

participants with lower education levels were more worried about getting infected when showing symptoms associated with the novel coronavirus, which was statistically significant [Table 3].

The majority of the study subjects in all the participated age groups showed worried about symptoms associated with COVID-19 which showed no significant results. But older age respondents showed more often back pain/chest distress, which was statistically significant ($P \leq 0.042$). [Table 4].

Insignificant results were found among gender in which the majority of both male and female participants often showed nervousness and anxiety about COVID-19. Statistical difference was found between male and female participants about back pain

and chest distress, which was more often in male participants than female subjects [Table 5].

Discussion

The majority of respondents in the present study had an education level of degree and above, while most of them were employed. The reason may be because of online study, most of the educated people might have shown interest to participate. This is similar to the study by Hyejung Yoon *et al.*^[2] and Harshal Sri Ram Sathe *et al.*^[3] Of the total sample, more than half the study subjects were males, which was in contrast to the study done by Vikram Ramasubramanian *et al.*^[13] and similar to the study by Harshal Sri Ram Sathe *et al.*^[3]

In the present study, most of the respondents were from 25–34 years of age group and study subjects of 35 years and above were scared to watch the news regarding novel coronavirus when compared to younger age groups this may be due to the fact that responsibilities in family and work tensions makes them scared to watch COVID-19-related news. But studies done by Naina Wakode *et al.*^[14], Sikandar A. Qalati *et al.*^[15], and Vikram Ramasubramanian *et al.*^[13] were in contrast to the present study, while similar results were reported by Mostafa A. Abolfotouh *et al.*^[16]

The major part of the participants felt sympathetic towards COVID-19 patients and their families, because of unexpected situations which effected families, financial strain and the lack of support will effect psychological conditions, so it is important for the primary care physicians to practice empathy during pandemic situations, which was on agreement with the study done by Dhar Bahadur Shrestha *et al.*^[17] and the results were contradictory to the study done by Vikram Ramasubramanian *et al.*^[13]

When asked about irritable and frequent conflicts with their family, 36.9% said that they never had any conflicts and 34.9% had occasionally. The coronavirus has led to sweeping changes and disrupting in nearly every aspect of daily life, loss of job, financial matters might be a source of stress contributing to

Table 1: Distribution of study participants according to their demographic details and overall stress level

Demographics	Frequency	Percentage
Age		
<25 years	59	6.1
25-34 years	414	42.8
35-44 years	220	22.8
45-54 years	274	28.3
Gender		
Male	636	65.8
Female	331	34.2
Occupation		
Student	16	1.7
Unemployed	39	4.0
Employed	912	94.3
Education		
Intermediate	66	6.8
Degree and PG	901	93.2
Are you a healthcare worker?		
No	954	98.7
Yes	13	1.3
Overall peritraumatic stress		
Mild stress (score 1-20)	128	13.2
Moderate stress (score 21-40)	838	86.7
Severe stress (score 41-60)	1	0.1

Table 2: Percentages of study participants according to their perceptions on COVID-19

Question	Never	Occasionally	Sometimes	Often	Most of the time
Compared to usual, I feel more nervous and anxious?	9.7%	23.7%	15.8%	46.4%	4.3%
I feel sympathetic to COVID-19 patients and their families?	1.6%	7.1%	32.8%	20.7%	37.8%
I feel helpless and angry about people around me, governors and media?	3.8%	25.0%	65.7%	2.9%	2.6%
I will believe the COVID-19 information from all sources without any evaluation?	38.2%	28.6%	14.4%	17.7%	1.1%
I am constantly sharing news about COVID-19?	22.1%	46.2%	19.0%	6.5%	6.1%
I avoid watching COVID-19 news since I am too scared to do so?	13.7%	12.0%	44.8%	15.8%	13.8%
I am more irritable and have frequent conflicts with my family?	36.9%	34.9%	28.2%	0.0%	0.0%
During this COVID-19 period, I often feel dizzy or have back pain and chest distress?	63.4%	10.0%	11.9%	14.7%	0.0%
I have changes in my eating habits?	20.4%	27.8%	48.5%	2.3%	1.0%
During this COVID19 period, I concentrated more on exercises/yoga than before?	14.0%	35.6%	32.3%	12.7%	5.5%
I feel uncomfortable when communicating with others during COVID-19?	8.1%	6.6%	15.8%	14.4%	55.1%
I am worried about getting infected when showing symptoms associated with novel coronavirus?	0.5%	5.8%	6.2%	1.7%	85.8%

Table 3: Perceptions of the participants toward COVID-19 according to their educational level

Questions	Educational level	Never	Occasionally	Sometimes	Often	Most of the time	R	P
Compared to usual, I feel more nervous and anxious?	Intermediate	12.1%	24.2%	13.6%	45.5%	4.5%	0.010	0.956
	Degree	9.5%	23.6%	16.0%	46.5%	4.3%		
I avoid watching COVID-19 news since I am too scared to do so?	Intermediate	16.7%	13.6%	33.3%	18.2%	18.2%	0.108	0.011*
	Degree	13.4%	11.9%	45.6%	15.6%	13.4%		
I am more irritable and have frequent conflicts with my family?	Intermediate	36.4%	30.3%	33.3%	0.0%	0.0%	0.019	0.583
	Degree	37.0%	35.2%	27.9%	0.0%	0.0%		
During this COVID-19 period, I often feel dizzy or have back pain and chest distress?	Intermediate	68.2%	13.6%	7.6%	10.6%	0.0%	0.041	0.378
	Degree	63.0%	9.8%	12.2%	15.0%	0.0%		
I am worried about getting infected when showing symptoms associated with novel coronavirus?	Intermediate	0.0%	0.0%	4.5%	4.5%	90.9%	0.010	0.043*
	Degree	0.6%	6.2%	6.3%	1.4%	85.5%		

Chi-square test, R=Pearson's correlation, *statistically significant

Table 4: Age-wise comparisons of the participants toward COVID-19 distress

Questions	Age	Never	Occasionally	Sometimes	Often	Most of the time	R	P
Compared to usual, I feel more nervous and anxious?	<25 years	5.1%	35.6%	16.9%	42.4%	0.0%	0.041	0.057
	25-34 years	8.7%	24.4%	12.3%	49.0%	5.6%		
	35-44 years	11.8%	22.7%	17.3%	43.2%	5.0%		
	45-54 years	11.8%	20.8%	19.7%	46.0%	2.9%		
I avoid watching COVID-19 news since I am too scared to do so?	<25 years	27.1%	8.5%	42.4%	5.1%	16.9%	0.102	0.003*
	25-34 years	16.9%	11.1%	43.0%	17.6%	11.4%		
	35-44 years	10.5%	13.2%	47.7%	13.6%	15.0%		
	45-54 years	8.4%	13.1%	45.6%	17.2%	15.7%		
I am more irritable and have frequent conflicts with my family?	<25 years	22.0%	42.4%	35.6%	0.0%	0.0%	-0.059	0.098
	25-34 years	37.0%	33.1%	30.0%	0.0%	0.0%		
	35-44 years	40.9%	31.4%	27.7%	0.0%	0.0%		
	45-54 years	36.9%	38.7%	24.5%	0.0%	0.0%		
During this COVID-19 period, I often feel dizzy or have back pain and chest distress?	<25 years	71.2%	15.3%	0.0%	13.6%	0.0%	0.058	0.042*
	25-34 years	64.5%	9.2%	11.1%	15.2%	0.0%		
	35-44 years	65.5%	8.6%	15.0%	10.9%	0.0%		
	45-54 years	58.4%	11.3%	13.1%	17.2%	0.0%		
I am worried about getting infected when showing symptoms associated with novel corona virus?	<25 years	0.0%	1.7%	6.8%	1.7%	89.8%	0.045	0.926
	25-34 years	0.2%	5.6%	5.6%	1.7%	87.0%		
	35-44 years	0.9%	5.5%	6.4%	1.4%	85.9%		
	45-54 years	0.7%	7.3%	6.9%	1.8%	83.2%		

Chi-square test, R=Pearson's correlation, *statistically significant

Table 5: COVID distress of the participants according to their gender

Questions	Gender	Never	Occasionally	Sometimes	Often	Most of the time	R	P
Compared to usual, I feel more nervous and anxious?	Male	8.6%	23.0%	16.8%	47.8%	3.8%	0.049	0.205
	Female	11.8%	25.1%	13.9%	43.8%	5.4%		
I avoid watching COVID-19 news since I am too scared to do so?	Male	13.4%	11.9%	42.9%	15.9%	15.9%	0.058	0.105
	Female	14.2%	12.1%	48.3%	15.7%	9.7%		
I am more irritable and have frequent conflicts with my family?	Male	38.5%	34.4%	27.0%	0.0%	0.0%	0.048	0.314
	Female	33.8%	35.6%	30.5%	0.0%	0.0%		
During this COVID-19 period, I often feel dizzy or have back pain and chest distress?	Male	58.6%	11.3%	13.4%	16.7%	0.0%	0.124	0.001*
	Female	72.5%	7.6%	9.1%	10.9%	0.0%		
I am worried about getting infected when showing symptoms associated with novel coronavirus?	Male	0.3%	5.2%	6.9%	1.9%	85.7%	0.014	0.311
	Female	0.9%	6.9%	4.8%	1.2%	86.1%		

Chi-square test, R=Pearson's correlation, *statistically significant

mental health problems. This was in accordance with the study done by Yan Guo *et al.*^[18] and different from the study done by Vikram Ramasubramanian *et al.*^[13]

Only meager of the study populations are health care workers while majority was general public. Though nearly half of

them often felt nervous and anxious toward COVID-19, and majority of the participants are worried most of the time about getting infected when showing symptoms associated with the novel coronavirus. The difference was seen among educational qualifications, where participants with lower education levels showed more worried about getting infected when showing



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symptoms associated with novel coronavirus than higher education levels, this may be the reason that higher level of education might practice better coping strategies, which was relevant to a study done by Mogesie Necho *et al.*^[19] Naina Wakode *et al.*^[14] and which was in contrast to the study by Dhar Bahadur Shrestha *et al.*^[17] and Jianyin Qiu *et al.*^[1]

In the present study, 35.6% occasionally concentrated on exercises/yoga than before, maybe due to the reason that being physically and mentally fit can fight against stress which is necessary for everyone and majority of the respondents also changed their dietary habits, this might be due to awareness given by government and health care workers to improve immunity against COVID-19, which was in contrast to a study done by Dhar Bahadur Shrestha *et al.*^[17]

More than half of the respondents reported that they could not stop themselves from collecting information about coronavirus and share information with others, as access to information is greater through media, “We’re not just fighting an epidemic; we’re fighting an infodemic”, as said by WHO Director-General Tedros Adhanom Ghebreyesus. While the results were in contrast to a study done by Priscilla Samson *et al.*^[7]

The majority of the participants often showed nervousness and anxiety about COVID-19. But when asked about back pain and chest distress, which was seen more often in male participants than female subjects, which was different from the study done by Mark Shevlin *et al.*^[20] which could be because of the fact that males being a head of the family, they experience more pressure and stress with financial and family responsibilities which might increase in this perplexing COVID situations. Overall, 86.7% of participants had mild to moderate levels of stress which was, in contrast to a study done by Vikram Ramasubramanian *et al.*^[13] and a total of 65.7% of participants felt helpless, angry about people around, government, and media which was similar to the study by Priscilla Samson *et al.*^[7] Plethora of psychosocial issues, such as contamination fears, dissatisfaction, fatigue, lockdown sway, a flood of deception (infodemic), bits of hearsay, different paranoid ideas, lacking supplies, monetary misfortune, and social stigma about the disease have negatively affected psychological well-being all around the world during this pandemic.

Results of the present study depict that the stress was higher among the study participants as the second wave was characterized by high morbidity rates, oxygen supply shortage, availability of meager beds in hospitals, finally with the emergence of mental health problems.

Mental health is an essential component of overall health. In India, counseling about mental health issues is still viewed as a social stigma, which is why people avoid seeking treatment for mental illness. The mental health of people prone to depression

and anxiety disorders should not be overlooked and efforts to varying degrees should be made to identify and maintain morale. In the era of COVID-19, panic disorder is very common among the public and there is a need to regulate schools, offices and homes to provide counseling services to all age groups so that all Indians can benefit healthy mental health and well-being.

Key points

- Participants with lower education level are more scared to watch the news, and were more worried about getting infected when showing symptoms associated with novel corona virus.
- Regardless of age, almost all the participants worried about symptoms associated with COVID-19, while back pain and chest distress was more common in males than females.

Conclusion

The COVID-19 emergency and the going with lockdown have without a doubt influenced each person in for sure and mental sufferings in human existence everywhere. The current investigation shows that practically almost all the study population are stressed and felt apprehensive and frightened of watching the news and stressed over manifestations related with COVID-19. It would likewise be valuable to analyze the content that individuals are getting from social networks and assist them with fostering the important abilities to have the option to channel such information. Likewise, the public authority should share however much data as could reasonably be expected with the general population, such as knowledge about COVID-19, every day flare-up status, and the public authority’s pandemic counteraction technique; this might assist with reducing psychological stress. Multisectoral comprehensive strategies and resilient system are required to tackle the direct and indirect effects of pandemic.

Limitations

- This study has some limitations. First, there are always limitations in the use of self-report questionnaires.
- Questionnaires are not diagnostic tools. The choice of the cut-off point is arbitrary and depends on the subjective of the participants.
- On the one hand, anonymity has allowed people to express themselves more freely; on the other, we have no data on the truthfulness of the information.
- The study cannot be generalized as it is an online survey, only educated and those who has access to the devices and internet has answered the questionnaire.

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Conflicts of interest

There are no conflicts of interest.



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Discernment in Practicing Nonpharmacological Modes of Intervention in Combating SARS COVID-19: Reflections of Dental Patients in India

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Abstract

Background: As a massively polarizing practice, concealing or face covering has emerged in the midst of the coronavirus disease 2019 (COVID-19) pandemic. Given the increasing speculation regarding therapeutic control measures, nonpharmacological approaches offer promising successful mitigation in the battle against severe acute respiratory syndrome (SARS) COVID-19. The evaluation of public capacity, prospects, and actions offers recommendations for public oversight of the implementation of prevention activities. Hence, this investigation was aimed to explore the discernment in practicing nonpharmacological modes of intervention in combating SARS COVID-19 among a group of people, dental patients. **Materials and Methods:** A pictorial questionnaire survey was done using convenience sampling, aimed at participants visiting the triage facility of the institution to capture their discernment in practicing nonpharmacological modes of intervention for 3 months. **Results:** A total of 6015 individuals with a mean age of 35.44 ± 14.20 years participated in the study. The majority of the participants were in the age group of 18–30 years ($n = 2340$, 38.9%) with female ($n = 3105$, 51.6%) and urban ($n = 3745$, 62.3%) predominance. Most of the study participants had purchased cloth masks ($n = 2980$, 49.5%) and had spent about 10–50 INR per mask ($n = 4050$, 80.6%). Patients have reported that the usage of a mask ($n = 1330$, 22.1%) was the most difficult measure to follow and also stated that sanitizing hands ($n = 2505$, 41.6%) was the easiest measure to follow during this pandemic. Crowding in the market ($n = 2255$, 37.5%) and placing masks around the neck ($n = 735$, 12.2%) were infringements served in their surroundings. Irrespective of educational qualification touching the outer area of the mask was the most common noncompliance reported by the participants which were statistically significant ($P = 0.0001$). **Conclusion:** The study participants have divulged that usage of masks and following social distance in public transport were the most difficult preventive measures of SARS COVID-19 to follow. Evaluating the attitudes of public in preventive procedures for COVID-19 can pave the way to make comprehensive guidelines.

Keywords: COVID-19, dental school, noncompliance, nonpharmacological interventions, pandemic, patient

INTRODUCTION

Having affected more than 72,196,732 confirmed cases and 1,630,521 deaths worldwide, the severe acute respiratory syndrome (SARS) coronavirus disease 2019 (COVID-19) pandemic continues to evolve at a rapid pace.^[1] The vulnerability index for the management of and response to the SARS COVID-19 epidemic in India shows that Andhra Pradesh score as 0.714, where 1.0 is the most vulnerable and 0.0 least vulnerable.^[2] The spread of the new COVID has influenced individuals everywhere in the world, state and Local governments are making clearing moves

to stop the spread of the illness and alleviate the general well-being and financial effect of the flare-up.^[3] Analysts and legislative authorities consider this pandemic as challenging with huge legal and procedural concerns including basic security, legitimate perspective, opportunities, and financial impact as communities, states, and countries respond to the scourge.^[4]

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Concealing or face-covering among the SARS COVID-19 pandemic has arisen as a profoundly polarizing practice. With given uncertainty mounting on therapeutic measures in controlling, nonpharmacological methods offer promising effective mitigation in combating SARS COVID-19.^[5] Assessing public capability, opportunity, and behavior provide a guide to monitor the public in adopting preventive practices. One such method of assessing is through questionnaire surveys. Validated and short questionnaire surveys that are aimed at capturing behavior can offer an insight into issues concentrated in adopting preventive behavior.^[6]

Fearing transmission of disease in the dental practice majority of the dentists are yet to resume full-fledged practice.^[7] Among these circumstances and in the wake of public interest, Sibar Institute of Dental Sciences, Guntur, Andhra Pradesh, opened its complete oral health services in August 2020, whereas in earlier months of lockdown it provided emergency services. To minimize the need for personal protective equipment, a Kiosk center with glass supported by indoor and outdoor audio for Triage purposes has been erected under Public Health Dentistry Department in the portico of the college to screen patients through health interviews to rule out SARS COVID-19 before they are sent to the Oral Medicine and Radiology Department for oral examination. Hence, with this background, a study was done to explore practices of nonpharmacological modes of intervention in combating SARS COVID-19 by dental patients visiting institution.

MATERIALS AND METHODS

Owing to the fact that pictures are better at communication than written words, as one picture is worth a thousand words, pictographs were used as a medium to discern preventive measures in practicing nonpharmacological modes of intervention in combating SARS COVID-19. This was a cross-sectional pictorial questionnaire survey done on convenience sampling, which was aimed at participants visiting dental institutions to discernment in practicing nonpharmacological modes of intervention. The participants are reflective of the general population of this area in terms of their nativity and belongingness to all walks of life. This study was done over 3 months (September 2020 to November 2020) on 6015 individuals who were chosen on a random basis among 300 patients on an average visiting per day for oral health needs, interviews were made at the triage section of the institute asking for their perception's and practices regarding the various preventive measures after obtaining consent.

The questionnaire consisted of demographic data and items related to preventive measures followed and noncompliance observed concerning SARS COVID-19. Two panels were utilized for this purpose. The first one with color pictographs attached on SARS COVID-19 preventive measures, and on the other panel, the pictures of noncompliance were placed. The panels consisting of pictographs of preventive measures followed and noncompliance practices observed in the family

and surroundings were finalized from the pilot study done among 54 participants with good reliability (Cronbach's alpha [α] = 0.82).

Ethical approval

A detailed review of the multifaceted aspect of the research and about the consent of the participants was elaborated for approval from the Institutional Ethical Committee (Pr. 255/IEC/SIDS/2020), while the anonymity of the study participants was secured by following the ethical principles of the World Medical Association Declaration of Helsinki.

RESULTS

A total of 6015 individuals with a mean age group of 35.44 ± 14.20 years participated in the study. The majority of the participants belonged to 18–30 years' age group ($n = 2340$, 38.9%) with female ($n = 3105$, 51.6%), urban ($n = 3745$, 62.3%), and marital status ($n = 4025$, 66.9%) predominance. It was found that 49.5% of the individuals in the study were illiterates ($n = 2975$) followed by participants with high school education ($n = 805$, 13.4%) while with reference to occupation majority of them fell under semi-profession category ($n = 1845$, 30.7%) [Table 1].

Most of the study participants used purchased cloth mask ($n = 2980$, 49.5%) followed by custom made/free cloth mask ($n = 2220$, 36.9%) [Figure 1]. Regarding the amount spent on purchasing each mask, the leading percentage of the individuals spent about 10–50 INR (Indian rupees) ($n = 4050$, 80.6%) [Figure 2]. Many individuals reported that maintaining social distance in the public transport ($n = 1530$, 25.4%) and the usage of the mask ($n = 1330$, 22.1%) were the most difficult practices to follow during this pandemic. They also perceived that sanitizing hands ($n = 2505$, 41.6%) and using hand wash ($n = 1835$, 30.5%) were the easiest practices to follow [Table 2].

Many participants observed that crowding in the market ($n = 1300$, 21.6%) and touching the outer area of the mask ($n = 940$, 15.6%) were the derelict of the COVID-19 measures by their family members, while it was warranted that crowding in the market ($n = 2255$, 37.5%) and placing mask around the neck ($n = 735$, 12.2%) were infringements observed in their surroundings [Table 3]. When comparing the noncompliance practices of the participants with their educational level, almost all the participants with various education levels have acquiesced that touching the outer area of the mask as the most disregard practice followed by them, except for the participants with primary school level education, where $P = 0.001$ which was statistically significant [Table 4].

DISCUSSION

This study was done to explore the reflections of the society through dental patients visiting a dental organization in rehearsing nonpharmacological methods of mediation in battling SARS COVID-19. Surveying participants who are

going to dental offices on identifying with receiving preventive conduct in battling COVID-19 can help in better treatment conventions and furthermore provide some insight into the

Table 1: Demographics details of the study participants

Variables	n (%)
Age (years)	
18-30	2340 (38.9)
31-40	1560 (25.9)
41-50	955 (15.9)
51-60	545 (9.1)
61-70	470 (7.8)
71-80	145 (2.4)
Gender	
Male	2910 (48.4)
Female	3105 (51.6)
Education	
Illiterate	2975 (49.5)
Primary school	235 (3.9)
Middle school	545 (9.1)
High school	805 (13.4)
Intermediate or post high school diploma	755 (12.6)
Graduate or postgraduate	405 (6.7)
Professional degree	295 (4.9)
Occupation	
Unemployed	1190 (19.8)
Unskilled worker	315 (5.2)
Semi-skilled worker	700 (11.6)
Skilled worker	1035 (17.2)
Clerical, shop-owner/farm	795 (13.2)
Semi-professional	1845 (30.7)
Professional	135 (2.2)
Place	
Urban	3745 (62.3)
Rural	2270 (37.7)
Marital status	
Married	4025 (66.9)
Unmarried	1905 (31.7)
Divorced	50 (0.8)
Widowed	35 (0.6)

unsafe conduct of the subject, encouraging dental specialists in better managing through appropriate guidelines and alterations.^[8] The desire for the prevention by an individual relies on the psychological need maintain which is influenced by means of autonomic and controlled motivation which accordingly depends on their attitude, subjective guidelines, and perceived behavioral control. Segregation toward the estrangement of and the making of people who wear a face mask in public areas or social gatherings that empower the obliviousness of social separation measures may sabotage a person’s relatedness and emotional standards with regard to COVID-19 avoidance.^[9] The absence of network selection to preventive measures for shortening coronavirus is because of fluctuated reasons which incorporate regulatory omissions, being critical, dread and nervousness, counterfeit news, accepted practices, culture, and governmental issues.^[10,11]

One of the reasons for failure in preparedness for epidemics is the lack of a comprehensive National Public Health Act in India. The number of enactments gives gigantic capacity to general well-being functionaries that should be figured out. General health authorities, such as examiners and well-being officials working with different assignments not only have a few essential requirement paraphernalia but are also influenced by defilement, similarity, giving grants, licenses, and enlistments; giving regulatory requests; forcing common punishments, and directives. Conclusively, there are one or different purposes behind not executing these well-being enactments in a genuine sense and general well-being stays defenseless against social, mental, and actual danger factors.^[12-14] Three principal factors hold up traffic of counteraction; individuals do not appreciate the dangers they run, it conflicts with human instinct for individuals to quiet themselves down in inflexible disengagement as a method for securing others, what’s more, individuals regularly unwittingly go about as a proceeding with risk to themselves as well as other people.^[15]

We are at a point in the development of living through a pandemic where the individuals who have returned in the operatory are getting more acclimated with giving patient consideration inside the bounds of what is called “the new

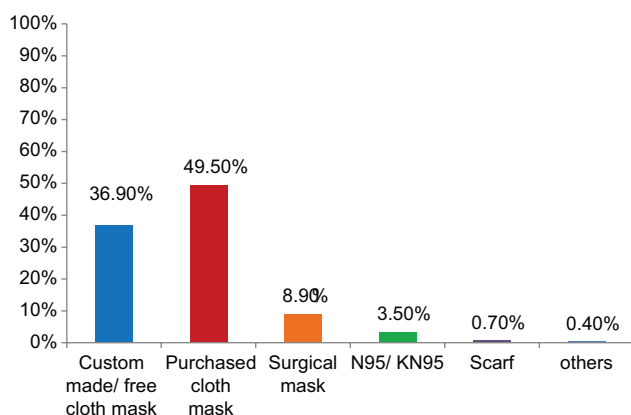


Figure 1: Types of masks used by the study participants

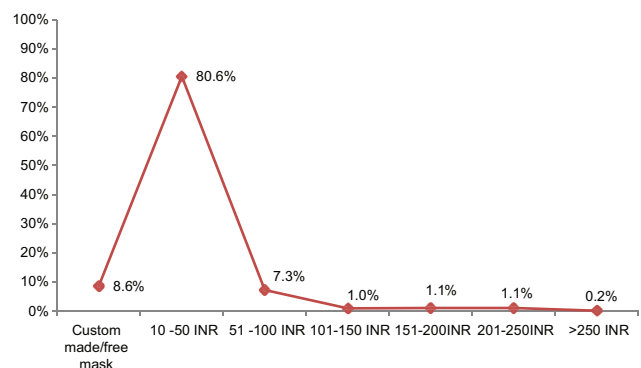


Figure 2: Amount spent on each mask by the individuals (in Indian rupees (INR))

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Table 2: Percentage of participants that expressed most difficult and easiest preventive measure to follow in combating severe acute respiratory syndrome coronavirus disease-2019

	Most difficult to follow?, n (%)	Easiest to follow?, n (%)
Hand wash	635 (10.6)	1835 (30.5)
Sanitizer	455 (7.6)	2505 (41.6)
Coughing/sneezing etiquette	290 (4.8)	110 (1.8)
Following social distancing circles	1030 (17.1)	280 (4.7)
Following social distance in public transport	1530 (25.4)	170 (2.8)
Usage of mask	1330 (22.1)	715 (11.9)
Maintaining social distance	745 (12.4)	400 (6.7)
Total	6015 (100)	6015 (100)

Table 3: Observations of the individuals on infringement of coronavirus disease 2019 measures in their family and surroundings

	Family, n (%)	Surroundings, n (%)
Social distancing without mask	275 (4.6)	390 (6.5)
Informal way of covering face with mask	265 (4.4)	285 (4.7)
Hanging mask to nearby objects	535 (8.9)	460 (7.6)
Frequently adjusting mask	665 (11.1)	205 (3.4)
Touching outer area of the mask	940 (15.6)	265 (4.4)
Nose/eye touching while wearing the mask	305 (5.1)	165 (2.7)
Crowding in markets	1300 (21.6)	2255 (37.5)
Crowding near delivery/cash counters	345 (5.7)	535 (8.9)
Chin strap	325 (5.4)	355 (5.9)
The ear hanger	375 (6.2)	350 (5.8)
Neck warmer	640 (10.6)	735 (12.2)
Wadded mask in their pocket	45 (0.7)	15 (0.2)
Total	6015 (100)	6015 (100)

Table 4: Noncompliance practices reported by the participants according to their level of education

Education	Noncompliance practices (%)											
	1	2	3	4	5	6	7	8	9	10	11	12
Illiterates	4.6	4.2	2.5	15.1	26.5	3.4	8.0	3.8	6.3	6.7	10.1	8.8
Primary school certificate	3.2	0.0	7.9	7.9	12.7	3.2	11.1	0.0	19.0	27.0	6.3	1.6
Middle school certificate	3.6	4.3	0.7	8.6	32.9	6.4	15.0	3.6	7.9	3.6	13.6	0.0
High school certificate	4.8	3.4	13.5	9.2	25.1	2.9	9.2	3.9	4.8	5.8	13.5	3.9
Intermediate or post high school diploma	1.9	4.4	7.5	6.3	20.8	5.7	10.1	1.9	13.8	3.1	15.7	8.8
Graduate or Postgraduate	1.6	4.1	8.9	11.1	24.7	4.1	6.0	4.6	9.5	6.8	15.4	3.3
Professional degree	0.0	0.0	14.8	22.2	22.2	0.0	7.4	11.1	7.4	3.7	11.1	0.0
Total	3.1	3.7	7.4	10.7	24.9	4.1	8.8	3.7	8.9	6.7	13.3	4.7

Chi-square test, $P < 0.001$ (Highly statistically significant). 1 - Social distancing without mask, 2 - Informal way of covering face with mask, 3 - Hanging mask to nearby objects, 4 - Frequently adjusting mask, 5 - Touching outer area of the mask, 6 - Nose/eye touching while wearing mask, 7 - Crowding in markets, 8 - Crowding near delivery/cash counters, 9 - Chinstrap, 10 - The ear hanger, 11 - Neck warmer, and 12 - Wadded mask in their pocket

normal.” Depending on convictions, comfort level, and neighborhood guidelines, a portion of our patients is returning with a “the same old thing” mentality. Others are careful, however agreeable, and afterward, there are the individuals who are not prepared to return by any stretch of the imagination. The examination applies an unmistakable investigation to dental patients’ information in the extraordinary period and presents notable and important findings of dental visits relating to socioeconomics. There is a clear contrast between the

general numbers of people even in these emergency times in looking for dental administrations which mirrored that females use more dental administrations than men inferable from different reasons. In the current observation, the proportion was 51.6% females to 48.4% males.^[16]

Purchased/custom made/free cloth masks were the personal choices of the study participants as a nonpharmacological means of a preventive measure adopted as they are affordable, available, accessible, reusable, and recyclable due to

which they are widely used as a public health intervention strategy.^[17]

In this study, it was observed that crowding in the market, touching the outer area of the face mask, and usage of the mask as a neck warmer were the infringements observed by the participants in their family and surroundings. Isolation and social distancing measures, including edicts to stay at home, have been brought into place globally to reduce the transmission of the virus, but at a huge cost to individuals and society. In addition to these measures, other effective interventions to increase adherence to behaviors that individuals in communities can enact to protect themselves and others: as reported by Glynda Alves that 90% of Indians are aware of wearing a mask as a preventive measure, but only 44% were wearing a mask where discomfort was revealed as a key reason for noncompliance.^[18] This study provides the evidence to start applying behavioral science methods and models to understand and modify the individual's perceptions and nonpharmacological practices.^[19]

The amount spent on each mask by the individuals was about 10–50 INR which might be due to the reason that the amount India spends on public health per capita every year is 1112 INR less than the cost of a single consultation in the country's top private hospitals or roughly the cost of a pizza at many hotels, that comes to INR 93 per month or INR 3 per day.^[20] The average cost of manufacturing these surgical face masks was around 40–45 paisa, while that for N95 mask is about ₹10–12, but due to market dynamics, these were being sold at a much higher price which was not affordable.^[21]

For the vast majority of people, wearing a mask is a new thing. While we as a whole conform to wearing masks out in the open, it is significant we attempt to expect as meager as conceivable about others, dependent on whether they are wearing one. It is anything but difficult to make a hasty judgment. As wearing a mask in broad daylight turns out to be more normal in numerous parts of the world, either on the grounds that it's obligatory where you live or on the grounds that you decide to wear one, it very well may be enticing to expect individuals who do not wear masks are reckless, misinformed, or egotistical. Dental specialists and individual dental patients are no exemption from this philosophy as the authors perceived. Understanding public capacity, opportunity, and motivation in receiving preventive strategy are the critical hotspots for conduct change; if not, any endeavor to change the conduct will just turn into purposeful publicity rather an adjustment in conduct.

Individual social desirability bias cannot be excluded owing to the fact that questionnaire studies elicit a conscious positive response; however, when asked about the most common preventive measure in combating SARS COVID-19 was not followed in the surroundings, and the family was found to be crowding in markets which reflected the lapse in following preventive measure as a whole in the community.

CONCLUSION

One of the most basic needs for a dental patient is the availability of transport in availing oral health care, with the pandemic majority of the participants reported that following social distancing in the public transport is the most difficult preventive measure reflecting the need to address the issue. While crowding in markets is the most common noncompliance observed within the family and in surroundings highlighting the importance of the need for strict implementation of legislative measures.

Overall, mask etiquette was the most difficult to follow hence required upliftment of cognitive skills on the usage of masks among the community. Noticing a patient's preventive conduct is one of the vital accomplishments for productive dental administrations conveyance among the corona infection pandemic. Dental foundations may end up being upheavals of cases if patients who go into the grounds are not checked or perceived. Effective guidance and making to comprehend guidelines are conceivable just on investigating patient's mentalities toward adopting preventive protocols for SARS COVID-19.

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Conflicts of interest

There are no conflicts of interest.

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Care Sought and Normative Need Assessment for Dental Caries among Rural Adults in Jeelugumelli Mandal, Andhra Pradesh, India

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Abstract

Introduction: Different rates of dental caries prevalence are experienced among different populations due to the influence of various demographic, political, and socioeconomic factors. **Aim:** To assess the prevalence of dental caries, the type of self-care remedies utilized, and normative needs assessment among rural adults residing in Jeelugumelli Mandal, West Godavari District, Andhra Pradesh, India. **Materials and Methods:** A cross-sectional survey was conducted among 800 adults by using a multi-stage random sampling technique in Jeelugumelli Mandal, West Godavari District, India. Data were collected using a pretested pro forma consisting of questionnaire-related demographics, traditional care, and clinical examination which was investigated by a single examiner to record caries experience (Decayed, Missing, and Filled Teeth [DMFT]) using WHO 1997 criteria. The data were analyzed using IBM SPSS Statistics, Version 20.0. Mann-Whitney *U*-test, Kruskal-Wallis, and Multivariate Linear Regression analysis tests were used and the level of significance was set at $P \leq 0.05$. **Results:** Mean DMFT value of the study population was 10.29 ± 4.806 and 33.4% of the subjects had used home remedies related to plant origin. The majority of the study population required surgical need (Extractions) with a mean value of 4.23 ± 5.553 and compared with unskilled workers, unemployed workers had 2.8 units higher chances of having caries ($R = 2.810$ confidence interval [CI] 1.876–3.744) while it was 5.031 units lesser for professionals in having caries ($R = -5.031$ CI [-4.16] – [-5.901]). **Conclusion:** Dental caries prevalence and normative needs for dental caries were high in the adult population residing in Jeelugumelli Mandal and most of the individuals are depending on self-care procedures to relieve pain.

Keywords: Dental caries, medicine, needs assessment, rural health, traditional

INTRODUCTION

Different factors can influence dental caries prevalence including demographic, political, and socioeconomic factors.^[1-4] The common beliefs, customs, and practices related to health and disease, in turn, influence the health-seeking behavior of inaccessible rural communities. Despite remarkable worldwide progress in the field of diagnostics, curative, and preventive health, the rural people still live in isolation, in natural and unpolluted surroundings far away from civilization with their traditional values, customs, beliefs, and myth intact.^[5]

Research suggests that higher rates of oral diseases occur in rural areas where a lower number of dentists are available

which remains a major barrier to oral health-care access for rural village residents.^[6] Facing numerous barriers to oral health care, low-income rural residents often are forced to choose alternative strategies, forgo treatment, and/or use self-care remedies for the relief of dental pain.^[7] Self-care is the component of health self-management that includes behaviors undertaken to enhance health, prevent disease, limit illness or restore health, which is derived from the individual's knowledge and skills.^[8] Given the large number of rural people who may seek pain relief by means other than visiting a

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dentist, it is important to understand the “alternative” treatment methods or self-care they utilize for pain relief.^[9]

With this background, the present study was aimed to assess the prevalence of dental caries, type of self-care remedies utilized and normative needs among rural adult people residing in Jeelugumelli Mandal, West Godavari District, Andhra Pradesh, India. This study location is the border area between Andhra Pradesh and Telangana states of India.

MATERIALS AND METHODS

A population-based cross-sectional survey was conducted among people residing in a rural locality of a state in south India. Jeelugumelli, a rural Mandal (administrative division), was selected for analysis in the present study. It is one of the 46 mandals of the West Godavari district in the south Indian state of Andhra Pradesh and the demography of the selected mandal represents the typical rural population of southern India. A total of 800 subjects were selected by using multi-stage random sampling technique and the adult rural population of Jeelugumelli Mandal was divided into three age groups, i.e., (a) 18–27 years (b) 35–44 years (c) 65–74 years to document the age-group-wise variation in the profile of dental caries. Through multi-stage random sampling technique, 8 villages from the Jeelugumelli mandal were included in the present study. From the selected villages every alternate house was considered randomly and all the individuals present at the time of examination, falling in the specified age groups were included. The area for conducting examinations was planned and arranged for maximum efficiency and ease of operation. The subjects were allowed to sit on a chair or stool as per availability, where, sufficient natural daylight was available while avoiding discomfort from direct sunlight on either the subject or the examiner. A table to place instruments and supplies was placed within easy reach of the examiner. The recording clerk was allowed to sit close enough to the examiner so that instructions and codes could be easily heard and the examiner could see that findings were being recorded correctly.

Individuals who were non co-operative, mentally challenged, edentulous were excluded. The present study was conducted during 2017 for 4 months (June–September) and data collection was done using a specially designed pretested proforma consisting of two parts. The first part included information on demographic data and questions related to the utilization of traditional remedies. The second part included clinical examination by a single examiner who was trained and calibrated by a standard examiner to record caries experience (Decayed, Missing and Filled Teeth [DMFT]) using WHO 1997 criteria. Before the field study, the questionnaire validity was accomplished through the revision of the questionnaire by two senior experts in Public Health Dentistry and the revised questionnaire was subjected to a pilot study on 20 individuals for evaluating the psychometric properties. The intra-examiner reliability was measured by

repeated examinations performed on rural adults to assess the intra-examiner agreement of caries experience, using Cohen’s Kappa statistics. The intra-examiner reliability in recording the caries experience was 0.8 and 0.9, respectively.

Sample size estimation

The sample size was estimated based on the prevalence of dental caries determined through the National Oral Health Survey and Fluoride Mapping, India, 2003.^[10] In that survey, the prevalence of dental caries above 18 years was 70%. As dental caries is the most prevalent disease, the least percentage of caries prevalence (50%) was taken to get the maximum sample size. Based on the prevalence the sample size was calculated using the formula

$$\text{Sample size } (n) = (Z\alpha + Z\beta)^2/d^2$$

Ethical clearance and consent

Ethical clearance was obtained from the ethical committee of the SIBAR institute of dental sciences. The study protocol was later approved by the Dr. NTR University of Health Sciences (Pr. 37/IEC-SIBAR/CIR/15), Vijayawada, Andhra Pradesh, India, and voluntary written informed consent was obtained from all the individuals participating in the study before examination after discussing in detail about the purpose of the study.

Statistical procedures

The collected data were analyzed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA: IBM Corp.^[11] Descriptive and inferential statistics were used to summarize the results and tests such as the Mann-Whitney *U*-test, Kruskal-Wallis, and Multivariate Linear Regression were used. The level of significance was set at $P \leq 0.05$.

RESULTS

The mean DMFT value of the study population was 10.29 ± 4.806 and was highest among 64–75 years age group (12.79 ± 4.265) followed by 35–44 years (10.20 ± 4.284) and 18–27 years age group (6.40 ± 3.483) which is statistically significant ($P \leq 0.001$). Mean DMFT values were higher among unemployed (17.25 ± 4.494), unskilled workers (11.40 ± 3.896) compared to skilled (4.54 ± 0.503), clerical/shop owner (8 ± 0.001), and professionals (5.02 ± 4.075) which is statistically significant ($P \leq 0.001$) [Table 1].

The majority of the study population required surgical need (Extractions) with a mean value of 4.23 ± 5.553 followed by prosthetic need (4.12 ± 1.337), pulp care and restorations (2.66 ± 3.349), one surface filling (2.18 ± 2.145), two surface fillings (1.29 ± 1.595). Mean surgical need (10.07 ± 4.067), and prosthetic need (9.80 ± 4.320) were higher for the 64–75 years age group [Table 2]. 33.4% of the subjects had been using home remedies related to plant origin for the treatment of dental caries and it was higher among 64–75 years age group (56%) [Graph 1]. Lack of financial support (50.8%) and decreased accessibility to the

dentist (14.6%) were the two main reasons for not consulting dentists for the treatment of dental caries [Graph 2].

Table 1: Mean Decayed, Missing and Filled Teeth scores of the study subjects according to demographic variables

Variables	Frequency	DMFT (mean±SD)	P
Age group (years)			
18-27	205	6.40±3.48	0.001 ^{###,*}
34-45	267	10.20±4.28	
64-75	328	12.79±4.26	
Gender			
Male	418	10.82±5.19	0.024 ^{#,*}
Female	382	9.71±4.27	
Occupation			
Unemployed	36	17.25±4.49	0.001 ^{###,*}
Unskilled	586	11.40±3.89	
Skilled	56	4.54±0.50	
Clerical, shop owner	22	8.00±0.001	
Professional	100	5.02±4.07	
Socioeconomic status			
Upper	94	3.11±2.09	0.001 ^{###,*}
Upper middle	151	8.47±3.30	
Middle	64	10.25±2.20	
Lower middle	491	12.23±4.26	

[#]Man-Whitney U test, ^{###}Kruskal-Wallis test, ^{*}Statistically significant. SD: Standard deviation, DMFT: Decayed, Missing and Filled Teeth

Table 2: Age-wise distribution of study subjects based on normative need (treatment required)

Normative need	Age	Frequency	Treatment in need (mean±SD)	P
Sound tooth (years)	18-27	205	27.12±2.86	0.001*
	34-45	267	22.19±4.92	
	64-75	328	19.30±4.43	
	Total	800	22.27±5.28	
One surface filling (years)	18-27	205	0.58±1.00	0.001*
	34-45	267	0.65±0.47	
	64-75	328	0.09±0.28	
	Total	800	0.40±0.66	
Two surface filling (years)	18-27	205	2.18±2.14	0.001*
	34-45	267	1.64±1.16	
	64-75	328	0.44±0.95	
	Total	800	1.29±1.59	
Pulp care and restoration (years)	18-27	205	1.85±2.55	0.001*
	34-45	267	5.88±3.33	
	64-75	328	0.55±0.97	
	Total	800	2.66±3.34	
Prosthetic need (years)	18-27	205	0.18±0.38	0.001*
	34-45	267	1.35±1.50	
	64-75	328	9.80±4.32	
	Total	800	4.12±5.51	
Surgical need (years)	18-27	205	0.00±0.001	0.001*
	34-45	267	0.31±0.97	
	64-75	328	10.07±4.06	
	Total	800	4.23±5.55	

Kruskal-Wallis test, ^{*}Statistically significant. SD: Standard deviation

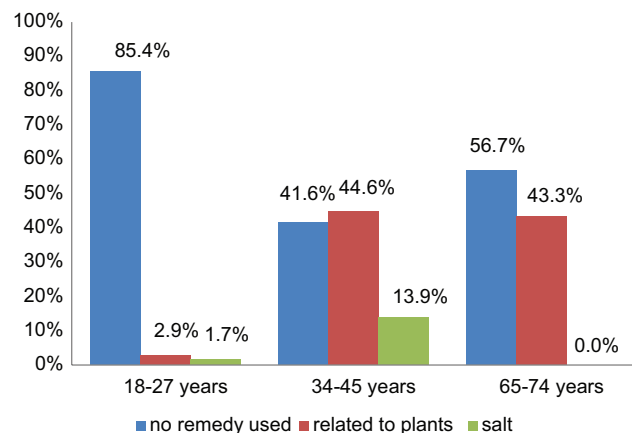
Compared with unskilled workers unemployed workers had 2.8 units higher chances of having caries ($R = 2.810$ confidence interval [CI] 1.876–3.744) while it was 5.031 units lesser for professionals in having caries ($R = -5.031$ CI [-4.16] – [-5.901]). Compared to the 64–75 years age group, the 18–27 years age group had 7.196 units lesser chances of having caries ($R = -7.196$ CI [-7.780] – [-6.612]), while it was 4.164 units lesser for 35–44 years age group. Compared to the lower middle class, the upper-middle class had 11 units lesser chances of having caries ($R = -11.102$, CI [-12.097] – [-10.17]) while it was 5.192 units lesser for the upper-middle class [Table 3].

DISCUSSION

Despite its known multifactorial etiology and prevention possibility, dental caries still represents the most widespread disease in the world, affecting various populations at different age groups.^[12,13] The current study was executed among rural areas of Jeelugumelli Mandal where there are various influential factors responsible for the higher prevalence of dental caries. As a result, these subjects are forced to use nontraditional providers to forgo treatment or use alternative strategies or self-remedies for the relief of dental pain.

In the present study, there was preponderance in male participation compared to females which was in accordance with Pashayev *et al.* The traditional patriarchal norms and limited education have relegated women to secondary status within the society could be the reason behind the less participation of females.^[3,14] Males had higher caries experience compared to females which was in contrast with the study done by Shaffer *et al.* Females are more concerned for esthetics and consume fewer amounts of refined sugars compared to males, which could be the reason for less caries in them in the present study.^[15-18]

There is an increase in mean DMFT values with an increase in the age which is in accordance with Kamberi *et al.* The decayed component was higher among 35–44 years age



Graph 1: Age-wise distribution of subjects according to home remedies they followed. $\chi^2 = 101.859, P = 0.001$

Table 3: Multivariate linear regression analysis between various demographic variables and decayed, missing and filled teeth scores

Variables	OR	95% CI		P
		Lower	Upper	
Age	3.868	3.626	4.110	0.001*
Age groups (years)				
18-27	-7.196	-7.780	-6.612	0.001*
35-44	-4.164	-4.601	-3.726	0.001*
65-74 (constant)	1.00	-	-	-
Gender				
Males (constant)	1.00	-	-	-
Females	0.503	0.085	0.921	0.018*
Occupation				
Professional	5.031	4.161	5.901	0.001*
Clerical/shop owner	1.690	0.588	2.791	0.003*
Skilled	1.501	0.610	2.393	0.001*
Unskilled (constant)	1.00	-	-	-
Unemployed	2.810	1.876	3.744	0.001*
Socioeconomic status				
Upper class	-11.102	-12.097	-10.107	0.001*
Upper middle class	-5.192	-5.808	-4.576	0.001*
Middle class	-2.685	-3.425	-1.944	0.001*
Lower middle class (constant)	1.00	-	-	-

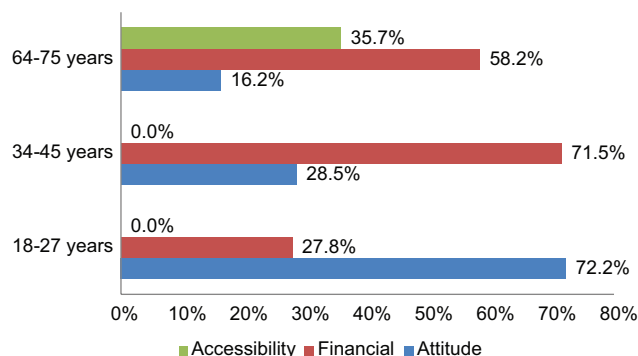
*Statistically significant. OR: Odds ratio, CI: Confidence interval

group, missing component was higher for 65–74 years, and filled component was comparatively higher for 18–27 years age group which are in accordance with Pashayev *et al.*^[19] An increase in age directly increases the exposure to a cariogenic environment might be a reason for increased DMFT levels in the present study.^[20]

Individuals suffering from dental caries do not always seek relief by visiting a dentist. For individuals who have low income who do not seek treatment from a dentist self-care plays a palliative role.^[7] 79.6% of the study participants had utilized at least one type of self-care to overcome the painful experience.^[21] 21.5% of Respondents more frequently reported using nonprescription medicines or over-the-counter medication for pain relief and this in-contrast with the findings of Locker D *et al.* where most of the participants received care from the dentist.^[22] The frequent use of nonprescription medicines suggests that there is a need for a greater understanding of how rural people learn about the effectiveness and safety of nonprescription medications.

Home remedies of plant origin are used by the majority of respondents to get relief from oral pain. According to the respondents, the sap which is coming out from the barks of *Jatropha Gossypifolia* will relieve the teeth as well as gum pain. This finding is in agreement with a study done by S Ganesan.^[23]

The plant's *Jatropha curcas* Linn (called “*mepala*” in telugu), *Jatropha Gossypifolia* Linn (called “*chima mepala*”) belonged to Euphorbiaceae family. The young stem, latex of these plants



Graph 2: Age group-wise distribution of subjects based on barriers for not visiting the dentist

are used to get relief from teeth pain, fetid smell, mouth, and gum ulcers.^[24] Along with these plants, *Andrographis paniculata* (green Chirayta in English language, *Nelavemu* in the Telugu language) is also being used by natives for relief of tooth pain which is naturally grown in Jeelugumelli environmental conditions.^[21]

Regarding the normative needs of the population, the majority need extractions as a treatment option for dental caries followed by prosthetic need, pulp care, two surfaces, and one surface filling respectively. These findings are in disagreement with the study done by Maru *et al.*, Duraiswamy *et al.*, where there is a major need for one surface filling. The need for extractions and replacement of missing teeth are higher among the 65–74 years age group, while pulp care and restorations were higher among 35–44 years age group.^[3,25]

While the effect of sociodemographic factors on oral health and dental care-seeking behavior has been well characterized in developed countries, this is not the case in developing countries.^[26] Compared to developed countries, the limited number of oral health care institutions and the less coverage of community oral health services combine to compromise access to oral health care services in India.^[26] In addition, health insurance coverage is higher in urban areas compared to rural areas in India. Furthermore, medical insurance is not nationalized, and coverage is mainly provided by employers. As a result, many patients have to pay for oral health care themselves.^[26] These prospects explained well the reasons for accumulated needs among elderly age groups compared to younger age groups.

Limitations

1. The cross-sectional design of the study inherits a limitation; it does not allow the determination of a causal relationship between the variables investigated and the outcome
2. Though a short time frame was used, recall bias was inevitable.

CONCLUSION

The high prevalence of dental caries found in the adult population residing in Jeelugumelli mandal should be

considered a relevant dental public health problem. Most of the individuals are depending on their self-care procedures to relieve pain and there is a high accumulation of normative needs in the current population.

Recommendations

1. Additional research to explore the safety and effectiveness of plant *Andrographis Paniculata* which has an immense significance in treating medical conditions is recommended for its application in treating dental pain
2. Dental doctors should be appointed in primary health centers along with medical staff to reduce the accessibility barrier in the utilization of dental services among rural residents
3. Should work on mobile and portable dental services through a public-private partnership among inaccessible areas
4. Additional advanced research like phytochemical analysis on self-care medicinal plants should be encouraged to rule out its safety and efficacy in treating dental diseases.

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Conflicts of interest

There are no conflicts of interest.

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Antiplaque Efficacy of Toothpaste – A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Abstract

Background: Different toothpastes of the corresponding active ingredients could have variable extent of effectiveness; this raises the amount of choices with the marketplace for toothpaste containing these agents and makes it more difficult to gauge their effectiveness. Therefore, the purpose of this systemic review and meta-analysis was to gauge the antiplaque efficacy of different toothpaste agents in studies involving randomized clinical trials published over a span of January 2015-May 2020. **Materials and Methods:** A comprehensive and systematic electronic search via PubMed, TRIP, Cochrane, and Google scholar prompted in 593 articles. Subsequently screening titles and abstracts for applicability, 31 Randomized controlled trial (RCT) articles were enclosed in the review. With the aid of the Cochrane Collaboration's tool, the risk of bias of each study was assessed. **Results:** A total of 2467 individuals partake in 31 RCT studies. Triclosan toothpaste was superior over sodium fluoride/monofluorophosphate toothpaste (standard mean difference, SMD – 1.36, 95% confidence interval [CI] (2.81–0.10), $P = 0.007$) in plaque reduction. Long-term utilization of stannous fluoride toothpastes reduced dental plaque superior than monofluorophosphate toothpaste (SMD – 0.61, 95% (CI 1.04–0.18), $P = 0.005$). There was no discernible difference in plaque reduction. **Conclusion:** Within the confines of the current study's limitations (which embody the severe imbalance within the quantity of evidence), once scrutiny among products, triclosan and stannous fluoride in dentifrices seem to be the foremost efficacious plaque controlling active agents.

Keywords: Antiplaque efficacy, randomized controlled trial, toothpaste

INTRODUCTION

Dental plaque is a biofilm harboring innumerable microorganisms and is a requisite factor for the inception and furtherance of periodontal diseases.^[1] The pioneer cariogenic microorganisms strains present in the plaque are the strains of *Streptococcus mutans*. They bring about alterations in the plaque metabolism, causing demineralization to predominate. Interruption of dental biofilm by mechanical debridement is pivotal to overseeing plaque and gingivitis. The two standard compelling techniques are mechanical plaque and chemical plaque control.^[2]

One of the most quintessential oral hygiene care strategies is to use a toothbrush in conjunction with a dentifrice to limit plaque accumulation and the risk of plaque-related diseases, including periodontitis and caries.^[3] Microbial infections are liable for a substantial percentage of dental problems. Dental issues are predominantly of development of dental plaque, dental caries, and periodontal diseases.^[4]

Different components have been incorporated as active ingredients in toothpaste concoctions, pertaining to the therapeutic claim.^[5] Contingent upon their activity, they can be assorted as antimicrobial, plaque inhibitory, antiplaque, or anti-gingivitis. Antiplaque agents are those able to impinge gingivitis and plaque significantly, and they ought to be favored in the therapeutics of gingivitis and the interception of periodontal diseases.^[6]

The precise formulation of the active agents into toothpaste is essential to maintain the agents' bioaccumulation and, in certain instances, to amelioration their substantivity. Thus, different concoctions of the corresponding active ingredients may have varying extent of effectiveness, this

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raises the number of options in the marketplace for products containing these agents and makes it more difficult to gauge their effectiveness.^[7] Therefore, the purpose of this systemic review and meta-analysis was to gauge the antiplaque efficacy of different toothpaste agents in studies involving randomized clinical trials published over a span of January 2015–May 2020.

MATERIALS AND METHODS

Framing of a research question and protocol registration

Using the PICO plan, the pursuit technique was done by an inquiry mirroring the point of the investigation: “what is the comparative efficacy of various toothpastes on the reduction of dental plaque in different groups of the population?”

Protocol registration: CRD42021246121 (<https://www.crd.york.ac.uk/prospero/#myprospero>).

Search strategy

The search was confined to randomized controlled trial (RCT) articles published from 2015 until 2020. This data confinement was preselected due to the rapid advancement of new toothpaste formulations. A comprehensive and systematic electronic search via PubMed, TRIP, Cochrane, and Google scholar using combinations of keywords antiplaque, efficacy, toothpaste, dentifrices, plaque resulted in 593 articles. Two reviewers removed duplicates from emanate obtained and independently scrutinized the remaining articles in terms of title and abstract. Subsequent to screening titles and abstracts for applicability, 31 RCT articles were enclosed in the review [Figure 1].

Inclusion criteria

- Research limited to RCTs
- Articles published in English
- Articles published within the span of 5 years, i.e., 2015-2020
- The publications which were available as full reports
- Studies conducted in any country on the general population with no restrictions on age and gender.

Exclusion criteria

- *In vitro* studies, abstracts, case reports, pilot studies, narrative reviews, and letters to editors were excluded.

Assessing the quality of the included studies

An aggregate of 31 articles have been chosen per the defined criteria. All the 31 articles were assessed with respective CONSORT checklists. With the aid of the Cochrane Collaboration tool, the risk of bias of each study was evaluated. Cochrane Review Manager Version 5.4 was used to generate risks of bias figures.

Statistical analysis

Cochran's Q coefficient, a Chi-square test, and a significance cutoff of 10% were used to determine the data's heterogeneity. For continuous data, a random-effects model was accustomed to combine the primary outcome variables from each study.

To assess the consistency of the results, the I² statistic and forest plots were employed. Overall outcome were assessed as standardized mean differences (SMD) (95% confidence interval [CI]). Review manager (RevMan), version 5.4.1 for Windows, The Cochrane Collaboration, Copenhagen, Denmark has been used to analyze data.

RESULTS

A total of 2467 individuals took part in 31 RCT studies. A conspectus of the selected studies and their characteristics is presented in Table 1.

Study design and subject characteristics

The proportions of researches conducted using parallel and cross-over designs were 90% and 10%, respectively. Only one study^[31] was triple blinded, 17 studies^[8,9,11-14,21,22,24,26,28-30,32,34-36] were double blinded, four studies^[7,16,23,37] were single blinded, whereas nine studies^[10,15,17-20,25,27,33] had not mentioned blinding. Twenty-eight studies were organized in clinical settings with adult participants, while in contrary two studies^[10,20] included children as participants and one study^[8] with dental students.

Risk of bias

The quality assessment is depicted in Figure 2.

For random sequence generation, no bias was ascertained. Blinding of personnel and participants, as well as incomplete outcome data, showed a substantial risk of bias more than 75%. Blinding of outcome assessment and allocation concealment disclosed a >50% unclear risk of bias 26% low-risk bias was seen for selective reporting [Figure 2].

Interventions and regimens

Out of 31 studies, 16 were herbal interventions, four were Triclosan interventions, three were stannous fluoride interventions, two were Probiotics interventions, two were plaque indicating dye interventions, and one was chlorhexidine intervention. Out of 16 herbal intervention studies, 11 studies were compared with conventional toothpaste. In all the studies, manual toothbrushes were used. Most of the researches forbade the utilization of any auxiliary oral hygiene practice.

Meta-analysis

As the outcome evaluation methods were different and there was a loss of quantitative data, only 18 articles were included in the meta-analysis. Triclosan toothpaste was superior over sodium fluoride/monofluorophosphate toothpaste (standard mean difference [SMD] -1.36 , 95% CI (2.81–0.10), $P = 0.007$) in plaque reduction [Figure 3]. Long-term use of stannous fluoride toothpastes reduced dental plaque superior than monofluorophosphate toothpaste (SMD -0.61 , 95% CI 1.04–0.18), $P = 0.005$) [Figure 4]. There was no significant difference (SMD -0.38 , 95% CI -0.82 , -0.06), $P = 0.009$) in plaque reduction between herbal and conventional toothpaste [Figure 5].



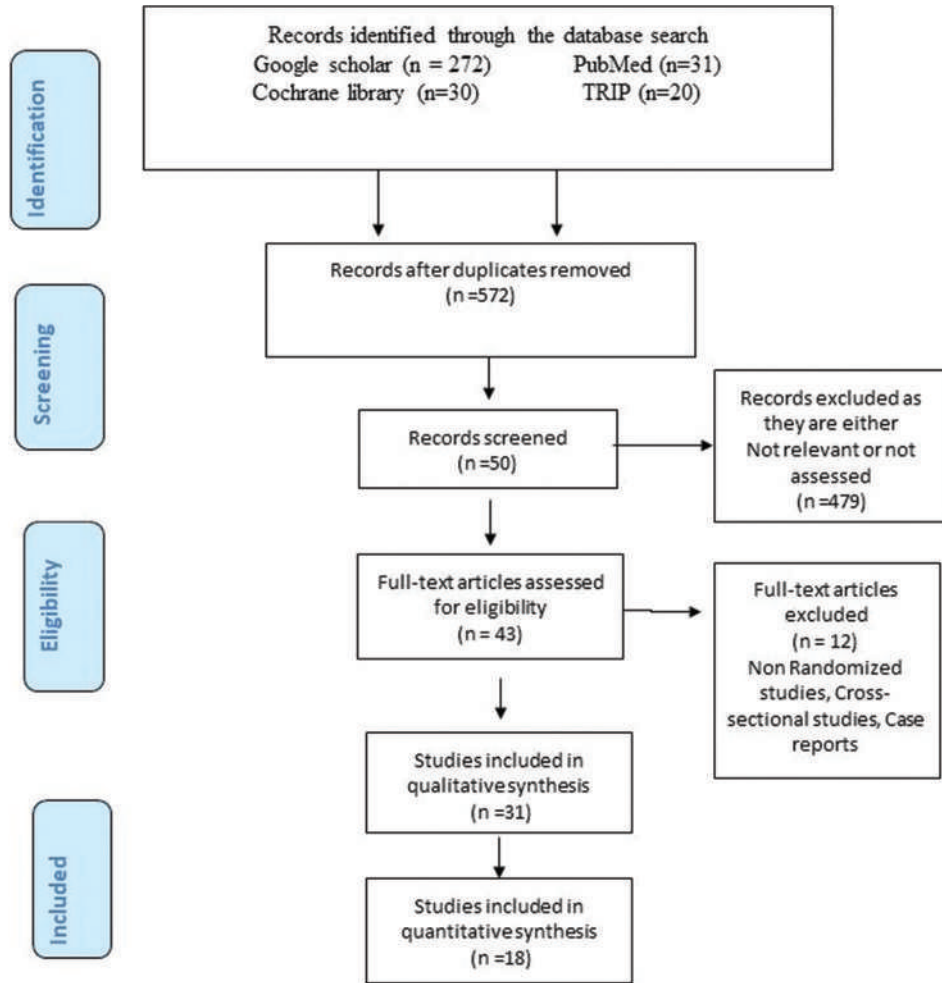


Figure 1: PRISMA flow diagram



Figure 2: Risk of bias graph and summary: review authors' judgments about each risk of bias item presented as percentages across all included studies


Dr. L. KRISHNA PRASAD
DEAN

Table 1: An overview and characteristics of included studies

Year, author, and project location	Duration of study	Intervention/comparison	Statistical inference	Conclusion/recommendation
2015 Abhishek <i>et al.</i> ^[8] Karnataka, India	30 days	Conventional toothpaste (<i>n</i> =15) versus neem containing toothpaste (<i>n</i> =15)	<i>t</i> -test <i>P</i> =0.001	Within the limitations (small sample size and Hawthorne effect), regular use of neem containing toothpaste provided a significant reduction of participants' dental plaque
2015 Mitra <i>et al.</i> ^[9] Mumbai, India	4 weeks	Herbal toothpaste (<i>n</i> =50) versus triclosan-containing toothpaste (<i>n</i> =50)	<i>t</i> -test <i>P</i> >0.05	Herbal toothpaste is equally efficient in the control of plaque as triclosan-containing toothpaste
2016 Sälzer <i>et al.</i> ^[10] Netherlands	8 weeks	Colgate caries protection toothpaste (without SLS, <i>n</i> =57) versus Zendium classic toothpaste (with SLS, <i>n</i> =58)	ANCOVA independent <i>t</i> -test paired <i>t</i> -test <i>P</i> =0.690	No statistically significant difference in plaque scores between the control group (with SLS) and the test group (SLS)
2015 Bayoumi <i>et al.</i> ^[11] Egypt	3 months	Colgate total containing sodium fluoride and 0.3% triclosan (<i>n</i> =25) versus Colgate max fresh containing 0.22% sodium fluoride (<i>n</i> =25)	Chi-square Mann-Whitney Friedman test Wilcoxon signed ranks test <i>P</i> =0.001	Triclosan toothpaste proved to have marked antiplaque and antigingival effect
2017 Tadikonda <i>et al.</i> ^[12] India	1 month	Colgate healthy teeth toothpaste (<i>n</i> =25) versus Glodent dentifrice containing papain, bromelain, neem, and miswak with 1000 ppm fluoride (<i>n</i> =25)	Paired <i>t</i> -test independent <i>t</i> -test <i>P</i> <0.001	Significant reduction in plaque score in the test (golden dentifrice) group compared to the control group (Colgate healthy teeth toothpaste)
2015 Ayad <i>et al.</i> ^[13] Canada	4 weeks	Toothpaste containing triclosan, PVM/MA polymer and 0.243% sodium fluoride i.e., Colgate total advanced whitening toothpaste (<i>n</i> =55) versus toothpaste containing 0.76% of sodium monofluorophosphate i.e., Colgate cavity protection toothpaste (<i>n</i> =60)	Independent <i>t</i> -test paired <i>t</i> -test <i>P</i> <0.05	The test regimen has significantly greater efficacy in controlling established dental plaque and gingivitis than the negative control regimen
2016 Liu and Yin ^[14] China	12 weeks	Toothpaste containing 0.6% <i>Ilicis Rotundae</i> cortex in a calcium carbonate base (<i>n</i> =25) versus toothpaste without any active ingredients (<i>n</i> =25)	Chi-square Paired <i>t</i> -test <i>P</i> <0.01	0.6% <i>Ilicis Rotundae</i> cortex was effective in reducing plaque compared to toothpaste without any active agents
2015 Shanmugapriya <i>et al.</i> ^[15] India	72 h	Herbal toothpaste (<i>n</i> =15) versus commercially available toothpaste (<i>n</i> =15)	Unpaired <i>t</i> -test paired <i>t</i> -test <i>P</i> =0.001	Plaque inhibitory action of herbal toothpaste was marginally less when compared to commercial toothpaste
2016 Stevens <i>et al.</i> ^[16] USA	7-10 days	Toothpaste without plaque indicating dye (<i>n</i> =20) versus toothpaste with a plaque indicating dye (<i>n</i> =20)	Paired <i>t</i> -test Independent <i>t</i> -test <i>P</i> =0.03	Mechanical plaque removal brushing with toothpaste containing plaque indicating dye showed significantly reduced plaque than toothpaste without dye
2017 Mina <i>et al.</i> ^[17] Iran	12 weeks	Masdent toothpaste containing <i>Pistacia lentiscus</i> (<i>n</i> =30) versus crest complete toothpaste (<i>n</i> =30)	ANCOVA Mann-Whitney <i>U</i> -test Wilcoxon test <i>P</i> =0.855	The Masdent group has higher plaque reduction efficacy than the crest group, which was not statistically significant
2017 Singh ^[18] Jammu, India	6 weeks	Herbal toothpaste (<i>n</i> =65) versus Colgate sensitive toothpaste (<i>n</i> =65)	<i>t</i> -test Chi-square test <i>P</i> >0.05	Both the dentifrices are equally effective in controlling plaque
2017 Arabsolghar <i>et al.</i> ^[19] Iran	14 days	Crest anti-cavity (without chlorhexidine, <i>n</i> =10) versus kin gingival (with chlorhexidine, <i>n</i> =10) versus orthokin (with chlorhexidine and zinc acetate, <i>n</i> =10)	ANOVA <i>P</i> >0.05	Orthokin had a greater but no significant antiplaque effect than kin gingival and crest toothpaste
2017 Sangeetha ^[20] India	30 days	Triclosan-containing toothpaste (<i>n</i> =28) versus fluoride-containing toothpaste (<i>n</i> =28)	Independent <i>t</i> -test paired <i>t</i> -test <i>P</i> =0.001	Triclosan-containing toothpaste significantly reduced plaque compared to conventional fluoride toothpaste
2017 Arat Maden <i>et al.</i> ^[21] Turkey	6 weeks	Xylitol (<i>n</i> =16) versus xylitol probiotic (<i>n</i> =16) versus fluoride dentifrice (<i>n</i> =16)	Kruskal-Wallis Mann-Whitney <i>U</i> -test <i>P</i> =0.006	Probiotic toothpaste showed statistically significant plaque reduction compared to xylitol and fluoride toothpaste groups with a 6 weeks follow-up period



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Table 1: Contd...

Year, author, and project location	Duration of study	Intervention/comparison	Statistical inference	Conclusion/recommendation
2018 Hosadurga <i>et al.</i> ^[22] Karnataka, India	28 days	Colgate herbal toothpaste (<i>n</i> =20) versus parodontax (<i>n</i> =25)	<i>t</i> -test Chi-square test <i>P</i> <0.001	Significant reduction in the test and control groups' plaque score within the limitations (small sample size, sampling frame may not represent the general population and dropouts in the control group)
2018 Rusminah <i>et al.</i> ^[23] Indonesia	7 days	Miswak toothpaste (<i>n</i> =15) versus non-Miswak toothpaste (<i>n</i> =15)	Paired <i>t</i> -test <i>P</i> =0.01	Toothpaste containing Miswak was more effective in reducing plaque compared to non-Miswak toothpaste
2019 Daly <i>et al.</i> ^[24] UK	3 months	Toothpaste containing enzymes and proteins i.e., Zendium-1450 ppm sodium fluoride, lactoferrin, lactoperoxidase, colostrum, amyloglucosidase, glucose oxidase, and potassium thiocyanate (<i>n</i> =113) versus Sensodyne Pronamel – sodium fluoride 1450 ppm (<i>n</i> =116)	Independent <i>t</i> -test paired <i>t</i> -test <i>P</i> =0.01	Toothpaste containing enzymes and proteins had efficacy in reducing plaque compared to the control group
2018 Rangrej <i>et al.</i> ^[25] India	15 days	PatanjaliDantKanti (<i>n</i> =17) versus Colgate herbal (<i>n</i> =17)	Independent <i>t</i> -test Paired <i>t</i> -test <i>P</i> =0.016	Within the limitations (small sample size, short duration of the study), the study concluded that the regular use of these herbal dentifrices significantly reduced the plaque accumulation and improved the participants' gingival health
2019 Hu <i>et al.</i> ^[26] China	6 months	0.457% stannous fluoride stabilized zinc phosphate in a silica base (<i>n</i> =49) versus 0.76% sodium monofluorophosphate in a phosphate dehydrate base (<i>n</i> =49)	Independent <i>t</i> -test Chi-square test <i>P</i> <0.001	The test group significantly reduces plaque compared to the control group as stannous fluoride acts as an antimicrobial agent
2019 Lorenz <i>et al.</i> ^[27] Germany	12 weeks	Monofluorophosphate (<i>n</i> =120) versus. amine fluoride/stannous fluoride (<i>n</i> =120)	Unpaired <i>t</i> -test <i>P</i> <0.001	The test group (amine fluoride/stannous fluoride) showed a statistically significant reduction in plaque score than the control test group (monofluorophosphate)
2018 Menon <i>et al.</i> ^[28] Kerala, India	12 months	Calcium phosphate-containing toothpaste, (<i>n</i> =15) versus calcium sucrose phosphate toothpaste (<i>n</i> =15)	ANOVA Student's <i>t</i> -test <i>P</i> =0.044	Significant reduction (<i>P</i> =0.044) in the plaque amount in calcium sucrose phosphate toothpaste group compared to calcium phosphate-containing toothpaste group
2019 Pentapati <i>et al.</i> ^[29] Bangalore, India	One month	100 ppm fluoridated toothpaste, i.e., Colgate, (<i>n</i> =38) versus herbal nonfluoridated toothpaste, i.e., Sudanta toothpaste, (<i>n</i> =37)	ANCOVA independent <i>t</i> -test paired <i>t</i> -test <i>P</i> <0.001	Significant decrease in mean plaque score in both the test (Sudanta Toothpaste) and control group (Colgate)
2019 Seriwatanachai <i>et al.</i> ^[30] Thailand	6 months	A regular fluoride toothpaste (<i>n</i> =45) versus crest pro-health, stannous fluoride with zinc lactate (<i>n</i> =45) versus Colgate total, stannous fluoride dentifrice with zinc phosphate (<i>n</i> =45)	ANCOVA Independent <i>t</i> -test Chi-square test <i>P</i> <0.001	Statistically significant efficacy of plaque control is seen in test dentifrice (stannous fluoride with zinc lactate) and commercial stannous fluoride-containing dentifrice
2019 Cheng <i>et al.</i> ^[31]	12 weeks	Pudilan extract containing toothpaste (<i>n</i> =59) versus Pudilan toothpaste simulator (<i>n</i> =55)	Chi-square Group <i>t</i> -test <i>P</i> <0.01	The Toothpaste containing Pudilan extract could effectively control plaque and can improve and prevent chronic gingivitis
2019 Koshy <i>et al.</i> ^[32] India	10 days	Patanjali Dant Kanti (<i>n</i> =15) versus Colgate dental cream (<i>n</i> =15)	Paired <i>t</i> -test Mann-Whitney <i>U</i> -test <i>P</i> =0.016	Within the limitations (short duration for the course, the tooth brushing technique's credibility, and toothbrush) concluded that herbal toothpaste has significantly higher plaque scores than conventional toothpaste
2019 Valones <i>et al.</i> ^[33] Brazil	1 month	<i>Rosmarinus officinalis</i> Linn. (<i>n</i> =55) versus Colgate-Palmolive (<i>n</i> =55)	Wilcoxon test <i>P</i> >0.005	<i>Rosmarinus officinalis</i> was effective in controlling plaque when compared to conventional fluoridated



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Table 1: Contd...

Year, author, and project location	Duration of study	Intervention/comparison	Statistical inference	Conclusion/recommendation
2019 Singh <i>et al.</i> ^[34] India	30 days	Dant kanti (<i>n</i> =19) versus Colgate total (<i>n</i> =19)	Independent <i>t</i> -test Paired <i>t</i> -test <i>P</i> =0.315	Within the limitations (shorter duration of the experiment), the study concluded that clinically herbal dentifrice is as effective as a nonherbal dentifrice in reducing plaque
2019 Cunha <i>et al.</i> ^[35] Brazil	18 weeks	0.3% triclosan, 2% copolymer and 0.243% sodium fluoride (<i>n</i> =25) versus BlueM group (hydrogen peroxide, sodium perborate, honey, xylitol, and lactoferrin (<i>n</i> =25)	ANOVA Mann–Whitney <i>U</i> -test <i>P</i> >0.005	The toothpaste containing oxygen and lactoferrin have comparable efficacy in controlling plaque as triclosan-containing toothpaste
2019 Shenoy and Prabakar ^[36] India	15 days	Toothpaste without plaque indicating dye (<i>n</i> =10) versus toothpaste with a plaque indicating dye (<i>n</i> =10)	Wilcoxon signed rank test Mann–Whitney <i>U</i> -test <i>P</i> =0.002	Regular use of plaque indicating dye-containing toothpaste significantly increases plaque removal efficacy
2020 Ramesh <i>et al.</i> ^[37] India	60 days	Herbal-based commercial toothpaste (<i>n</i> =20) versus homeopathic-based commercial dentifrice (<i>n</i> =20) versus conventional dentifrice (<i>n</i> =20) versus prepared placebo dentifrice (<i>n</i> =20)	ANOVA <i>P</i> =0.001	Herbal dentifrices have equivalent efficiency to conventional dentifrice in plaque reduction
2020 Kaur <i>et al.</i> ^[38] India	45 days	No intervention continued to use the same toothpaste (<i>n</i> =15) versus probiotic toothpaste (<i>n</i> =15)	One-way ANOVA Paired <i>t</i> -test <i>P</i> <0.01	Statistically significant reduction in plaque in both the groups

P<0.05 – Statistically significant. SLS: Sodium lauryl sulfate, PVM/MA: Polyvinyl methyl ether/maleic acid

DISCUSSION

Although the fact that most populace assert to brush their teeth at least once daily, gingivitis and chronic periodontitis are still pervasive in the majority of the population. Maintaining an adequate degree of plaque control with conventional mechanical procedures and dentifrices is manifestly challenging; nonetheless, it is presently the sole realistic means of enhancing community periodontal health from a preventive standpoint.^[28] In an attempt to find answers for clinical enquires, it is necessary to conduct an evidence-based literature search.

The active ingredients in the herbal dentifrices used in the present systematic review are myrrh, sage, chamomile, eucalyptus, essential oils, 4% Tulsi extract, 0.6% *Ilicis Rotundae* cortex, *Pistacia lentiscus*, papain, bromide, miswak, and neem acts by penetrating the biofilm, resulting in disruption of plaque and has antibacterial properties. There is substantiation that herbal dentifrices may be efficacious toward dental plaque, so one would anticipate accord on which dentifrice to choose. The review enclosed eleven randomized control trials encrusting a total populace of 703 qualified for the assessment of mean plaque depletion. The presence of predisposition in 11 enclosed studies, three of which had a low risk of bias, can be attributed to the existence of heterogeneity. Apart from blinding of participants and personnel, which showed a low risk of bias across all studies, all domains demonstrate a high risk of bias. None of the studies have a follow-up period of >3 months. With a high *I*² esteem (*I*² = 87%), the results of various studies can be inconclusive. The estimates favored neither herbal nor

conventional dentifrices because the risk of predisposition was detected to be high and the methodological quality was identified to be low. Ultimately, there is inconsistency in documenting any adverse effects associated through use of exploratory herbal products. These findings are similar to the Mehta *et al.*^[39] (SMD 2.14; 95% CI: 0.88–3.41, *P* = 0.0009), which delineated there is no high-quality evidence supporting or contradict the herbal dentifrice's antiplaque efficacy.

There was moderate quality evidence that toothpastes containing triclosan/copolymer with fluoride reduced plaque in comparison to fluoride toothpastes that did not contain triclosan/copolymer (SMD 1.36, 95% CI (2.81–0.10), *P* = 0.007).

Although the findings of discrete trials in the meta-analyses exhibited significant heterogeneity, the SnF2 dentifrice performed better than other dentifrices (SMD –0.61, 95% CI (1.04–0.18), *P* = 0.005). The findings of the current research are consistent with those of the Johannsen *et al.*^[40] research which found that SnF2-containing dentifrices diminished dental plaque and gingivitis (SMD –0.63, 95% CI (1.11–0.15), *P* = 0.010).

The methods used to gauge the plaque vary widely across the included studies: 26.5% of researches used Silness and Loe plaque index (PI); 55.9% utilized Turesky–Gillmore–Glickman modification of Quigley–Hein PI; 5.9% of studies used MGMPI; 2.9% studies used O'Leary PI; 5.9% used Turesky–Gillmore–Glickman PI and 2.9% used image analysis, whereas a study done by Mitra *et al.* did not specify the type of plaque scoring index.^[8] Significant statistical heterogeneity due to

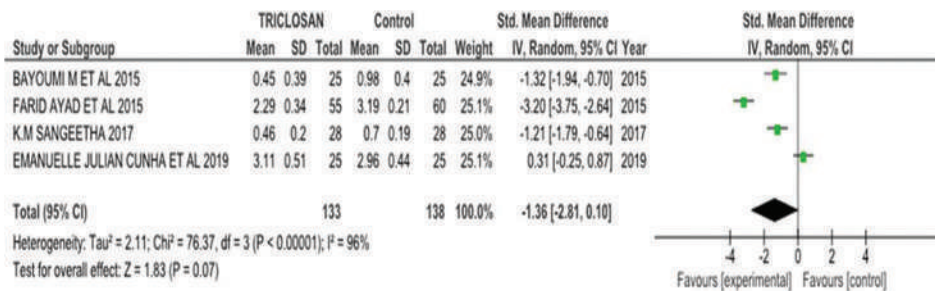


Figure 3: Forest plot of comparison: Triclosan versus control toothpaste

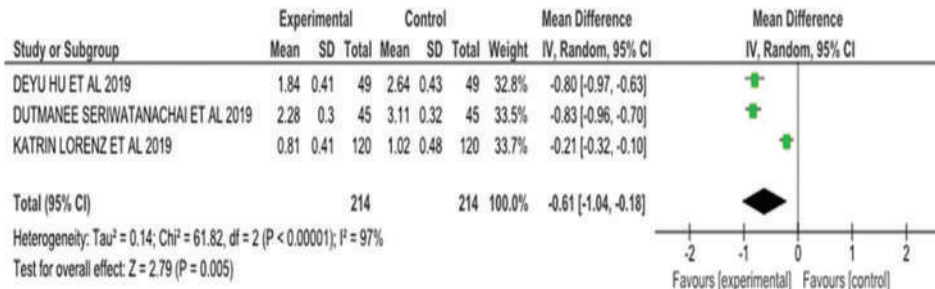


Figure 4: Forest plot of comparison: Stannous fluoride versus control toothpaste

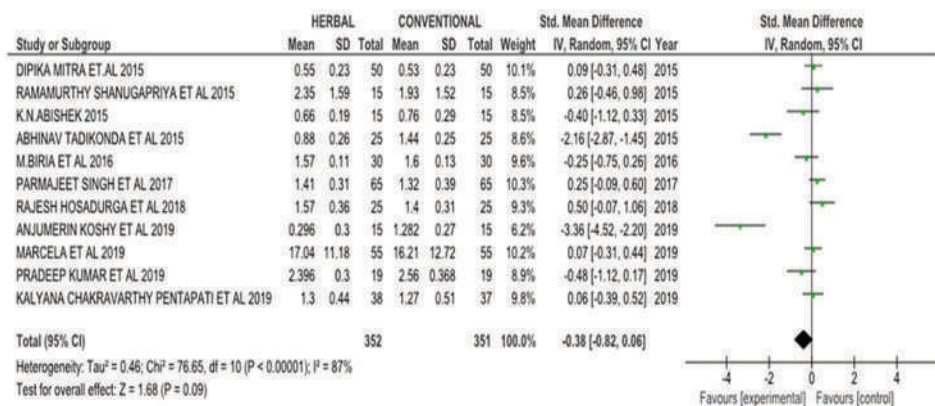


Figure 5: Forest plot of comparison: Herbal versus conventional toothpaste

methodological diversity in outcome assessment tools indicates that the researches are not all reckoning the same quantity, but it does not certainly imply that the actual intervention effect varies.^[41]

The majority of researchers (76.4%) did not use proper sample size calculations when enlisting the research populace. The findings of the meta-analysis may be influenced by the limited sample size. Apart from the toothpaste interference, the provisions were distinctive since various sorts of manual toothbrushes have been used, which may have contributed in an additional disparity in all of the research findings. Different concoctions of the test and control group have varying efficacy levels and would have affected intervention outcomes. Furthermore, a thorough review of the data revealed variations within the consistency of methodological

approaches, populace, and rate of attrition. About 17.8% of the study population was lost in follow-up, which could have influenced the study results.

Limitations of this review

The considerable variance in the quantity of researches collating each active agent and the various risks of bias necessitates caution when analyzing the results. A severe imbalance in the amount of evidence for each intervention may affect the overall analysis's power and reliability.

The implication for future research

Future RCTs evaluating auxiliary chemotherapeutics must include at least a 6-month follow-up period to establish the efficacy and safety of patients' chemical agents and compliance and compensate for the Hawthorne effect, with outcome measures assessed at baseline, 6 months, and an intermediate

time point. It is foremost to maintain a proper interpretation of the baseline clinical idiosyncrasy and periodontal diagnosis, as well as the pertinent outcome measures reported.

CONCLUSION

Within the confines of the current study's limitations (which embody the severe imbalance within the quantity of evidence and data confinement to time restraint), once scrutiny among products, triclosan and stannous fluoride in dentifrices seem to be the foremost efficacious plaque controlling active agents. The literature supporting the use of herbal-based antiplaque preparations is generally weak, since most observations suffered from a lack of experimental design and a dearth of pertinent controls and comparisons. It is critical to understand that, in the absence of a negative monitor, head-to-head distinctions with existing products with known competence are not an adequate experimental design.

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Conflicts of interest

There are no conflicts of interest.

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Streptococcus Mutans-Specific Antimicrobial Peptide C16G2-Mediated Caries Prevention: A Review

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ABSTRACT

Oral biofilms are a group of healthy synergistic organisms, that on interplay with the immune system undergo transition and colonize the pathogenic bacteria, leading to various diseases like dental caries, gingivitis, periodontitis and a few systemic conditions. Dental caries being the most common disease of the oral cavity, comprise a heterogeneous group of bacteria that can cause imbalance in the biofilm. Caries prevention has been in research for decades, where antibiotics, chemical biocides and fluoride-antimicrobial approaches have not been adequate for this multifactorial disease. In recent years, the major focus of caries prevention has been shifted to plaque-biofilm modification as an ecological approach that would prevent bacterial colonization. Saliva produces various natural antimicrobial peptides that can regulate biofilm modification. Synthetic production of antimicrobial peptides concentrates on selective elimination and a targeted approach towards cariogenic pathogens, precisely *Streptococcus mutans* (*S. mutans*). A search in Medline/PubMed, EBSCO and ScienceDirect databases on C16G2, antimicrobial peptides (AMPs) and *S. mutans*, using MeSH (Medical Subject Heading) terms was performed and papers published until 2020 were included for further evaluation. A total of eight articles written in English with available full texts were selected based on the search strategy. They included four publications on AMPs against *S. mutans* and another four articles on AMPs in caries prevention. This review focuses on C16G2 antimicrobial peptide and its potential to modify biofilm and inhibit the targeted bacteria causing dental caries.

Key words: Antimicrobial Peptides; *Streptococcus Mutans*; Mouthwashes; Dental Caries; Anti-Bacterial Agents

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INTRODUCTION

Antibiotics, the epitome of “wonder drugs”, have a major role in the control and prevention of microbial infections, predominantly bacterial pathogens [1]. However, drug resistance, overuse and misuse are growing concerns in the

management of microbial diseases pushing the world on to the cusp of a post-antibiotic era, where formerly efficient treatment procedures have become inapplicable.

Antibiotics, despite having a significant role in modern medicine, have mostly failed to control

and/or prevent many infections due to drug resistance, accentuating the need for developing newer antimicrobial medication management [2]. Recently, antimicrobial peptides (AMPs) have been recognized as potential antibiotic substitutes because of their broad-spectrum killing activity, including the drug-resistant strains [3]. AMPs are genetically common molecules of innate immunity observed mainly within neutrophil granules and in epithelial cell secretions of skin and mucosal surfaces of mammals [4,5]. AMPs are small, amphipathic molecules that possess both hydrophilic and hydrophobic areas with variable amino-acid structure/length and an overall cationic charge [6].

Based on their secondary structures, they can be categorized into four different types: beta-sheet, alpha-helix, extended and loop [7]. These are versatile, highly specific antimicrobial compounds that have a broad range of antibacterial, antiviral and antifungal activity [8]. A healthy cluster of synergistic microbial organisms amalgamate to form an oral biofilm. Microbes in the oral biofilm interact with the host immune system and can colonize, which may lead to dental caries, gingivitis, periodontitis or spread to distant organs causing systemic conditions [9,10]. Dental caries, the most prevalent disease of dental structures, are considered to be a multifactorial disease with predominant pathogenic events lead by *Streptococcus mutans* (*S. mutans*) [11]. Cariogenic bacterial pathogens rapidly metabolize carbohydrates and subsequently produce organic acids and reduce plaque pH, which leads to demineralization of inorganic components of dental hard tissues and irreversible loss of tooth structure [12,13]. This substantiates the concept of endogenous, biofilm mediated disease where acidogenic/aciduric elements inhabiting the oral flora gain an ecological advantage over other species and interrupt the homeostatic balance in the plaque biofilm, ultimately leading to commencement of the disease process [12].

DISCUSSION

Antimicrobial Peptides

Research studies on prevention of caries are

ongoing for decades with multiple modalities such as various types of sodium fluoride and dental sealants. The motive of current research studies shifted to modification of oral ecology-plaque biofilm with a specific aim to prevent bacterial colonization. AMPs are an adverse group of molecules with exceptional antimicrobial features and a great capacity for controlling bacterial infections and altering biofilm environment [8]. AMPs demonstrate a net positive charge and a high ratio of hydrophobic amino acids allowing them to selectively bind to negatively charged bacterial membranes. The ability of AMPs to kill bacteria usually depends on their capability to interact with the bacterial membrane or cell wall [11]. They display a direct and rapid antimicrobial property that can be categorized into two basic groups: The disruptive mechanisms, which cause disruption of physiological integrity of microbial membrane and the membrane undisruptive mechanisms, which act on targets within the cell [5,13].

Independent of the proposed group, the initial interaction between the cationic AMPs and the negatively charged bacterial surface is electrostatic [14].

In most AMPs, the interaction between cationic residues of peptides and the negatively charged moieties of a bacterial membrane can form pores that obliterate the membrane integrity, facilitating targeted microbe lysis [11].

There are four classical models of disruptive mechanisms: (1) Toroidal; (2) Carpet; (3) Aggregate; and (4) Barrel. Lately, novel disruptive models or models indirectly related to membrane disruption have been defined. These models are as follows: (1) Disordered toroidal; (2) Membrane thinning/thickening; (3) Charged lipid clustering; (4) Non-bilayer intermediate; (5) Oxidized lipid targeting; (6) Anion carrier; (7) Non-lytic membrane depolarization; and (8) the Electroporation model [14].

In the case of undisruptive mechanism, they traverse the lipid bilayer without any destruction but destroy bacteria through prevention of intracellular functions [11]. The undisruptive mechanisms rely on AMP passage through the membrane as a result of a combination of characteristics including AMP sequence and composition of the membrane. A number of

cellular metabolism reactions are prevented, which ultimately leads to cell death. Two mechanisms of AMP cell entry have been described including a spontaneous membrane translocation or crossing as a result of a secondary AMP structure that drives permeabilization of the membrane [13,14]. After crossing the membrane, multiple intracellular sites like gene promoters and coding sequences, mRNA-binding sites, enzyme regulatory sites, and protein pre-folding sites can be targeted by AMPs. Inhibition occurs through impeding DNA transcription and/or RNA translation, or causing breakdown of metabolic pathways and cell death by poor protein folding [15].

The main distinctions between microbial and mammalian cells such as membrane composition, polarization, transmembrane potential and structural features protect mammalian cells against AMPs [6].

In contrast to the bacterial membrane, the cytoplasmic membrane of the mammalian cell is loaded with zwitterionic phospholipids, producing a membrane with neutral net charge [16,17]. Thus, hydrophobic interactions are the major promoter of mammalian cell membrane

and AMP interplay. In comparison, the electrostatic interactions between AMPs and bacterial membranes are stronger [5]. Furthermore, mammalian cell membranes, differ from microbial membranes in that they possess a large amount of cholesterol [16-18] and are suggested to decrease AMP activity by stabilizing the phospholipid bilayer [19]. Mammalian cells have a lower negative transmembrane potential between (-90 to -110 mV) in contrast to bacterial cells (-130 and -150 mV) [16,17,20]. Strong negative membrane potential in bacteria may likewise assist in selectivity of AMPs between bacterial and mammalian cells [16]. Apart from limited number of pathogens, most indigenous oral microorganisms are beneficial. Broad spectrum killing activity exhibited by the currently available AMPs disrupts the ecological balance of the indigenous microbiota leading to unidentified clinical implications [21,22]. Therefore, establishment of new narrow-spectrum treatments that can preserve the protective advantages of the normal microflora during therapy is essential [23]. Promising studies on AMPs against *S. mutans* are listed in Table 1.

Table 1: Antimicrobial peptide (AMP) studies against *Streptococcus mutans* (*S. mutans*)

Study outline and conclusion	Author(s)/year
Potent 1 antibiotic component Mutacin B-Ny266 is efficient against <i>S. mutans</i> identified in the oral cavity. Antibiotics targeted against microbes in dental plaque is a promising strategy for controlling pathogenic oral microbial flora.	Dufour et al. 2020 [24]
Inhibitory action against adhesion, virulence associated genes and enzymes of <i>S. mutans</i> was observed in pomegranate-derived AMPs; Pug-1, Pug-2, Pug-3 and Pug-4. Antimicrobial pathway of Pomegranate against carious pathogens occurs due to anti-adherence properties. Pomegranate AMPs are non-toxic to human keratinocytes.	Kokilakanit et al. 2020 [25]
Biochemical contents produced from <i>Hylarana guentheri</i> such as Temporin-GHc and temporin-GHd renders antimicrobial action against bacteria and fungi. The antimicrobial action is achieved by downregulating glucosyltransferases enzyme in <i>S. mutans</i> . Selective and targeted antimicrobial action against <i>S. mutans</i> occurs in the presence of human erythrocyte.	Zhong et al. 2019 [26]
STAMP molecules are Specifically Targeted AMP synthetic molecules prepared to render antimicrobial property with a specific target against a pathogen. C16G2 is a STAMP molecule prepared by the combination of the killing domain from the antimicrobial agent Novispirin G10 with a targeting domain from <i>S. mutans</i> pheromone. C16G2 is prepared to selectively act against <i>S. mutans</i> to render anti-carious action in oral cavity without disturbing non-pathogenic resident bacteria in oral flora.	Baker et al. 2019 [27]

Recently, an initiation of a targeted approach to manage oral microbial pathogenesis using a newer version of AMPs called specifically targeted antimicrobial peptides (STAMPs) was developed [22,28].

A typical STAMP molecule requires two functionally independent peptide domains, a killing moiety comprised of a non-specific AMP that can rapidly destroy bacteria, and a targeting moiety comprised of a species specific, high affinity binding peptide [28,29].

The two moieties are subsequently integrated through a small linker, producing a fusion AMP without detrimental changes in the independent functions of two domains [13]. The AMP dimers (fusion peptides) compounded as single linear molecules, frequently possess higher killing dynamics in comparison to their parental peptides [4]. Randomized clinical trial studies against cariogenic microbes and caries management are shown in Table 2.

Table 2: Randomized clinical trials on antimicrobial peptides and caries management/prevention

No.	Study hypothesis	Study findings, inference, & clinical relevance	Author(s)/year
1	Gaseous ozone effect on deep carious pathogens identified from incompletely excavated carious lesions was evaluated. In addition, Vascular endothelial growth factor (VEGF) from pulpal tissue, neuronal nitric oxide synthase and superoxide dismutase (SOD) was also investigated in this study	Gaseous ozone reduced microbial count of bacteria that includes lactobacillus. The levels of VEGF were higher in pulp tissue and SOD activity was lower in the study group than controls. The findings of the study confirmed that gaseous ozone has a biocompatible effect on pulpal tissue by rendering antimicrobial action	Krunić et al. 2019 [30]
2	Salivary human neutrophil peptide 1-3 (HNP 1-3) levels were compared in the study group with probiotic supplements and in the control group. The results of the study showed higher levels of salivary HNP 1-3 among caries resistant children than caries susceptible	<i>Lactobacillus paracasei</i> probiotics enhance HNP 1-3 temporarily with significant statistical correlation elevated <i>Lactobacillus</i> spp. counts. The study also showed reduced <i>S. mutans</i> but did not show statistical correlation	Wattanarat et al. 2015 [31]
3	Importance of salivary proteins adsorbed on enamel surface and its demineralization effect was evaluated	Surfaces of enamel coated with whole saliva, parotid saliva, dialyzed whole and dialyzed protein saliva showed significantly higher levels of protection than uncoated enamel. The parotid and whole saliva rendered better protective action than dialyzed saliva. The findings of the study denoted that ionic contents of saliva provide protection against acid related enamel demineralization	Martins et al. 2013 [32]
4	<i>S. mutans</i> adhesion to cellular surface inhibition was studied by plasmon resonance method using synthetic peptide (p1025) to residues of 1025-1044 adhesions	In-vivo study model on streptococcal adhesion investigation revealed prevention of recolonization of <i>S. mutans</i> but not <i>Actinomyces</i> spp. The results of the study confirmed that peptide molecules inhibited microbial adhesins and prevented colonization	Kelly et al. 1999 [33]

Specifically Targeted Antimicrobial Peptides Targeting *S. mutans*

As discussed earlier, *S. mutans* is considered the primary etiological agent of dental caries. It is well known that, absence of *S. mutans* has been associated with areas containing healthy dentition, while progressively increasing levels of *S. mutans* are found in regions of caries development [34]. A study had mentioned that patients with dental plaque consisting of small amounts of *S. mutans* are resistant to exogenous colonization from cariogenic organism which have shown reduction on dental caries [35]. Thus STAMPs aimed at *S. mutans* can serve as an alternative therapy in preventing dental caries. Development of resistance to AMPs may occur due to contributions from both bacterial cell changes and host tissue alterations which eventually lead to inability of the host to act against bacterial infections. The resistance of the oral bacteria in prolonged AMPs may occur due to changes occurring in the organism such as surface remodelling, biofilm pathogen alterations and host tissue changes like efflux pump mechanism of organisms and proteolytic degradation. Understanding these mechanisms will assist researchers in developing new therapeutic strategies while formulating AMP mouth rinses [1]. Thus, the number of STAMPs targeting *S. mutans* were researched for selecting inhibition of pioneer caries bacteria [36]. C16G2 is a STAMP designed with antimicrobial specificity for *S. mutans* [37]. C16G2 utilizes *S. mutans* produced pheromone (competence stimulating peptide) which acts as the STAMP targeting domain against the cell surface of *S. mutans* [28]. The term C16G2, denotes the 16 amino-acid (C16) sequence of TFFRLFNRSFQTALGK molecule [38]. Whereas, the killing domain is designated as G2, which is derived from a broad spectrum antimicrobial peptide. These 2 peptide sequences are conjoined by a sequence of 3 glycine residues [37]. As per sequence analysis, C16G2 is an amphipathic and cationic alpha-helical peptide which is comparable with the conventional AMPs [39].

The hydrophobic property of C16G2 is substantially greater than that of individual moieties because of the stacking of hydrophobic residues [40]. CSPC16-*S. mutans* is a species-specific AMP but is separate from the ComD surface receptor [28]. A natural *S. mutans*-specific targeting sequence in this pheromone may become attached to a different receptor (lipids, exopolysaccharides, teichoic acid) on the bacterial surface before interaction with ComD. According to recent studies, C16G2 exerts bactericidal action by a mechanism involving interference with the cytoplasmic membrane. C16G2 build-up on *S. mutans* cell surface results in loss of membrane potential followed by efflux of intracellular contents and disruption of membrane integrity finally causing cell death. The mutual amphipathic characteristic of C16G2 and AMPs gives rise to the STAMP acting as a membrane damaging peptide but with higher target specificity [40]. It has been proposed that C16G2 specifically eradicates *S. mutans* from multispecies biofilms without impacting closely associated non-cariogenic oral streptococci in saliva-derived and planktonic biofilm systems [28,41].

Studies on C16G2 and oral biofilm:

An in-vitro study on human saliva-derived polymicrobial biofilms treatment with C16G2 showed reduction of *S. mutans* counts and revealed a benign oral microbial community with enhanced health-related bacteria populations and less detrimental gram-negative bacteria [42]. C16G2 is considered to be fast-acting against bacteria in under one minute of exposure. The advantage of rapid action in a shorter duration is adequate to us as oral hygiene rinses. C16G2 is soluble in aqueous solutions indicating that STAMP is easily amenable for use in the oral cavity through a mouth rinse medium [28,36,40,43]. Another in-vivo study evaluated efficacy of mouth rinse with C16G2 formulation showed reduction of both plaque and salivary *S. mutans* counts. The study findings also showed prevention of *S. mutans* regrowth despite frequent exposure to sugar.

This is because of reduction in lactic acid

production resulting in the elevation of pH which favors growth of healthy bacteria, thus rendering an unsuitable environment for growth of cariogenic bacteria. Thus, it might be feasible to develop a healthy non-cariogenic microbial ecosystem in the mouth through C16G2-mediated STAMP intervention in clinical practice [41]. An unimpaired dental biofilm without *S. mutans* resists later growth of exogenous *S. mutans* due to reduced sucrose intake of other microbes in the biofilm which delay cariogenesis. Thus the oral microbial community maintained by C16G2 treatment exhibit a healthy microbial population with non-cariogenic species such as *S. mitis*, *S. sanguinis* as well as reduced periodontitis associated gram-negative species *Fusobacteria* [42]. C16G2 was recognized by the US food and drug administration as an investigational drug for prevention of dental caries and has efficiently concluded phase 2 clinical trials [8].

CONCLUSION

C16G2 is an effective STAMP against dental caries that can be prepared in an aqueous formulation for use as an oral hygiene rinse. Its selective activity against *S. mutans* is helpful in caries preventive management. Development of C16G2 is a very significant advancement in oral biofilm management and caries prevention. Randomized clinical trials are required to evaluate the clinical utility of C16G2 and its effectiveness as a routine self-care practice.

CONFLICT OF INTEREST STATEMENT

None declared.

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Scientific Research Report

The Influence of Dissonance Induction and Assessment Reactivity in Improving Adherence to COVID-19 Precautionary Measures: A Cluster Randomised Controlled Trial

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ABSTRACT

Background: During the coronavirus disease 2019 (COVID-19) pandemic, adherence to suggested precautionary measures has been emphasized as important in preventing and curtailing its spread. However, strict adherence to precautionary measures can be demanding.

Methods: This cluster randomised controlled trial done among 1517 undergraduate dental students tested the effectiveness of 'dissonance induction' (DI) and 'assessment reactivity' (AR) in improving adherence to World Health Organization (WHO) measures as compared to a control group. At baseline, participants in the DI group were tested for their knowledge of precautionary measures, immediately followed by assessment of their adherence to them. This methodology was adapted to systematically reveal the poor adherence of the participants in their self-held cognitions, should there be any. The magnitude of dissonance was measured as the proportion of such dissonant cognitions held by an individual. In the AR group, at baseline, participants were asked about their attitudes alone toward measures. The control group was neither assessed for knowledge and adherence nor for attitudes toward the measures at baseline. Two weeks after the administration of these interventions in the DI and AR groups, the 3 study groups were assessed for adherence.

Results: The follow-up adherence scores in the DI group were found to be significantly higher (15.11 ± 4.1) compared to the AR (13.13 ± 2.01) and control (12.87 ± 2.97) groups as analysed by Kruskal-Wallis analysis of variance ($H = 243.5$; $P < .001$). Wilcoxon signed-rank test showed that the adherence scores significantly improved in the DI group from baseline to follow-up ($z = -8.84$; $P < .001$). Magnitude of dissonance at baseline was found to be a significant predictor of follow-up adherence scores ($R^2 = 0.255$).

Conclusion: This study found that DI is an easy intervention to bring an immediate and significant change in adherence to precautionary measures.

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Introduction

The fact that coronavirus disease 2019 (COVID-19) was declared a public health emergency of international concern reflects the magnitude of the global crisis caused by this viral pandemic.¹ Since severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, is a

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novel virus with recent emergence among humans, humans are completely immune-naïve and consequently vulnerable to infection.² International experiences suggest that the transmission of the disease can be rapid in rather short periods of time.^{3,4} Though COVID-19 is associated with mortality, the case fatality rate was reported to be lesser for COVID-19 (2%) compared with severe acute respiratory syndrome (SARS) (10%) and Middle East Respiratory Syndrome (MERS; 34%). However, it was reported that COVID-19 has been responsible for more deaths than SARS and MERS combined. These findings reflect the more contagious, though less severe, nature of COVID-19 compared with SARS and MERS.⁵ It is for this reason that the greatest challenge ahead for health care systems is to curtail the rapidity and magnitude of transmission. As on 24 December 2020, official reports from the Ministry of Health and Family Welfare, Government of India suggest that 10.1 million individuals have been confirmed positive among suspected cases and contacts of known cases.⁶ These numbers illustrate the nature of transmission of the disease. Responsibility has been placed on the citizens of the nation to prevent themselves from getting affected. The World Health Organization (WHO) suggested precautionary measures of frequent hand washing, social distancing, avoiding touching face, nose, or mouth, practising respiratory hygiene, maintaining 1-meter distance from those coughing and sneezing, and refraining from smoking and activities that weaken the lungs. These measures have been widely circulated in various media platforms for people to assimilate and adopt.⁷ However, literature suggests that though persuasive health messages are often successful in bringing attitudinal changes among people, these changes are short-lived. More importantly, it has been strongly established by social psychologists that positive attitudes do not necessarily translate to positive behaviours. Therefore, more thorough and scientifically informed behavioural interventions are warranted to improve adherence to precautionary measures and combat the outbreak of this and other infectious diseases.

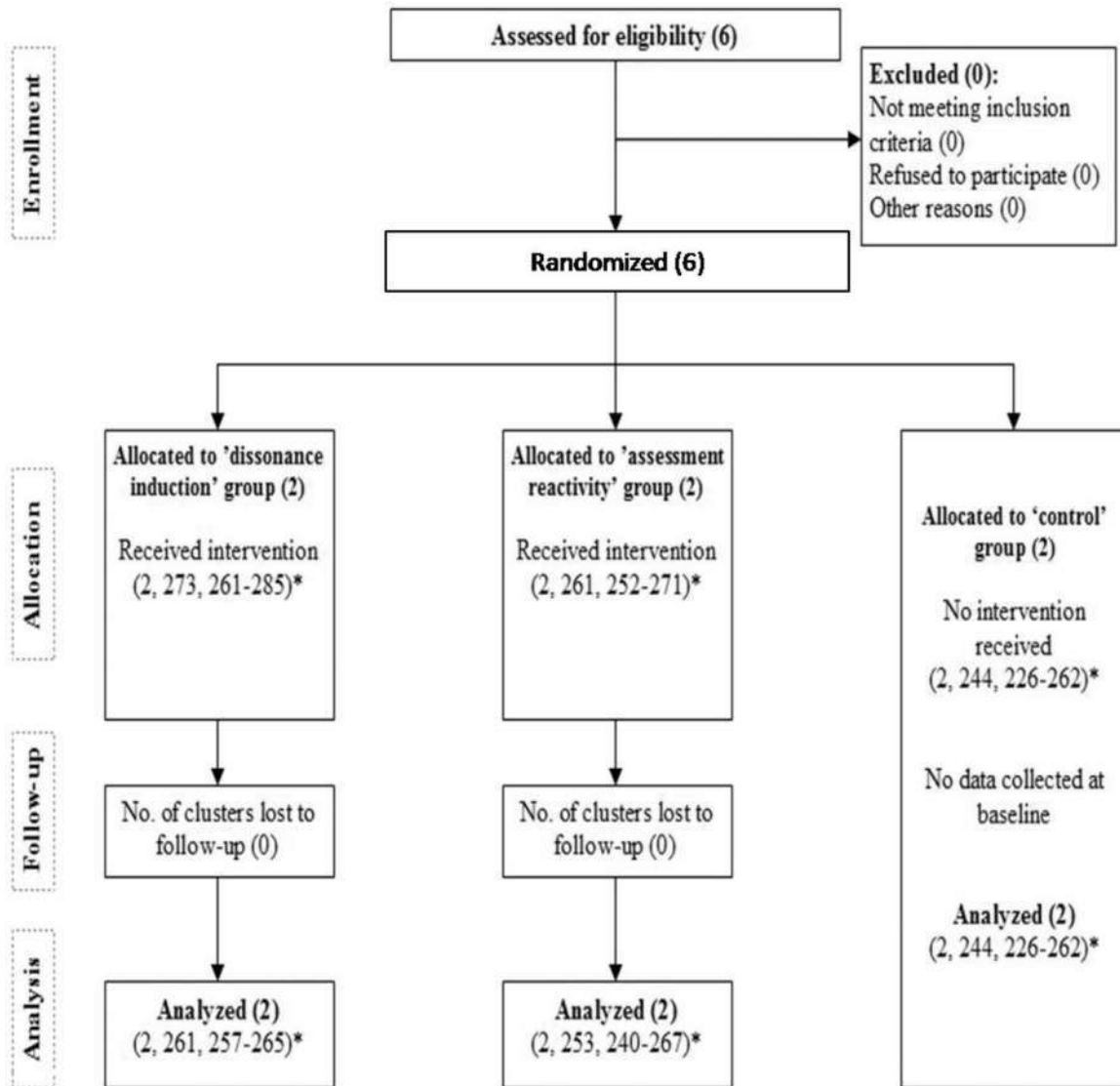
The benefits of answering questions, termed as 'question benefit effect' (QBE), about a behaviour in producing positive changes, though minor in magnitude, in that behaviour have been previously studied.⁸ Various theories have been proposed to explain the behaviour change following responding to questions on that behaviour. Sherman⁹ postulated that mental stimulation takes place while responding to questions about a behaviour that results in formation of cognitive representations or behavioural scripts. These cognitive representations get reactivated while the subject performs that behaviour and assists a positive behavioural change. Another explanation is the theory of attitude accessibility that proposes that the questions about behavioural intentions of individuals activate the attitudes intrinsic for that behaviour, making them more accessible in memory.¹⁰ This theory was tested with regard to healthy eating behaviours.¹¹ Cognitive dissonance is another explanation for QBE, which attempts to attribute the benefit to the cognitive conflict felt by reporting behaviours inconsistent with their beliefs. Cognitive dissonance, as proposed by Leon Festinger,¹² is an unpleasant drive state similar to hunger or thirst experienced when 2 psychologically inconsistent cognitions are simultaneously held by a person that is against the inner drive to maintain

harmony between one's attitudes/beliefs and behaviour. Aronson¹³ proposed that dissonance theory makes its clearest predictions when the people's self-concepts are violated by their own actions. Aronson also argued that passing on information to individuals in a persuasive manner would only result in attitudinal changes that are temporary. Dissonance-induced persuasion is more effective in bringing a behavioural change because individuals' inconsistent behaviour with their own self-concept creates a necessity to attain consistency between their self-concepts and behaviour. Assessment reactivity, on the other hand, is the influence of behavioural assessment at the present time on later behaviour. It was proposed that mere questioning about a behaviour influences an individual to change the behaviour in question.¹⁴ In the present study, the group where inconsistency of the subjects' behaviour with their own concepts is explicitly made evident to them by testing their knowledge and behaviour at baseline is identified henceforth as the 'Dissonance Induction' (DI) group. The group where mere questioning of attitudes was done at baseline is identified as the 'Assessment Reactivity' (AR) group. There is a control group which was neither tested for knowledge, behaviour nor for the attitudes at baseline. Our hypothesis (H1) was inducing dissonance among subjects by systematically making evident to them the poor adherence to their own cognitions results in better follow-up adherence to the precautionary measures. If the null hypothesis (H0) were to be true, there would be no difference in the follow-up adherence scores between the 3 study groups.

Methods

This cluster randomised controlled trial was conducted during the months of February and March 2020 following ethical approval from the Institutional Ethical Committee of SIBAR Institute of Dental Sciences (Pr.69/IEC/SIBAR/2020). The allocation ratio was 1:1:1 in the DI, AR, and control groups. Six teaching dental institutions (clusters) of the 16 functioning dental institutions in the southern Indian state of Andhra Pradesh were selected and 2 each were allocated to DI, AR, and the control groups after randomisation. The study details are depicted in Figure 1. All the study participants were provided with the necessary information on the purpose and process of the study without revealing the specific focus and objectives of the study. Consent was obtained prior to the subjects' participation in the study, and participation in the study was voluntary. Care was taken to ensure anonymity in the questionnaire, leaving no place for coercion. Since all the participants within a cluster received either the same intervention or no intervention, contamination between the study groups was not possible. Furthermore, study participants from each individual dental institution were neither aware of the alternative interventions tested in the study nor did they know about the parallel conduct of the study among students from other dental institutions.

DI at baseline: In the DI group, dissonance was induced at baseline by administering a self-administered, structured questionnaire that revealed the existing cognitive conflict, should there be any. This questionnaire consisted of 2



*(number of clusters, average cluster size, range of cluster size)

Fig. 1 – CONSORT flow diagram of the cluster randomized trial.

CONSORT = Consolidated Standard of Reporting Trials.

*Number of clusters, average cluster size, and range of cluster size.

sections: knowledge about COVID-19 precautionary measures and adherence to precautionary measures. To assess knowledge on COVID-19 precautionary measures, a combination of 6 WHO suggested precautionary measures and 6 distractor options was given for the participants to choose from. The adherence was assessed immediately after the participants had responded to the knowledge questions. This questionnaire served 2 purposes: making the subjects explicitly mention their self-concepts in the 'knowledge' section and making it evident for the subjects how consistent/inconsistent their actions were with their own cognition in the

'adherence section'. Dissonance was considered to be induced if a subject chose a WHO suggested precautionary measure in the knowledge section and reported his or her current adherence to that precautionary measure to be 'occasional' or 'never' as the poor adherence of the subjects with their self-held cognitions was systematically revealed to them. On the other hand, consonant cognitions refer to WHO suggested precautionary measures chosen by the subjects in the knowledge section for which they report their adherence to be 'often' or 'almost always'. The knowledge score (ks) of a subject 'i' was calculated using the formula $ks(i) = N - Z$, as

suggested by Kurz, where N is the number of correct responses chosen by that subject and Z refers to the number of incorrect responses.¹⁵ This ensures that a subject who chooses all the responses randomly will get a 'zero' knowledge score. The baseline adherence to WHO suggested COVID-19 precautionary measures was assessed on a 4-point Likert scale (0-3) with higher score indicative of better adherence. The magnitude of dissonance (M_D) was calculated as the number of dissonant cognitions divided by the sum of number of consonant and dissonant cognitions ($M_D = N_{dc} / (N_{cc} + N_{dc})$; where N_{cc} refers to number of consonant cognitions and N_{dc} refers to number of dissonant cognitions).

Baseline attitudinal measurement in the AR group: In the AR group, a self-administered questionnaire inquiring the attitudes of the subjects toward WHO suggested COVID-19 precautionary measures was administered. In contrast with the DI group, these participants were neither assessed on their knowledge of the precautionary measures nor were they asked about their adherence to precautionary measures at baseline. The attitudes were documented on a 4-point Likert scale (0-3) with higher scores indicative of more positive attitudes.

The control group was not assessed for either the knowledge and practice or the attitudes toward precautionary measures at baseline.

Assessment of adherence in the study groups at follow-up: During the follow-up, 2 weeks after the administration of corresponding questionnaires in the DI and AR groups, self-reported adherence to the WHO suggested COVID-19 precautionary measures was documented in all the 3 study groups on a 4-point Likert scale (0-3) with '0' indicating nonadherence and '3' indicating perfect adherence. Adherence score of a subject at the follow-up was calculated as the sum of scores obtained for the responses to the 6 precautions of interest. Therefore, the follow-up adherence scores range from 0 to 18, with '0' indicating nonadherence to all the 6 precautionary measures and '18' indicating perfect adherence to all the precautionary measures. A total of 60 students were lost to follow-up across both the intervention groups and were excluded from the analysis. The final sample included 1517 subjects with 522 in the DI group, 507 in the AR group, and 488 in the control group.

Statistical Analysis: SPSS version 2.0 software was used for descriptive statistics and data analysis. The Wilcoxon signed rank test identified the change in adherence scores from baseline to follow-up in the DI group, Spearman's correlation test evaluated the correlation between attitudinal scores at baseline and follow-up adherence scores in the AR group, Kruskal-Wallis analysis of variance tested the differences in follow-up adherence scores between the study groups, and simple linear regression analysis was used for the DI group with follow-up adherence score as the dependent variable and magnitude of dissonance as the explanatory variable.

Results

The response rate was 68.4% in the DI group, 66% in the AR group, and 63.2% in the control group. There was no significant difference in the gender distribution between the study

Table 1 – Distribution of responses to the knowledge test on COVID-19 precautionary measures in the Dissonance Induction group at baseline (n = 522).

Precautionary measure	Response	n (%)
Use prophylactic antibiotics	Yes	47 (9)
	No	475 (91)
Eat plenty of garlic	Yes	112 (21.45)
	No	410 (78.55)
Avoid touching your face	Yes	425 (81.41)
	No	97 (18.59)
Refrain from smoking and other activities that weaken the lungs	Yes	259 (49.61)
	No	263 (50.39)
Use hand dryers after washing your hands	Yes	97 (18.58)
	No	425 (81.42)
Cover your mouth and nose when coughing or sneezing	Yes	499 (95.6)
	No	23 (4.4)
Maintain at least 1 meter distance between you and people sneezing or coughing	Yes	495 (94.82)
	No	27 (5.18)
Take regular hot water baths	Yes	73 (13.98)
	No	449 (86.02)
Practice social distancing and avoid unnecessary travel	Yes	485 (92.91)
	No	37 (7.09)
Stay in hot and humid climates	Yes	51 (9.77)
	No	471 (90.23)
Stay away from mosquitoes as they can transmit SARS-CoV-2	Yes	45 (8.62)
	No	477 (91.38)
Wash your hands frequently with alcohol-based hand rub	Yes	510 (97.7)
	No	12 (2.3)

COVID-19 = coronavirus disease 2019; SARS-COV-2 = severe acute respiratory syndrome-coronavirus 2.

groups with female students representing 80.07% (418) of the DI group, 77.51% (393) of the AR group, and 78.07% (381) of the control group.

Baseline observations in the DI group: Table 1 shows the participants' responses to knowledge questions in the DI group at baseline. The mean knowledge score of COVID-19 precautionary measures among the participants from the DI group was 3.23 ± 2.15 (95% CI 3.04-3.41), after adjusting for the incorrect responses. The mean score for magnitude of dissonance was observed to be 0.26 ± 0.18 , which suggests that the proportion of dissonant cognitions on an average was slightly more than 25%. The mean adherence to precautionary measures score in the DI group at baseline was 13.02 ± 3.74 (95% CI 12.69-13.34).

Baseline observations in the AR group: The mean attitudinal score toward COVID-19 precautionary measures in the AR group at baseline was 14.02 ± 3.6 , where a score of 18 was indicative that the subject is most likely to practice all 6 precautionary measures suggested.

Follow-up observations in the study groups: An apparent difference was noted among the 3 study groups with regard to their mean follow-up adherence scores in practicing the COVID-19 precautionary measures, with the DI group demonstrating higher scores compared to AR and control groups (Figure 2). Significantly higher adherence scores in DI group were suggestive of the more thorough adherence to precautionary measures in the DI group compared to AR and control groups at follow-up. No significant differences were noted between the AR and control groups in post hoc tests for

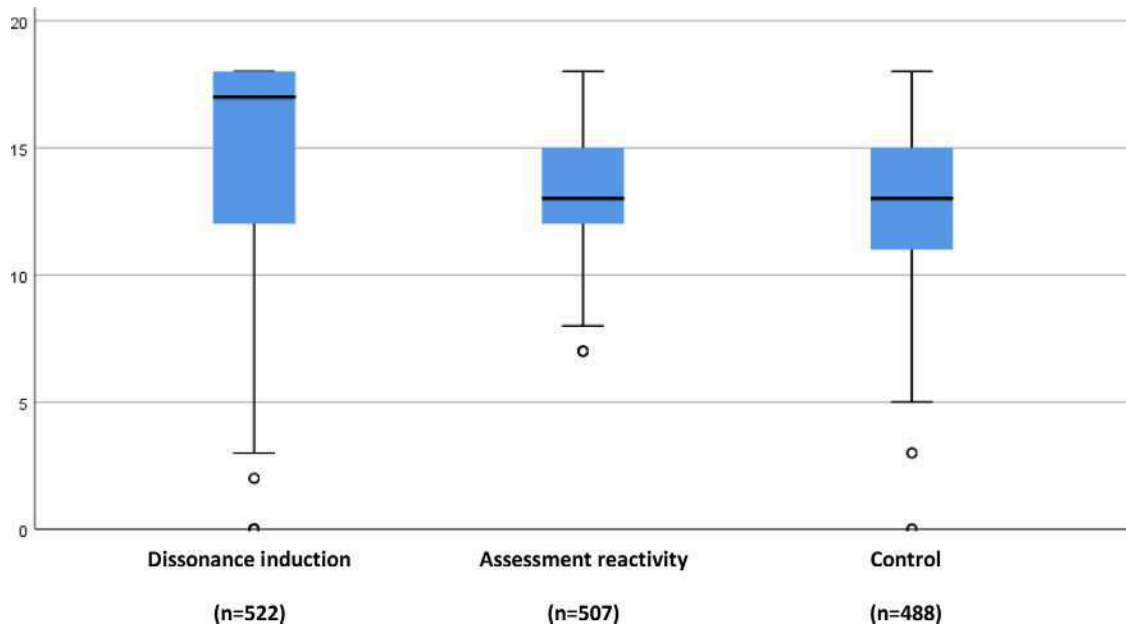


Fig. 2 – Box plot showing differences in the follow-up adherence scores between the study groups.

Table 2 – Differences in follow-up adherence scores to COVID-19 precautionary measures between the study groups.

Group	N	mean ± SD	95% CI	Mean rank	H (df, N)	P value	P value (post hoc tests)		
							DI - AR	DI - C	AR - C
DI	522	15.11 ± 4.1	14.75-15.47	999.4	243.5 (2, 1517)	.001*	.001*	.001*	.27
AR	507	13.13 ± 2.01	12.96-13.31	639.97					
C	488	12.87 ± 2.97	12.6-13.14	625.51					

AR = assessment reactivity; C= control; CI = confidence interval; COVID-19 = coronavirus disease 2019; df = degrees of freedom; DI = dissonance induction; SD = standard deviation.

Kruskal-Wallis ANOVA; P ≤ 005 considered statistically significant.

* Denotes statistical significance.

multiple pair wise comparisons, while statistically significant differences were noted for DI group compared with both the AR and control groups (Table 2).

Observations in DI group from baseline to follow-up: The adherence scores in the DI group significantly improved from baseline to follow-up (Wilcoxon Z statistic = -8.84; P < .001) (Table 3). It was noted in this group that the follow-up adherence scores exhibited a positive linear relation with the

Table 3 – Differences in adherence scores to COVID-19 precautionary measures in the DI group from baseline to follow-up.

Time	mean ± SD	95% CI	z-statistic	P value
Baseline	13.02 ± 3.74	12.69-13.34	-8.84	.001*
Follow-up	15.11 ± 4.1	14.75-15.47		

CI = confidence interval; COVID-19 = coronavirus disease 2019; DI = dissonance induction; SD = standard deviation.

Wilcoxon signed rank test; P ≤ .05 considered statistically significant.

* Denotes statistical significance.

magnitude of dissonance at baseline (Spearman’s ρ = 0.505; P < .001). Magnitude of dissonance was found to be a significant predictor of the follow-up adherence scores in linear regression analysis (Table 4).

Observations in the AR group from baseline to follow-up: In the AR group, there was no correlation between the adherence scores of the study participants at follow-up and their attitudinal scores at baseline (Spearman’s ρ = -0.006; P = 0.88).

Table 4 – The relation between magnitude of dissonance and practice precautionary measures during follow-up in the DI group.

Predictor	β - coefficient	95% CI	P value	R ² value
Constant	11.7	11.1 – 12.3	.001*	0.255
Magnitude of dissonance	11.93	10.1 – 13.6	.001*	

CI = confidence interval; DI = dissonance induction; R² = coefficient of determination.

Linear regression.

* Denotes statistical significance.

Discussion

DI was found to be an effective way to bring positive changes in adherence to COVID-19 precautionary measures, demonstrating significant positive differences with the AR and control groups, and consequently, the null hypothesis (H_0) can be rejected in support of H_1 . This study attempted to make subjects in the DI group experience the cognitive conflict at baseline by testing their knowledge on and immediately inquiring about their practice of COVID-19 precautionary measures. Such inquiry results in induction of dissonance, a state of cognitive conflict, by making the discrepancy between cognition and behaviour explicitly evident to the respondent. This may influence people to adopt behaviours that they believe are health-promoting in nature. Aronson et al¹⁶ referred to this method as creation of dissonance by hypocrisy induction. Aronson et al¹⁶ successfully tested the effectiveness of this method in overcoming denial and improving the intentions to use condoms, where hypocrisy was induced by having the subjects publicly advocate condom use and then systematically making the subjects aware of their own previous failures in condom use. Dickerson et al¹⁷ reported behaviours conducive to water conservation among the hypocrisy-induced group. Aronson argued that the change in people's attitudes through informational campaigns is short-lived because such a change is brought about by an external source. They proposed that little investment of the self in formation of the attitude is the reason behind the impermanent nature of these changes and such attitudes are vulnerable for change if there is a stronger counterargument by a different external source in the future. Aronson et al¹⁸ suggested that dissonance-generated persuasion is effective in resulting a long-term change as there is the opportunity for critical reflection and investment of the self in the process of attitudinal or behavioural change. In the present study, magnitude of dissonance explained more than a quarter of the variance in the follow-up adherence scores in the DI group adding strength to the argument that induction of dissonance is effective in bringing positive health behavioural changes. Wilding et al¹⁹ reported that the dissonance-enhanced QBE condition was more effective in health behavioural modification compared to a standard QBE intervention. Dissonance was also discussed as the most plausible mechanism explaining QBE in the meta-analyses conducted by Wood et al¹¹ and Spangenberg et al.²⁰

Besides induction of dissonance, another intervention tested in this study was mere questioning about the attitudes toward COVID-19 precautionary measures at baseline without assessing the knowledge and practice of these measures. The existing evidence regarding whether mere questioning of the attitudes relating to a behaviour may bring a positive behavioural change is equivocal.^{14,18,21} In the present study there was no significant difference between the AR and control groups in the follow-up adherence scores. Moreover, the attitudes toward practice of the precautionary measures at baseline in the AR group demonstrated no correlation with the follow-up adherence scores. Spangenberg et al²² also reported no differences between participants who were asked and not asked to predict their behaviour. These findings, however, were inconsistent with those reported by Wood

et al,¹¹ in which participants who were asked to report their intentions demonstrated more accessible attitudes compared to those who were not. Ayres et al²³ proposed that QBE alone is insufficient in promoting health behaviours; a combination of motivation and QBE was reported to be effective in significantly increasing behaviour in a randomised controlled trial. Thus, the present study adds strength to the existing QBE research and postulates DI, over mere questioning about attitudes, as an efficient intervention to promote healthy behaviours with regard to COVID-19 precautionary measures.

Possessing concrete insight into positive health behaviours does not mean practice of these behaviours.²⁴ Also, it was reported in the literature that people believe their behaviour to be better than their actual behaviour.²⁵ Therefore, one of the fundamental goals of health behavioural research is to close the gap between knowledge and behaviours. As a cost-effective alternative to close this gap, QBE was previously tested in different domains of health care such as health screening,²⁶ health check-ups,²⁷ vaccination,²⁸ adoption of health-promoting behaviours, and reduction of risk behaviours.¹⁹ However, this is the first time, to our knowledge, that QBE has been tested for behavioural change with respect to limiting the spread of an infectious disease where strict adherence to suggested precautionary measures is regarded as the best way to combat the spread. It is evident from this study that though the study population possesses good knowledge of the COVID-19 precautionary measures to be followed; they also held some misconceptions among which eating plenty of garlic was the most common. Other common misconceptions identified were the need to take regular hot water baths; the need to use hand dryers after hand wash; and the need to stay in hot and humid climates. Refraining from smoking was identified by only less than half of the participants in the intervention group as a precautionary measure. The problem with holding erroneous beliefs is that the unwarranted practice of these beliefs may act as a compensatory mechanism for people to ignore the actual precautionary measures to be followed. For instance, a person from India who holds a notion that staying in hot and humid climates is protective against severe acute respiratory syndrome-coronavirus 2 may not feel the necessity to practice social distancing. This study highlights some of the erroneous notions held by the dental students and provides an indication of the necessity to more effectively communicate the precautionary measures to reduce the transmission of COVID-19.

The limitations of this study are randomisation at the level of the dental institution; short follow-up time; increased accessibility to information related to COVID-19 through the study period. Randomisation was not done at the participant level to prevent contamination bias.²⁹ Students from the same institution randomised to any of the 3 study groups may share their experiences with colleagues in a different study group than theirs; this is the reason why cluster randomisation was preferred. However, cluster randomisation carries the risk of reduced statistical power. A follow-up time of only 2 weeks was considered in the present study. It was reported in the literature that QBE decays with time. However, in the context of epidemics, even short-term changes in behaviours toward the positive are of tremendous importance. In the literature, follow-up times after administering

the questionnaire varied over a wide range in the previous QBE research with Van Kerckhove et al³⁰ measuring the dependent variable immediately after questioning and Murray et al³¹ reported a time interval of 5 years between administration of questionnaire and measurement of the outcomes. It is important to point here that all study participants, regardless of the study groups, had increased accessibility to COVID-19 information through the study period, which may have an influence on their adherence to the precautionary measures. Nevertheless, access to information is common for participants in all study groups and any possible influence could have affected the follow-up adherence scores in all groups. Another limitation in this study is the gender imbalance with 78.5% of the study participants being females. However, this imbalance is consistent with the existing gender-based imbalance in enrolment into dentistry in India.³² DI was found to be a low-cost, rapid, and effective intervention in this study to improve adherence to COVID-19 precautionary measures. We propose that a more definitive argument in favour of making use of DI as a cost-effective method can be made by comparing the adherence levels to healthy behaviours between DI and strict auditing. Such establishment of DI as a cost-effective alternative goes a long way in the determination of the choice of interventions for behaviour change and not just with regard to COVID-19 but also in the broader context of various health behaviours. Future research on QBE in health behavioural research must also attempt randomisation at an individual level and optimum follow-up times.

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Conflict of interest

None disclosed.

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Article

The Post-Vaccination Quantitative Total Immunoglobulin Levels against SARS-CoV-2 in Healthcare Workers: A Multi-Centric Cohort Study in India

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Abstract: Healthcare workers (HCWs) in India received the AZD1222 and BBV152 vaccines from January 2021 onwards. The objective of this study was to compare the immune response (seropositivity rate and geometric mean titer (GMT), and 95% confidence interval (CI)] against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in HCWs who received these vaccines, after the first and second doses. Therefore, the total immunoglobulin (Ig) levels specific to SARS-CoV-2 were measured using quantitative enzyme-linked immunosorbent assay (ELISA). The study population of 133 HCWs consisted of two groups in which the immune response was measured for the AZD1222 and BBV152 vaccines. Data collection was performed from 6 February to 20 August 2021. Four weeks after the first and second dose, the odds ratio of seroconversion for AZD1222 and BBV152 vaccine was 10.3 times (95% CI: 4.5–23.7) and 15.9 times (95% CI: 6.3–39.9), respectively. The GMT was 6392.93 and 6398.82 U/mL for AZD1222 and 1480.47 and 990.38 U/mL for BBV152 after the first and second doses, respectively. Both vaccines elicited an immune response, but the seroconversion rate and GMT after each dose were significantly higher for AZD1222 than those for the BBV152 vaccine in this study.

Keywords: SARS-CoV-2; immune response; immunoglobulins; vaccination; antibodies; enzyme-linked immunosorbent assay; seroconversion

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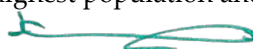
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1. Introduction

COVID-19 infection is a severe acute respiratory syndrome caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. SARS-CoV-2 is a newly emergent coronavirus, first recognized in Wuhan, Hubei province, China, in December 2019. This is a positive-sense, single-stranded, highly contagious RNA virus that can be transmitted among humans. According to an announcement made by the Health Ministry of India, the COVID-19 vaccination drive started with two vaccines: AZD1222 and Bharat Biotech BBV152 vaccines, in January 2021. The two vaccine products were the non-replicating chimpanzee adenovirus vaccine vector (ChAdOx1) from the Serum Institute of India (SII), Pune, India (in collaboration with the University of Oxford, U.K., and pharma giant, AstraZeneca) and an inactivated virus vaccine from the Bharat Biotech Ltd., Hyderabad, India (in collaboration with the National Institute of Virology of Indian Council of Medical Research (ICMR), India). Priority for vaccination was given to an estimated thirty million healthcare and frontline workers [2]. Experts in disease control, immunization, public health, and information technology recommended strategies to be employed in India's COVID-19 vaccination program. According to the strategy for COVID-19 vaccination in India, the country with the second-highest population and cases of infected patients, two



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vaccines, AZD1222 and BBV152, were approved by the Drugs Controller General of India (DCGI), based on results from clinical trials [3].

In India, the first round of vaccination was administered to current healthcare workers, and the second round to priority groups, such as the elderly population, people with comorbidities, pregnant women, and children. Vaccination of people older than 60 years and those older than 45 years with comorbidities began on 1 March 2021 [4].

In an earlier study, researchers evaluated the diagnostic performance of seven rapid IgG/IgM tests and the EUROIMMUN IgA/IgG ELISA for antibodies against SARS-CoV-2 in patients with COVID-19. Their results demonstrated that commercial automated assays and ELISAs are suitable for the detection of IgG and total Ig antibodies against SARS-CoV-2 [5]. The present study mainly focused on evaluating post-vaccination immune responses in healthy individuals.

This study aimed to determine immune response and seroconversion after two doses of the recommended vaccines in a study group of healthcare workers by estimating the total immunoglobulin specific to the spike protein of SARS-CoV-2. Based on the literature on the methodology for the quantitative estimation of total immunoglobulin, we chose enzyme-linked immunosorbent assay (ELISA) to estimate the level of antibodies against the recombinant spike protein of SARS-CoV-2 [6–8].

2. Materials and Methods

2.1. Study Institutions

This study was conducted at the All India Institute of Medical Sciences Mangalagiri (AIIMS, M.G.), Andhra Pradesh, in collaboration with the SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh.

2.2. Study Design and Participants

This was a prospective cohort study.

2.3. Study Cohorts

For group 1, we recruited adult healthcare workers aged >18 years who received the AZD1222 vaccine at the COVID-19 vaccination center at AIIMS, M.G. The AstraZeneca AZD1222 vaccine was recommended by the government of India for patients aged \geq 18 years. As per the recommended schedule, 2 doses (0.5 mL each) of the vaccine were administered at an interval of 4 weeks to all study participants of one group [9]. The first dose of both vaccines was administered to the study groups of healthcare workers on January 18, 2021. The immune response to the vaccine was measured 4 weeks after administration of the first and second doses, and then 24 weeks after the second dose, to verify the rate of seroconversion. This study cohort consisted of 88 participants (N = 88).

For Group 2, we recruited adult healthcare workers aged >18 years who received the BBV152 vaccine at the COVID-19 vaccination center at the SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh. The BBV152 vaccine was developed in India by Bharat Biotech, in collaboration with the Indian Council of Medical Research (ICMR)—National Institute of Virology (NIV). This group received the second dose of the BBV152 vaccine 28 days after the first dose, as per the recommendations [9].

Seropositivity to the vaccine was measured at 4 weeks after administration of the first and second doses and 24 weeks after the second dose to verify the rate of seroconversion. This study cohort consisted of 45 participants (N = 45).

A total of 133 HCWs who, reportedly, did not have a previous COVID-19 infection—that is, who were SARS-CoV-2 naïve—were enrolled into the study. HCWs on steroids, severe comorbid illnesses, children, and pregnant women were excluded from the study.

Data collection for this study began 6 February 2021 and ended 20 August 2021.

2.4. Study Group for Cut-Off Value Determination for ELISA Testing

Twenty-five healthy volunteers who had never been exposed to or immunized against COVID-19 were also enrolled to confirm the optical density (450 nm) 0.02, with the standard high control (units/mL) of “0” stated in the test kit manufacturer’s instruction manual.

As per the Indian Council of Medical Research (ICMR)’s COVID-19 testing guidelines, twenty-five healthy patients were not tested, since they lacked COVID-19 symptoms, and 5 mL whole blood samples were collected from these patients to determine the cut-off value for the total SARS-CoV-2 spike antibody level.

2.5. Blood Samples and Biosafety

Strict infection control measures were taken during blood collection, and complete personal protective equipment (PPE) was worn by all personnel involved in the study during collection and processing of samples and testing in the biosafety level 2 (BSL2) laboratory [10].

Whole blood samples (5 mL) were collected in plain vacutainer rapid serum tubes from each participant 4 weeks after the first dose of vaccination. During the follow-up period, a second blood sample was collected 4 weeks after the second dose of vaccination and again at 24 weeks after the second dose.

The serum was separated from whole blood immediately after collection and stored in separate aliquots at $-20\text{ }^{\circ}\text{C}$ in a deep freezer until further testing was performed in batches.

2.6. Antibody Measurement by Ig Total ELISA

The Human SARS-CoV-2 Spike (trimer) Ig Total ELISA Kit (Invitrogen-BMS2323, ThermoFisher Scientific Inc., Vienna, Austria) was used to detect the total Ig level in serum samples. The test was a solid-phase sandwich ELISA, which was designed to detect and quantify the level of total Ig against human SARS-CoV-2 in the serum.

Trimerized spike protein was pre-coated in the wells of the supplied microplate. Samples and controls, including a high control, were used as a standard; these were then added to these wells and bound to the immobilized (capture) spike protein. The wells were washed, and secondary Ig antibodies conjugated to horseradish peroxidase (HRP) were added and bound to the primary antibodies. The wells were washed, and a substrate solution was added to react with the enzyme complex to produce a measurable signal. The total Ig antibody assay recognizes IgG, IgM, and IgA. The intensity of this signal is directly proportional to the concentration of the antibodies present in the original specimen.

Each ELISA run was performed with standards and controls in duplicates. The absorbance was measured using a BioTek Epoch2 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Standard curves were plotted using four parametric log–log curves fitted via Gen5™ v3.11 Microplate Reader and Imager Software (BioTek Instruments, Inc., Winooski, VT, USA). The background absorbance was subtracted from all data points, including the standards, unknown serum samples, and controls, prior to plotting. The concentrations of unknown serum samples and controls were determined using the standard curve (four parameter logistic (4PL) regression). We read the absorbance at 450 nm and determined the results of the assay quantitatively, as per the kit instructions over the range of 0 to 4000 units/mL total human SARS-CoV-2 Ig.

To set the cut-off value for results analysis, we have used change-point analyses [11]. Therefore, the cut-off antibody level was determined using the following formula:

$$\text{Cut-off} = \text{Mean} + 3 \text{ times the standard deviation}$$

Based on the above calculation, a total SARS-CoV-2 spike antibody titer greater than the cut-off value of 907.58 U/mL was considered seropositive and confirmed seroconversion.

An additional third sample was collected from participants in both groups who did not demonstrate any detectable level of total spike antibody or whose antibody level was below the calculated cut-off value in the samples collected 4 weeks after the second dose.

2.7. Statistical Analysis

The data were entered into a Microsoft Excel (MS Excel) spreadsheet and were analyzed using Epi-Info, IBM SPSS, and Open Epi software. The primary outcome of interest was seroconversion. The immune response to the vaccine—that is, seropositivity—was treated as a nominal variable with the following categories: seroconverted (yes) and seroconverted (no) with antibody titers more than 907.58 U/mL and less than 907.58 U/mL, respectively. The key variables of interest were the immune response to each vaccine, AZD1222 and BBV152, 4 weeks after the first and the second dose and 24 weeks after the second dose, in individuals who did not have prior history of COVID-19.

The chi-square test and Fisher's exact test were used to test the statistical significance of the association between two categorical variables. In this cohort study, the odds ratio (OR) and relative risk (RR) with corresponding 95% confidence intervals (CI) were calculated.

The geometric mean titer (GMT) and standard deviation (SD) with 95% CI were calculated for the antibody titers four weeks after the first and second doses of AZD1222 and BBV152 vaccines. An independent sample t-test was applied to determine whether there was any significant difference in GMTs of AZD1222 and BBV152 vaccines four weeks after the first and second doses.

A difference was considered statistically significant if the *p*-value was less than or equal to 0.05.

3. Results

Table 1 presents the demographic characteristics of the study participants. The AZD1222 vaccination group consisted of 57.9% (51/88) males and 42.1% (37/88) females. In the BBV152 vaccination group, 46.7% (21/45) of the participants were males and 53.3% (24/45) were females. The age range was 18–56 years (median 34 years) in the AZD1222 group and 19–52 years (median 26 years) in the BBV152 group. The mean age of participants was 33.1 and 29.8 years for the receivers of the AZD1222 and BBV152 vaccines, respectively.

Table 1. Demographic data of the participants in this study.

Participants	Characteristics	Vaccine	
		AZD1222 N = 88	BBV152 N = 45
Age, years	Range	18.00–56.00	19.00–52.00
	Mean age (SD)	33.1 (6.7)	29.8 (9.4)
	Median age (IQR)	34 (29.00–37.00)	26 (22–36)
Age, years	<20 (N, %)	2 (2.3)	1 (2.2)
	20–29	22 (25)	25 (55.6)
	30–39	53 (60.2)	11 (24.4)
	>40 (N, %)	11 (12.5)	8 (17.8)
Gender	Male (N, %)	51 (57.9)	21 (46.7)
	Female (N, %)	37 (42.1)	24 (53.3)

Abbreviations: SD: standard deviation; IQR: interquartile range.

As seen in Table 2, 71 out of 88 individuals (80.7%) had seroconversion from the AZD1222 vaccine 4 weeks after the first dose, whereas only 13 out of 45 individuals (28.9%) had seroconversion from the BBV152 vaccine 4 weeks after the first dose, and the difference was found to be highly statistically significant. Four weeks after the first dose, the seroconversion from the AZD1222 vaccine was 10.3 times (95% CI for odds ratio: 4.46–23.67) greater than that from BBV152. Meanwhile, 79 out of 88 individuals (89.8%) had seroconversion from the AZD1222 vaccine 4 weeks after the second dose, whereas only

16 out of 45 individuals (35.6%) had seroconversion from BBV152 vaccine 4 weeks after the second dose, and the difference was highly statistically significant. Four weeks after administration of the second dose, the seroconversion from the AZD1222 vaccine was 15.9 times (95% CI for odds ratio: 6.33–39.96) greater than that from BBV152 vaccine.

Table 2. Comparison of seroconversion rate 4 weeks after the first dose and the second dose of AZD1222 and BBV152 vaccines in SARS-CoV-2 naïve participants (N = 133).

Characteristics	SARS-CoV-2 Naïve Participant #		Odds Ratio, [95% CI], <i>p</i> Value	Relative Risk [95% CI]
	AZD1222 Vaccine	BBV152 Vaccine		
4 weeks after the first dose Seropositivity rate (%)	71/88, (80.7)	13/45, (28.9)	10.28 [4.46, 23.67] * <i>p</i> < 0.0001	2.79 [1.75, 4.47]
4 weeks after the second dose Seropositivity rate (%)	79/88, (89.8)	16/45, (35.6)	15.91 [6.33, 39.96] * <i>p</i> < 0.0001	2.53 [1.69, 3.77]

May include asymptomatic COVID-19 participants. * 95% CI for odds ratio calculated by the Wald method using WINPEPI software.

As shown in Table 3, the GMT (and SD) of the antibody titer (U/mL) measured 4 weeks after the first dose of AZD1222 and BBV152 vaccines were 6392.93 (4.92) and 1480.47 (9.32), respectively. An independent samples *t*-test was applied, and the difference was found to be highly statistically significant. The GMT (and SD) of the antibody titer (U/mL) measured 4 weeks after the second dose of AZD1222 and BBV152 vaccines were 6398.82 (3.24) and 990.38 (5.26), respectively. An independent samples *t*-test was applied, and this difference was found to be highly statistically significant.

Table 3. Geometric mean (and SD) antibody titer after the first and second dose of AZD1222 and BBV152 vaccines in SARS-CoV-2 naïve participants.

Characteristics	SARS-CoV-2 Naïve Participant #		<i>p</i> Value
	AZD1222 Vaccine, N = 84	BBV152 Vaccine, N = 17 (Four Weeks after First Dose) N = 25 (Four Weeks after Second Dose)	
	Antibody Titer, Geometric Mean (SD) [95% CI], in U/mL	Antibody Titer, Geometric Mean (SD) [95% CI], in U/mL	
4 weeks after the first dose	6392.93 (4.92) [6391.88–6393.98]	1480.47 (9.32) [1476.04–1484.9]	0.0001
4 weeks after the second dose	6398.82 (3.24) [6398.13–6399.51]	990.38 (5.26) [988.31–992.44]	0.0013

May include asymptomatic participants with COVID-19. Differences were considered statistically significant at *p*-value < 0.05.

Relative risk = 0.71, 95% CI for relative risk= (0.20, 2.49). As seen in Table 4, in total, 3 out of 19 individuals (15.8%) and 6 out of 27 individuals (22.2%) had seroconversion from the AZD1222 and BBV152 vaccine, respectively, 24 weeks after the second dose, but the difference was not statistically significant.

Table 4. Seroconversion from the AZD1222 and BBV152 vaccines 24 weeks after the second dose (N = 46) without previous COVID-19 infection.

Immune Response to Vaccine Twenty-Four Weeks after the Second Dose of Vaccine	Seroconverted	
	Yes	No
AZD1222	3 (15.8%)	16 (84.2%)
BBV152	6 (22.2%)	21 (77.8%)

Fisher exact test, $p = 0.88$. Odds ratio = 0.66, 95% CI for odds ratio by the Wald method = (0.14, 3.03).

4. Discussion

The World Health Organization (WHO) accelerated the development of COVID-19 vaccines worldwide at the beginning of 2020 in order to control the COVID-19 pandemic. Among several vaccine preparations, the recombinant coronavirus spike (S) protein, a replication-deficient simian adenoviral vaccine (ChAdOx1-S), and a BBV152 inactivated viral vaccine were recommended by WHO [12]. The present cohort study evaluated the immune response of healthcare workers against SARS-CoV-2 spike protein by estimating the total Ig titer after the administration of AZD1222 and BBV152 vaccines. We found that the seropositivity rate four weeks after the first dose was significantly higher in AZD1222 than in BBV152 vaccine recipients.

Furthermore, we also found that the seropositivity rate four weeks after the second dose was significantly higher in AZD1222 vaccine vs. BBV152 vaccine recipients. Our study findings are similar to those of Singh et al. (2021), who found that the seropositivity rate of antispikes antibody was significantly higher in Covishield (AZD1222 vaccine) vs. Covaxin (BBV152 vaccine) recipients (98.1 vs. 80.0%; $p < 0.001$) [13].

We also found that the geometric mean (and SD) of the antibody titer measured 4 weeks after the first and second doses of AZD1222 and BBV152 vaccines was significantly higher with the AZD1222 than with the BBV152 vaccine. A study by Singh et al. (2021) found that, after the administration of a second dose of Covaxin, the GMTs with 95% CI and median (IQR) antispikes antibody titer only increased significantly after two doses. In contrast, the Covishield vaccine showed a greater than threefold increase in the antispikes antibody GMT, even after a single dose.

However, the seropositivity rates 24 weeks after the second dose of AZD1222 and BBV152 vaccines did not show any statistically significant difference. Individuals who were infected with SARS-CoV-2 prior to receiving the AZD1222 and BBV152 vaccines did not report a significantly different seropositivity rate 4 weeks after the first and second doses of the AZD1222 and BBV152 vaccines.

A study revealed that, in the first phase (1/2) of a UK clinical trial testing two doses of the ChAdOx1 nCoV-19 (Covishield) vaccine, there was a significant increase in anti-spikes IgG antibody after 56 days compared to its median titer after 28 days [14]. Voysey et al. (2021) reported the clinical efficacy of the above vaccine in phase 2/3 of trials conducted in the UK and Brazil and found that this vaccine was effective against symptomatic COVID-19 [15,16]. Indeed, the numerous freely available basic research data on the mechanisms of SARS-CoV-2 infection have convinced most developers of innovative vaccines to focus their efforts on inducing an immune response against the spike protein. The S protein of SARS-CoV-2 is the most suitable antigen for inducing neutralizing antibodies against the pathogen [17]. In one such study, the researchers evaluated antispikes protein receptor-binding domain (S-RBD) antibodies, which represent a useful means of estimating the individual protection against SARS-CoV-2 infection, and observed a significant decrease in anti-RBD IgG levels within a short period following a complete two-dose vaccination cycle [18].

We have not estimated the antibody level in the study groups before vaccination, because the virus-specific neutralizing antibodies (NAbs), induced through either infection or vaccination, have the ability to block viral infection. The level of NAbs has been used as the gold standard to evaluate the efficacy of vaccines against smallpox, polio, and

influenza viruses [19]. The rationale behind measuring antibodies before vaccination is because previous infection is associated with a stronger immune response after vaccination [20].

Various studies have reported increasing levels of neutralizing antibodies of IgM and IgG to SARS-CoV-2 at different time intervals. Long et al. (2020) reported that, in 285 patients with COVID-19 infection, there were acute antibody responses to SARS-CoV-2 [21]. Within 19 days, one hundred percent of the patients were positive for Immunoglobulin G (IgG). The seroconversion of IgG and IgM occurred either simultaneously or sequentially. In addition, after symptom onset, the proportion of patients with virus-specific IgG positivity reached 100% at approximately 17–19 days. Virus-specific IgG and IgM antibody titers increased during the first 3 weeks after symptom onset.

There are many different vaccines prepared using different platforms, including recombinant vectors, DNA, mRNA in lipid nanoparticles, inactivated viruses, live attenuated viruses, and protein subunits against SARS-CoV-2 [22]. India had approved AZD1222/Covishield, a monovalent vaccine, and BBV152/COVAXIN, a whole virion-inactivated SARS-CoV-2 vaccine [23].

Based on immunogenicity studies in humans, the rate of seroconversion (a four-fold or greater increase over baseline) to S-binding antibodies was over 98% and 99%, 28 days after the first and second dose, respectively, in participants who were seronegative at baseline. The rate of seroconversion, as measured in a live neutralization assay, was over 80% and over 99%, 28 days after the first and the second dose, respectively, in participants who were seronegative at baseline [24].

The SARS-CoV-2 spike protein gene is expressed in the AZD1222 vaccine [25] and instructs the host cells to produce the S-antigen protein, which is unique to SARS-CoV-2. This allows the body to mount an immune response, and this information is retained in memory immune cells. The results of a clinical trial based on a median follow-up of 80 days showed that the efficacy of two doses of vaccine was 63.1%, irrespective of the interval between the doses. Efficacy tended to be higher when the interval was longer. The data reviewed at this time support the conclusion that the known and potential benefits of the AZD1222-S/nCoV-19 (recombinant) vaccine outweigh the risks [26].

The present study estimated antispikes immunoglobulin levels in vaccinated populations to evaluate the immune response to vaccines as a convenient tool for assessing immunological response. This has been supported by a study conducted by researchers on the long-term persistence of SARS-CoV-2 spike protein-specific and neutralizing antibodies in recovered COVID-19 patients. Their findings corroborate the reliability of estimating the antispikes immunoglobulin levels as a convenient tool for assessing the immunological response of COVID-19-infected individuals to quantify the immunogenicity of the vaccines and therapeutic effects [27].

Finally, massive vaccination campaigns are expected to elucidate several critical aspects of the SARS-CoV-2 immunity. As larger populations get vaccinated, the durability of the immunity induced by the different vaccine strategies, as well as the finer details of the immune responses elicited, will emerge, including in those individuals with suboptimal immunity [28].

The limitations of this study included the small number of participants due to the high cost of the test kit and insufficient funds to support the testing of a larger number of participants. The lack of baseline antispikes (S) serology is also a limitation of the study.

5. Conclusions

We found that the seropositivity response was significantly better in the AZD1222 group than in the BBV152 group, 4 weeks after the second vaccine dose, in 133 HCWs. Seropositivity response was also improved 24 weeks after the second dose in the BBV152 group compared to the AZD1222 group amongst 46 HCWs, but the difference was not statistically significant. This study found a low seroconversion rate in the study group vaccinated with the inactivated viral vaccine compared to the live vaccine.

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Institutional Review Board Statement: All procedures were performed in accordance with the Indian National Ethical Guidelines and as per the 1964 Helsinki Declaration and its latest amendment. This study was approved by the Institutional Ethics Committee (IEC) of AIIMS Mangalagiri (AIIMS/MG//IEC/2021-22/102).

Informed Consent Statement: Written informed consent was obtained from all the participants. The study participants were informed about the publication of clinical data and images after the completion of the study. The data from this study were kept confidential.

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Leukocyte Adhesion Deficiency-1 with Oral Manifestations – A Rare Case Report

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Abstract

Leukocyte adhesion deficiency 1 (LAD) is a rare, inherited disorder portrayed by the inability of leukocytes to emigrate from the bloodstream toward sites of inflammation. LAD should be considered a rare but possible disorder in patients with persistent periodontal problems. This paper presents a case of leukocyte adhesion deficiency in an 18-year-old girl and a brief literature review.

Keywords: CD 18, leukocyte adhesion deficiency, periodontitis

INTRODUCTION

The immune system is a magnificent alliance between cells and proteins that work together to provide defense against infection. When this alliance is disturbed, this encounters a disease. These disturbances can be in the form of primary immune deficiencies. One such immunodeficiency is leukocyte adhesion deficiency (LAD 1), an autosomal recessive disorder.^[1] LAD 1 is estimated to occur in 1 per million people worldwide.^[2]

The present paper reports a rare case of LAD in an 18-year-old female patient who had presented with oral manifestations.

CASE REPORT

Clinical findings

An 18-year-old young female patient presented with a chief complaint of pain in the gums since two months. She revealed a history of pain which was gradual in onset, intermittent in nature, throbbing type, non-radiating, aggravated on taking food, and relieved gradually. On extra oral examination, both right and left submandibular lymph nodes were palpable with a size of 1 × 1 cm, and were non tender, soft in consistency [Table 1].

Intraoral examination showed pallor of buccal, labial, and lingual mucosa. Gingival and periodontal status revealed an erythematous, edematous marginal, and attached gingiva with erosive areas associated with loss of interdental papilla

and generalized gingival recession. On palpation bleeding on probing was noticed with generalized periodontal pockets and furcation involvement [Figure 1].

Based on the chief complaint and history and on considering clinical findings, we provisionally considered it to be desquamative gingivitis associated with aggressive periodontitis.

Diagnostic assessment

The patient was advised for radiographic and hematological investigations. Orthopantomograph revealed generalized horizontal bone loss and furcation involvement in all first and second molars [Figure 2].

On the recall visit, clinically the lesions were still persistent. She was again advised for a blood workup to assess the state of anemia and leukocytosis. But white blood cells count and, in particular, neutrophil count were still high.

Based on the clinical findings and laboratory investigations, a diagnosis of primary immunodeficiency was considered. So,

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Figure 1: Intraoral images depicting erythematous gingiva

the patient was advised for advanced laboratory investigations to assess the immunoglobulins and lymphocytes using immunoturbidimetry and flow cytometry, respectively [Table 2].

Therapeutic intervention and follow-up

Symptomatic management using topical anesthetic gel was advocated, and iron supplements were also prescribed to improve her hemoglobin levels. Oral prophylaxis was carried out with the appropriate use of antibiotics. Further clinical follow-up was hindered as the patient suffered from grave gastrointestinal tract infections and was under physician supervision at higher centers.

Considering the history and clinical findings and based on the laboratory findings, it was diagnosed as leukocyte adhesion deficiency type 1(LAD 1).

DISCUSSION

Interaction of leukocytes with vascular endothelial cells plays an important role to migrate the defense cells to the site of insult. This mechanism takes place in four steps: rolling, slow adhesion, firm adhesion, and transmigration.^[3,4]

LAD I is caused by mutations in ITGB2 (integrin beta-2), the gene located at 21q22 β 2 integrins, there by affecting the third phase of adhesion cascade.^[5]

LAD 1 subtypes include severe, moderate, or variable. In severe form, less than 2% of CD18- expressing neutrophils. In moderate form, 2-30% of CD18-expressing neutrophils in which patients survive childhood with recurrent infections and mortality was reported by 40 years.^[6,7]

Clinical features of leukocyte deficiency type 1 are delayed separation of the umbilical cord with severe omphalitis and recurrent severe infections that usually affect lungs, skin, and gingiva in children. The absence of pus at the infection site is the characteristic of LAD 1.^[8] Most frequently isolated microbes from these patients are Staphylococcus aureus and gram-negative pseudomonas.^[9]

Oral manifestations include oral ulcers, severe periodontitis, and gradual loss of permanent teeth. LAD

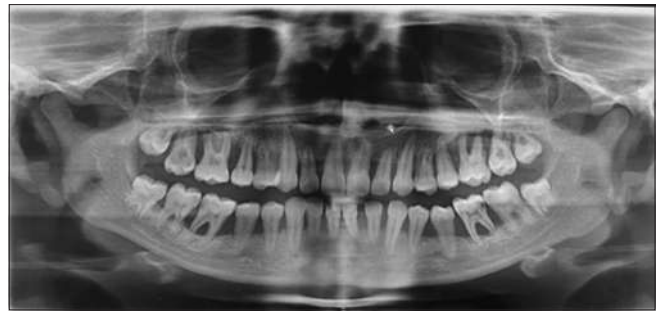


Figure 2: Panoramic radiograph reveals generalized horizontal bone loss

Table 1: Timeline of history

Age of the patient	18 years
Pain in gingiva	2 months
Past medical history	No relevant history
Family history	Parents consanguineous marriage

Table 2: Laboratory investigations of patient

Investigation	Result
Hemoglobin	7.7 gm%
Total leukocyte count	25.5 $10^3/\mu$ l
WBC count	17,800 cells/cumm
Neutrophils	77%
Lymphocytes	21%
ESR	75 mm/hour
Absolute lymphocyte count	4.9103/ μ l
Absolute CD3 count (T cells)	4277 cells/ μ l (87.24%)
Absolute CD 3 + CD4 + counts	1970 cells/ μ l
CD4%	40.19%
Absolute CD 3 + CD8 + counts	2098 cells/ μ l
CD8%	42.79%

sub gingival microbes are dissimilar to localized aggressive periodontitis.

Diagnosis is usually made based on clinical presentation, increased leukocyte count on laboratory investigations, and absence of CD 18, CD 11a, CD 11b, and CD 11c on flow cytometry analysis. The gold standard for diagnosis is the identification of a mutation in the ITGB2 gene by genetic analysis. Cordocentesis can also establish the diagnosis as leukocytes express CD18 on their surface from 20 weeks of gestation.^[10]

In this deficiency syndrome, we as dentists cannot change the root cause but can help the patient to lead a better life by providing supportive measures. In this case, the supportive treatment helped the patient, and the results were satisfactory.

CONCLUSION

Though LAD is rare, it should be considered a differential diagnosis when we come across patients with persistent clinical

signs which are recalcitrant to symptomatic management and periodontal therapy.

Declaration

Patient consent was obtained to use the relevant clinical details and images for the scientific publication without revealing the identity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Incidence of retromolar canals in a subset of Indian population

Running title: Incidence of retromolar canals

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Abstract

Background: Retromolar canals need consideration in surgical procedures involving the retromolar area such as third molar impaction, osteotomy etc.

Aim: The present study aimed to evaluate the retromolar region for the presence of retromolar canals in a subset of Indian population using CBCT imaging.

Methodology: 125 CBCT images of mandible were retrieved from archival records of a CBCT centre in Bangalore. The presence of retromolar canals was evaluated on sagittal, axial and coronal sections. The



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canals were categorized into different types as suggested by Ossenberg and this was assessed on the sagittal section. The diameter of retromolar canal at their exit and distance of retromolar foramen from the 3rd molar was measured on the axial sections. The results were tabulated and statistically analyzed.

Results: Of the 125 subjects examined, 46 subjects were found to have retromolar canals. Of the 250 sites examined, 54 sites presented with retromolar canals with an incidence of 21.6%. Majority of the canals were of the Type A (79.6%) category followed by Type B canals((20.3%). The diameter of the canal varied from 0.5mm to 1.4mm. The distance of the retromolar canal from the anterior border of the ramus was variable and not concentrated in a particular region.

Conclusion: An incidence of 21.6% compulsively justifies the need to recognize these anatomic variants in surgical procedures involving the retromolar area. It is important for radiologists to possess adequate knowledge on the presence and frequency of these retromolar canals and incorporate them in the interpretation reports to alert the surgeon.

Key words: Retromolar canal, CBCT, Retromolar foramen, Third molar

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Introduction

Anatomical variations are defined as normal flexibility in the topography and morphology of body structures. The retromolar canal is one such anatomic variant, classically described as a canal that branches off the inferior alveolar canal behind the 3rd molar and follows a recurrent path, curving in a posterosuperior direction to open into the retromolar fossa through the retromolar foramen, which is usually located on the alveolar and central part of the retromolar trigone. The retromolar fossa or trigone is a triangle-shaped region anteriorly limited by the third molar, medially by the temporal crest, and laterally by the anterior border of mandibular ramus.¹The constituents of these canals include neurovascular bundle originating from the inferior alveolar nerve, striated muscle fibers, thin myelinated nerve fibers, numerous venules and a muscular artery.²

Various imaging modalities such as panoramic radiography, computed tomography, cone beam computed tomography have been used to detect and evaluate these anatomical variations. However, Orthopantomographs (OPGs) provide primary and significant overview of the oral and maxillofacial region. Two

dimensional radiographic modalities like OPGs fail in the proper presurgical evaluation of the anatomical structures in the buccolingual aspect and crosssectional slices. OPG's also fall short in obtaining reliable measurements, and other factors which influence the diagnostic quality of two dimensional imaging such as patient positioning, magnification distortion and superposition of the anatomical structures are a major challenge to the clinician to evaluate these fine anatomical structures such as retromolar canals ,mandibular incisive canal, accessory mental foramen, accessory mandibular canal, etc.^{2,3}

In the recent times to overcome all these challenges Cone beam computed tomography (CBCT) technology can be used as a substantial modality of maxillofacial imaging extensively as it shows three-dimensional (3D) images of dental structures with clear structural images of high accuracy on which measurements can be easily made.³

There is variation in distribution of these anatomical variations in individuals with different racial and cultural backgrounds hence there is need to examine these canals anatomical variants to deliver quality patient care. This study was done to evaluate the retromolar region for the presence of retromolar canals in a subset of Indian

5341



population by CBCT imaging and to measure the distance from the distal alveolar crest of the 3rd molar to the exit point of retromolar canals

Materials and method

The retrospective study was done in the department of Oral medicine and Radiology after obtaining ethical approval from institutional ethics committee. A radiographic cross sectional study was designed and 125 CBCT images of the mandible were retrieved from the archival records of a CBCT centre (Fig 1). All images of subjects above 18 years were included and CBCT scans displaying developmental disorders, abnormal morphology resulting from trauma and pathologic conditions that potentially affected the area of interest and CBCT images of patients with history of surgery in the region of interest were excluded.

The images were taken with the Kodak 9300C CBCT machine with the following exposure parameters, tube voltage- 90 kVp, tube current - 5 mA and exposure time - 14 seconds. A cylindrical shaped field of view (FOV) measuring 40 x 50 mm with a voxel size of 200 microns will be used will be used for imaging.

The sagittal, coronal and axial images reconstructed from the projection data was used for assessing the retromolar canals. The images were examined under standard viewing conditions and exported and saved in a jpeg format. Copies of CBCT images was made, adjusted and exported to Microsoft Office PowerPoint to create a separate file set for observer evaluation. The file set did not contain any demographic data, and the images were assigned serial numbers. Images were examined by 2 observers; the first observer reviewed the images after an interval of one week. Type of retromolar canals evaluated was as shown in Fig 2.

Type A, The retromolar canal branches off the mandibular canal distal to the 3rd molar and courses superiorly to open into the retromolar fossa.

Type B The retromolar canal courses between the retromolar fossa and the radicular portion of third molar.

Type C The retromolar canal originates from the mandibular foramen in the ramus, it courses anteroinferiorly and then anterosuperiorly to open into the retromolar fossa.

The region of exit of retromolar canals in the retromolar fossa was noted on coronal sections by dividing the retromolar fossa into the buccal half and lingual half (Fig 3). The width at the point of origin from the mandibular canal and the width at the point of exit in the retromolar fossa were measured in sagittal sections. The distance from the distal alveolar crest of the 3rd molar to the mesial aspect of the exit point of all the 3 types of retromolar canals was also measured on sagittal sections.

The data was tabulated and statistically analyzed using SPSS statistical software version 20.0 of IBM, Chicago, USA with Chi-square and Fisher's exact test. Chi square test was used to assess the distribution of retromolar canals based on age, and Fischer's exact test was used to assess the distribution of retromolar canals based on gender and side of occurrence.

Results

125 CBCT images were obtained from a CBCT centre in Bengaluru, these subjects had underwent CBCT examination as a part of their routine radiographic examination . These images were obtained retrospectively analyzed for the presence and course of retromolar canals.

CBCT images of 125 subjects were examined bilaterally for the presence of retromolar canals, hence a total of 250 sites were examined. Out of 125 subjects examined, 46 subjects were found to have retromolar canals. Out of 250 sites examined, 54 sites



presented with retromolar canals. Thus an incidence of 21.6% was noted in the present study which is similar to the incidence reported in CBCT studies conducted in various countries such as Brazil, Switzerland and Turkey (24.6%,-25.6%,26.7% respectively)

Discussion

Retromolar canals need consideration in surgical procedures involving the posterior mandible such as third molar impactions, osteotomy and bone harvesting procedures. Complications such as sensory deficits hemorrhage and traumatic neuroma may arise in the absence of recognition of these variants. The impingement of the neurovascular bundle in the retromolar fossa from prosthetic appliances in the elderly due to the resorption of alveolar bone may result in patient discomfort. These retromolar canals may act as an alternative route for nerve fibers in the posterior mandibular region and evade anesthetization during routine mandibular blocks.¹

Patil et al found that out of the 160 subjects examined, 116 (72.5%) subjects presented with RMCs. It was found that 44 out of 72 males (45%) and 72 of 88 (68%) females presented with retromolar canals. Type B was the most common type. Based on the results of this study, retromolar canal should be considered as a normal anatomical variation rather than a rare finding. The incidence rates of retromolar canals were determined according to gender, location, and type of RMC.⁴ Siddiqui et al found retromolar foramen/canal in 6 mandibles (17.14%), bilaterally in 2 mandibles and unilaterally in 4 mandibles and concluded that retromolar foramen /canal is of profound clinical importance.⁵

de Gringo et al assessed the presence and characteristics of the retromolar canal and association with mandibular molars using cone-beam computed tomography (CBCT). They

found 24.5% prevalence of the retromolar canal. The oblique trajectory was the most common (33.3% on the right side and 50% on the left side).⁶

de Freitas et al evaluated the prevalence of retromolar canals in cone beam computed tomography (CBCT) images and to correlate it with their possible clinical repercussions. They found prevalence of retromolar canals was observed in 15 patients (5.0%), of which 06 were in males and 09 in females.⁷

Full understanding of the anatomy of the mandibular canal and its variations, such as bifid canals, foramina and RMC have great importance in the planning and execution of surgical and anesthetic procedures. Regarding anesthetic procedures, the presence of alterations in the mandibular canal and RMCs may result in anesthetic failures, since such canals may exit through accessory foramina and contain a neurovascular bundle. Failure to achieve adequate anesthesia may therefore be observed when performing the inferior alveolar nerve block for procedures in the region of the last mandibular tooth. Studies on the incidence of RMC are important to prevent failures in regional anesthesia of the inferior alveolar nerve and buccal nerve fibers as well as to minimize the occurrence of accidents and complications in the posterior region of the mandible.⁷

The diameter of the retromolar canals were found to be ranging from 0.5mm to 1.4mm with an average of 0.62mm. The diameter of RMC has been reported to range from 0.2 mm to 3.29 mm in literature. Males have been reported to have larger diameters of RMC, which can be explained by the fact that male mandibles are usually larger than in females.

5343



The drawback of present study was smaller sample size. Further studies are needed with larger sample size and different racial group.

Conclusion

In the present study out of 125, 46 subjects were found to have retromolar canals. Of the 250 sites examined, 54 sites presented with retromolar canals. The majority of retromolar canals in the present study belonged to the Type A (79.6%) category followed by Type B (20.3%). The diameter of the retromolar canals were found to be ranging from 0.5mm to 1.4mm with a mean of 0.62mm. Distance of the retromolar canal from the anterior border of the ramus was variable and not concentrated in a particular region (ranged from 3 -19.1 mm with an average of 9.08 mm) .

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Tables

Table 1: Distribution of retromolar canals based on age

Age groups	No of subjects examined	No of subjects with retromolar canals	% Of subjects with retromolar canals
<20	5	2	40%
20-39	22	9	40.9%
40-59	58	21	36%



>60	40	14	35%
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Table 2: Distribution of retromolar canals based on gender.

	No of subjects examined	No of subjects with retromolar canals
Males	70	26
Females	55	20

Table 3: Distribution of retro molar canals based on the side of occurrence.

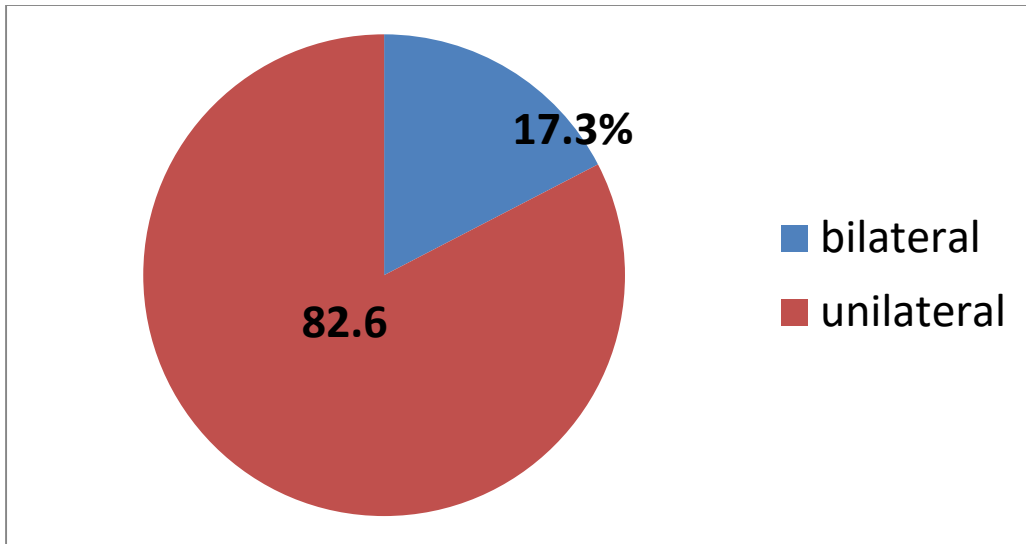
Sides		Number
Unilateral	Left	14
	Right	24
Total		38

5345

Table 4 The various types of canals encountered in the study.

No of sites examined	Type A		Type B
	Type A1	Type A2	
250	15	28	

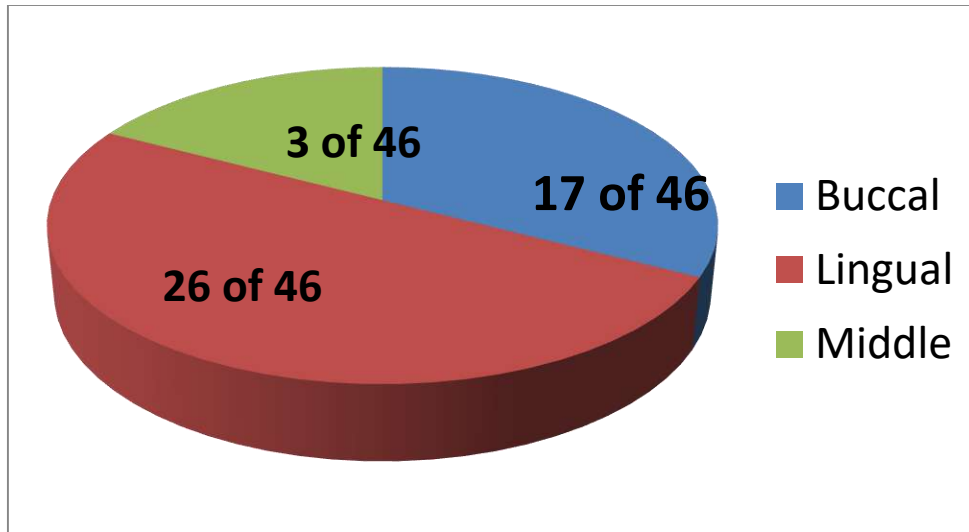




5346

Graph 1 : Unilateral and bilateral distribution of retromolar canals





Graph 2 Depicts the opening of the retromolar canal in the retromolar fossa.

5347

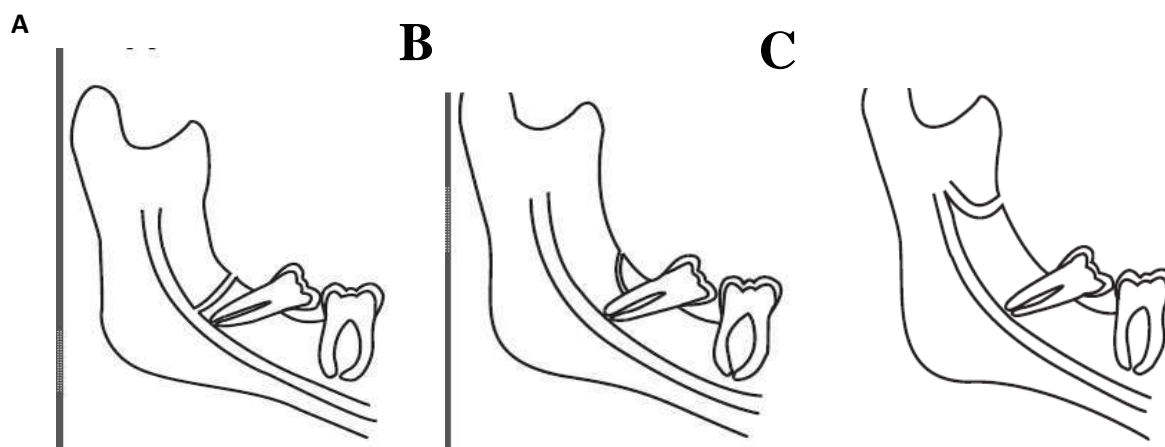
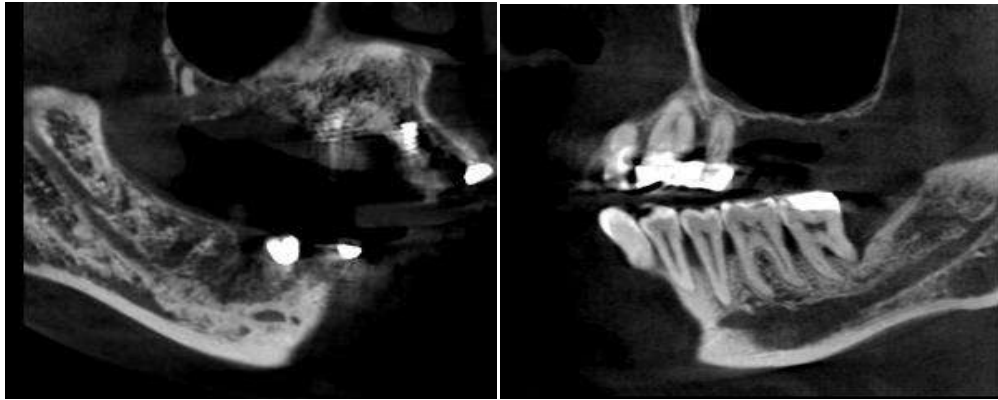


FIGURE 1. Pictorial representation of various types of retromolar canals.


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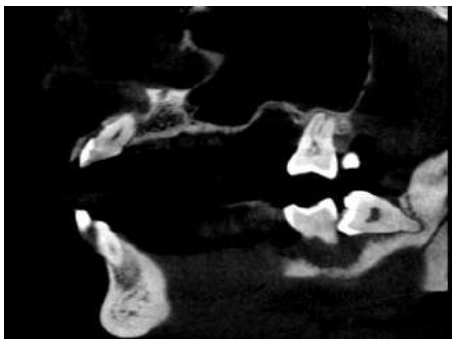


FIGURE 2: TYPES OF RETROMOLAR CANALS

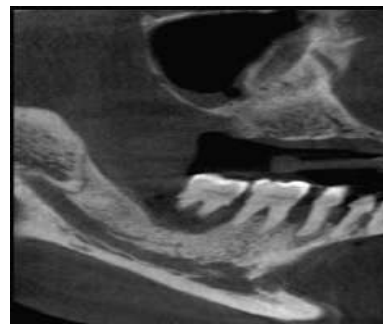


TYPE A1 RETROMOLAR CANAL

TYPE A2 RETROMOLAR CANAL



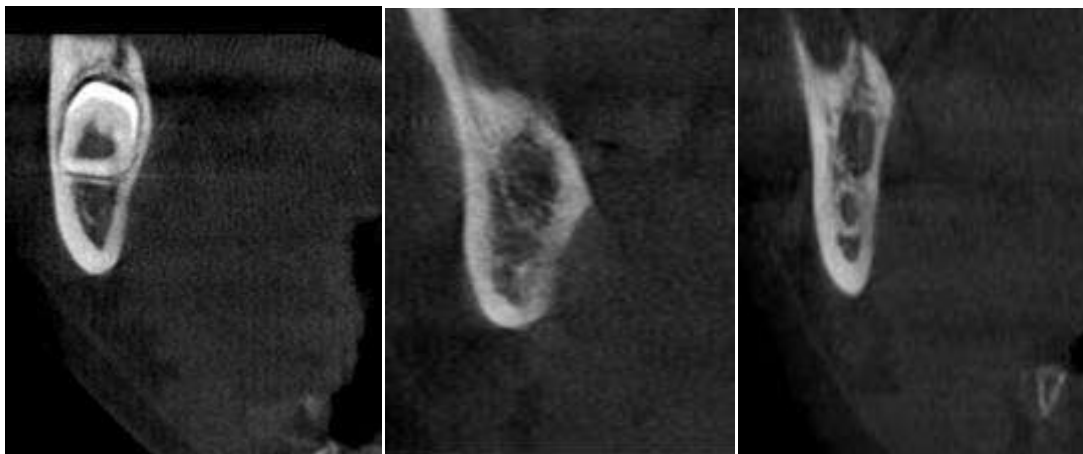
TYPE B RETROMOLAR CANAL.



TYPE C RETROMOLAR CANAL.

5348

FIGURE 3: OPENING OF THE RETROMOLAR CANALS IN DIFFERENT ASPECTS OF THE RETROMOLAR REGION.



LINGUAL SIDE

BUCCAL SIDE
RETROMOLAR FOSSA

MID PORTION OF THE



Article type: Original article

Association of salivary soluble CD44 in patients with oral leukoplakia: A case control study

Running title: Salivary soluble CD44 in patients with oral leukoplakia

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Abstract

Background: Leukoplakia is the most common potentially malignant disorder of the oral mucosa. Latest developments in the field of molecular biology have greatly increased our knowledge of the role of various bio markers in carcinogenesis and prognosis of precancerous and cancerous lesions.

Aim: The present study aims to assess the expression of CD44 in saliva of subjects with leukoplakia by quantitative Real Time-polymerase chain reaction.

Methodology: 30 subjects with leukoplakia (clinically and histopathologically confirmed) and 20 healthy controls were included in the study. Quantitative real time PCR was run on the salivary samples of these study subjects.

Results: An individual pattern of down regulation of CD44 was noted in 60% of the subjects with leukoplakia and upregulation was noted in 90% of controls by absolute quantification method. The gene fold expression ratio by relative quantification method between subjects with leukoplakia and controls delta ct method was found to be $5.777/5.827 = 0.9914$ and by double delta ct method was found to be $28.4151/28.0694 = 1.0123$. No statistical significant difference was noted in the expression of CD44 and grades of epithelial dysplasia.

Conclusion: Although an individual pattern of down regulation of CD44 was noted in subjects with leukoplakia, the gene fold expression ratio between subjects with leukoplakia and controls by both the methods was approximately 1 suggesting no difference in the gene fold expression ratio between subjects with leukoplakia and controls. CD44 was not found to be a reliable marker in determining the malignant transformation of leukoplakia.

Keywords: Leukoplakia, CD44, q RT-PCR, Bio-markers, Saliva.

Introduction

Oral cancer is the 15th most prevalent cancer with the age standardized incidence rate of 3.9 per 100,000 populations worldwide. The Indian subcontinent accounts for one-third of the world burden of this malignancy. Oral cancer is the most common form of cancer and accounts for increasing number of cancer related deaths among men in India.¹ Oral cancer mostly occurs in the buccal mucosa and of the Indian population as compared to the western countries where oral cancer mainly occurs in the lateral border of the tongue. This may be due to the fact that the Indian population is more prone to use of smokeless variety of tobacco.²

Leukoplakia is the most common potentially malignant disorder of the oral mucosa. The annual incidence of oral leukoplakia among subjects greater than 15 years of age ranges from 0.2% to 11.7% in different populations of India.³ It is defined by the World Health Organization in 2005 as a “A white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer”. In an explanatory note it has been explicitly stated that the term leukoplakia is unrelated to the absence or presence of epithelial dysplasia.⁴

Studies have shown that dentists and other health care providers are in a tremendous need of systematic educational updates in oral cancer prevention and early detection.²

The ability to clinically predict malignant transformation is difficult.⁵ A number of diagnostic kits have been developed for the early detection of oral cancer namely OralCDx Brush Biopsy, ViziLite Plus, toluidine blue staining etc. Although these tests can assist in identification of dysplastic features or frank malignancy, they are associated with either false positive or false negative results and are not conclusive.⁶ Hence a need for confirmation of their malignant potential by histopathological evaluation arises. However histopathological diagnosis has limited prognostic value and disadvantage of patient discomfort and fear.⁵

A latest development of various bio-markers in carcinogenesis of precancerous and cancerous lesions helps in prediction of rate of malignant transformation and prognosis of the lesion.⁶ These biomarkers have generally been detected either in the serum or tissue

specimens. Some of these biomarkers can also be detected in saliva as saliva may be considered as a reflection of serum.⁷ Various biomarkers namely Ki-67, argyrophilic nucleolar organizer regions, p53 helps to predict the malignant potential of and as a prognostic tool in oral cancer.⁸

CD44 is a biomarker expressed as a cell surface glycoprotein by a large variety of tissues such as colorectal tissues, urinary bladder, lungs head and neck epithelia etc. It is also released from mast cells, lymphocytes, and hence are detectable in circulation as well. CD44 acts as a receptor for the extracellular matrix components namely hyaluronate, laminin, collagen etc.^{9,10}

Studies have evaluated the role of CD44 in tissue samples of HNSCC with the help of various methods such as immunohistochemistry, PCR and flow cytometry. An increased expression of CD44 in saliva of subjects with HNSCC as compared to normal study subjects has been reported in four studies. However one study found no difference in expression of CD44 in both HNSCC and normal study subjects. This was attributed to the fact that HNSCC is a multifactorial disease which involves a number of risk factors.¹¹

There are limited studies on the expression of CD44 in saliva of subjects with leukoplakia. Hence this research was done to assess and compare the levels of CD44 in saliva samples of soluble CD44 in saliva of leukoplakia group with and controls by quantitative Real Time-polymerase chain reaction (RT-PCR) technique and to evaluate the correlation between the salivary soluble CD44 and epithelial dysplasia in subjects with leukoplakia.

Materials and method

Study design

This case control study was done in the department of Oral medicine and Radiology after obtaining the approval from institutional ethics committee.

In this study with Null hypothesis $H_0: m_1 = m_2$ vs. Alternative hypothesis $H_a: m_1 = m_2 + d$ where d is the difference between two means and n_1 and n_2 are the sample size for Group I and Group II such that $N=n_1+n_2$. The ratio $r=n_1/n_2$ is considered whenever the researcher needs unequal sample size due to various reasons, such as ethical, cost, availability etc.

Sample size estimation

In this study for an outcome variable CD44s with difference of 1.0(SD=1.40), 90% statistical power and 5% statistical significance, the total sample size of 50 can be derived by systematic random sampling.

Then the total sample size for the study is as follows

$$N = \frac{(r+1)(Z_{\alpha/2} + Z_{1-\beta})^2 \sigma^2}{r d^2}$$

Where Z_{α} is the normal deviate at α level of significance(Z_{α} is 1.96 for 5% level of significance and 2.58 for 1% level of significance) and $Z_{1-\beta}$ is the normal deviate at $1-\beta$ % power with β % of type II error(0.84 at 80% power and 1.28 at 90% statistical power). $r=n_1/n_2$ is the ratio of sample size required for two groups, generally it is one for keeping equal sample size for two groups, If $r=0.5$ gives the sample size distribution as 1:2 for two groups. σ and d are the pooled standard deviation and difference of means of two groups.

Methodology

Total 50 samples were divided into, 30 subjects with leukoplakia (clinically and histopathologically confirmed) and 20 healthy controls groups.

Non stimulated salivary sample was collected from both the groups and subjected for investigation. The expression of CD44 in saliva of subjects with leukoplakia was assessed by quantitative Real Time-polymerase chain reaction (PCR) method for both the groups.

Results

In the present study, the difference in CD44 gene fold expression ratio between subjects with leukoplakia and controls was calculated by the following two methods; Delta ct method: Delta ct mean of the subjects/ Delta ct mean of controls and Double delta ct method: RQ mean of subjects/ RQ mean of controls. In both the above mentioned methods, values <1 indicate that the gene fold expression of cases are less than controls where as values >1 indicate that the gene fold expression in cases is more than controls. Values =1 indicate no change in gene fold expression.

In the present study, the gene fold expression ratio by delta ct method was found to be $5.777/5.827= 0.9914$ and by double delta ct method was found to be $28.4151/28.0694=1.0123$. As the ratio between subjects with leukoplakia and controls was found close to 1 it can be interpreted as gene fold expression between the cases and controls is not significant / or no change is observed between the subjects with leukoplakia and control group in terms of gene fold expression.

Table 1 and 2 indicated distribution of subjects based on gender, age. Males were 27 with leukoplakia and 17 in control group whereas 3 each with leukoplakia and control group (Table 1). Subjects were evaluated with age range of 21-40, 41-60, and >60 (table 2).

Table 3 indicates distribution leukoplakia lesions based on their clinical form and location. Highest leukoplakia form was homogenous one 24 (Buccal mucosa) and 4 as non homogenous one (4 in buccal mucosa, 1 each in soft palate and gingival). There was 18 cases with no dysplasia, 6 mild for homogenous variety. In non homogenous variety, 3 were no dysplasia, 2 were mild and 1 moderate dysplasia (Table 4).

Based on the quantitative expression of CD44 gene, subjects with leukoplakia had 12 cases of upregulation and 18 with down regulation. In control group there was 18 upregulation and 2 down regulation (Table 5). CD44 gene expression based on clinical forms of leukoplakia lesions indicated in table 6 with highest cases with homogenous compared to speckled type.

Table 7 indicates CD44 gene expression based on degree of dysplasia. There was 9 cases diagnosed as no dysplasia, 3 with mild and 1 with moderate dysplasia.

Discussion:

Oral cancer is the most common type of cancer of the head and neck, with an annual worldwide incidence of 300 000 cases. The disease is an important cause of death and morbidity, with a 5-year survival of less than 50%. Most of the oral cancers arise from potentially malignant disorders. Leukoplakia is the most common potentially malignant disorder of the oral mucosa. The prevalence of oral leukoplakia ranges from 0.4% to 0.7% of the population worldwide.^{1,3}

The present study evaluates and compares the CD44 gene for diagnosis of leukoplakia. In the present study 16% of the subjects presented with epithelial dysplasia. The frequency of epithelial dysplasia in leukoplakia varies between < 1 and > 30% across literature. The lowest frequency of 0.3% was reported in a population-based study in India in which all clinically diagnosed leukoplakias were biopsied and a very small portion of the subjects presented with epithelial dysplasia.

In the present study, the age of the subjects with leukoplakia ranged between 25-77 years. This is in accordance with two Chinese^{12,13} studies in which the age of subjects with leukoplakia ranged from 21-84 years and 24-83 years respectively. In the present study the mean age of subjects with leukoplakia was found to be 48.4 years which is in similarity with studies conducted in India¹⁴, where in the mean ages were found to be 48, 47, years respectively. However studies in countries like Brazil¹⁵, China¹³, Taiwan¹⁶, Netherlands¹⁷ reported a slightly higher mean age of the subjects ie. 54 years, 55 years, 52 years, 51 years respectively.

In the present study, most of the subjects with leukoplakia were in the 5th decade of life. An extensive early survey in India and studies conducted in China¹³ and Taiwan¹⁶ reported a peak incidence of leukoplakia in the fifth decade of life in concurrence with the present study.

It has been reported that the age at which leukoplakia occurs in an individual is an important risk factor that affects the malignant outcome of leukoplakia. The malignant potential of leukoplakia was assessed in a Chinese study¹³ and it was found that the malignant transformation rate of leukoplakia was 72.6% higher in the elderly patients as compared to the younger age groups.

A Hungarian study¹⁸ reported a peak incidence of leukoplakia in the 6th decade of life. This could be due to the prolonged duration of exposure to tobacco amongst the older age groups.

Tobacco usage is the most common etiological factor in the development of leukoplakia. In the developing world, tobacco and areca nut use, either alone or in combination, accounts for the vast majority of leukoplakias. In the present study, 90% of the subjects were smokers and 6% of the subjects used smokeless tobacco habit alone and 3% of subjects used both. The findings of the present study are in agreement with previous reports from Brazil¹⁵, Hungary.¹⁸

C`ema et al evaluated the role of pan-CD44 protein expression in leukoplakia tissues. They concluded that CD9 antigen expression in the exosomes of the oral epithelium explained the intercellular flow of SolCD44 and other fluids in the leukoplakia area.¹⁹ Franzmann et al assessed salivary soluble CD44 (solCD44) expression in HNSCC patients and concluded that, salivary solCD44 ELISA seems to effectively detect HNSCC at all stages.²⁰ Gadge et al stated that among CD44s and its variant isoforms, v5, v6, variant isoform v6 may serve as a marker in detecting high-risk leukoplakias.²¹

Ghazi et al found that diagnostic test's accuracy for identification of OSCC and dysplastic leukoplakia from non-dysplastic leukoplakia and normal tissues and recognition of OSCC from dysplastic leukoplakia showed optimum sensitivity and specificity.²²

Eighty percent of the study subjects presented with a homogenous variety of the disease whereas 17% of subjects presented with a speckled variety of leukoplakia. One subject i.e., 3% presented with verrucous variety of leukoplakia.

In the present study 16% of the subjects presented with epithelial dysplasia. The frequency of epithelial dysplasia in leukoplakia varies between < 1 and > 30% across literature. The lowest frequency of 0.3% was reported in a population-based study in India in which all clinically diagnosed leukoplakias were biopsied and a very small portion of the subjects presented with epithelial dysplasia.

CD44 expression has been assessed in the head and neck region by various methods such as immuno-histochemistry, western-blot analysis, flow cytometry and polymerase chain reactions in various mediums such as the tissue samples, serum and saliva as a diagnostic and prognostic bio-marker. Discrepancies in the expression of CD44 are probably due to the use of different immunohistochemistry protocols and also due to the differences in the patient population examined.

It has been observed that in epithelia that show loss of integrity such as in HNSCC, low solCD44 levels are initially observed in serum and saliva, which leads to the hypermethylation of the CD44 promoter resulting in increased expression of CD44. Hypermethylation status together with increased CD44 levels may be used as an early marker for HNSCC in cases of oral potentially malignant disorders such as leukoplakia.

Hence, an attempt was made to assess the expression salivary soluble CD44 in subjects with potentially malignant disorders such as leukoplakia. The present study showed no change in CD44 gene fold expression when compared between subjects with leukoplakia and controls although an individual pattern of down regulation and up regulation was noted in the cases and the control population. The results of the present study could not be compared with studies in literature as no studies have assessed the expression of CD44 in serum and saliva samples of subjects with leukoplakia.

Further validation of role of CD44 expression in potentially malignant lesions can be substantiated by determining the role of CD44 in tissue samples of oral leukoplakia. The deterministic establishment of role of CD44 observed in tissue samples, would pave way for further studies in serum in saliva samples. This would help in clarifying the role of CD44 in predicting the malignant potential of premalignant lesions .Studies investigating the effect of smoking cessation and reversal of the lesion on the levels of CD44 are strongly recommended. Success in any of these areas could revolutionize oral cancer screening, by providing a simple and reliable measurement of oral cancer risk assessment that can alert primary care providers, dentists, and other frontline screeners to individuals most in need of skilled oral exam at a stage when the process can be more easily treated or perhaps even reversed with behavioral modification.

Conclusion

It was concluded that, an individual pattern of down regulation in 60% of the cases with leukoplakia and up regulation was noted in 90% of the controls by absolute quantification method, and the gene fold expression ratio by relative quantification method between subjects with leukoplakia and controls by delta ct method was found to be $5.777/5.827= 0.9914$ and by double delta ct method was found to be $28.4151/28.0694=1.0123$. There was no difference in the gene fold expression ratio of CD44 between subjects with leukoplakia and controls as the gene fold expression ratio value between subjects with leukoplakia and controls was found to be approximately 1. No statistical significant difference was noted in the expression of CD44 and grades of epithelial dysplasia. CD44 were not found to be a reliable marker in determining the malignant transformation of leukoplakia.

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Table 1: Distribution of subjects based on gender.

GENDER	MALES		FEMALES		TOTAL
	n	%	n	%	
SUBJECTS WITH LEUKOPLAKIA	27	90%	3	10%	30
CONTROLS	17	85%	3	15%	20

Table 2: Distribution of subjects based on age.

AGE RANGE	SUBJECTS WITH LEUKOPLAKIA		CONTROLS	
	n	%	n	%
21-40 YEARS	8	26.7%	5	25%
41-60 YEARS	16	53.3%	10	50%
>60 YEARS	6	20.0%	5	25%
TOTAL	30	100%	20	100%

Table 3: Distribution of leukoplakia lesions based on their clinical form and location.

TYPE OF LEUKOPLAKIA	BUCCAL MUCOSA		LABIAL MUCOSA		TONGUE		SOFT PALATE		GINGIVA	
	n	%	n	%	n	%	n	%	n	%
HOMOGENOUS (24)	24	100%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
NON-HOMOGENOUS (6)	4	66.6%	0	0.0%	0	0.0%	1	16.7%	1	16.7%

Table 4: Distribution of leukoplakia lesions based on degree of dysplasia.

TYPE OF LEUKOPLAKIA	NO DYSPLASIA		MILD DYSPLASIA		MODERATE DYSPLASIA	
	n	%	n	%	N	%
HOMOGENOUS (24)	18	75%	6	25%	0	0.0%
NON HOMOGENOUS (6)	3	50%	2	33.33%	1	16.66%

Table 5: Distribution of subjects based on the quantitative expression of CD44 gene.

	UPREGULATION		DOWNREGULATION	
	n	%	n	%
SUBJECTS WITH LEUKOPLAKIA (30)	12	40%	18	60%
CONTROLS (20)	18	90%	2	10%

Table 6: CD44 gene expression based on clinical forms of leukoplakia lesions.

SUBJECTS WITH LEUKOPLAKIA	UPREGULATION		DOWNREGULATION	
	n	%	n	%
HOMOGENOUS LEUKOPLAKIA (24)	10	41.6%	14	58.3%
SPECKLED LEUKOPLAKIA (6)	2	33.33%	4	66.66%

Table 7: CD44 gene expression based on degree of dysplasia.

SUBJECTS WITH LEUKOPLAKIA	UPREGULATION		DOWNREGULATION	
	n	%	n	%
NO DYSPLASIA (21)	9	42.85%	12	57.14%
MILD DYSPLASIA (8)	3	37.5%	5	62.5%
MODERATE DYSPLASIA (1)	1	100%	0	0.0%



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Comparative Evaluation Of Clinical And Radiographic Horizontal Condylar Guidance Angle Values By Using Two Different Interocclusal Recording Materials In Dentulous Patients

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Abstract

Introduction: The success of prosthodontic procedures can be accurately determined by the exact replication of the patient's condylar path using a semi-adjustable articulator. It allows the clinician to determine the morphology of the occlusal surfaces in relation to the condylar pathway during mandibular movements. If condylar guidance is not exactly registered, it might result in occlusal interferences during movements of the mandible and lengthen chairside adjustment time, which is inconvenient for the patient and dentist.

Materials and methods: A total of 20 dentulous patients from 20 to 30 years of age group attending the department of prosthodontics at Sibar institute of dental sciences were selected for the study. Alu wax and jet bite were used to obtain the protrusive interocclusal record (PIR). Protrusive records were then used to program the semi-adjustable articulator to obtain HCGA values on both sides. Using the same protrusive records CBCT radiograph was taken. Radiographic images were traced on tracing paper and HCGA values were measured using Frankfort's horizontal reference line (the line connecting porion and orbitale) and the mean curvature line (most-superior and most-inferior points of the curvatures) on both sides using a protractor. The data were analyzed by paired sample t-tests and Pearson's correlation tests.

Results: There was no significant difference between the right and left sides in the clinical and radiographic methods using Alu wax PIR. In the clinical method utilizing jet bite PIR, the right side had significantly higher HCGA values than on the left side. On CBCT, no such differences were found.

Conclusion: CBCT horizontal condylar guidance angle values were higher than those obtained using the clinical method by using both records. Values obtained from both the methods, i.e., protrusive interocclusal record and cone-beam computed tomography, were compared and correlated.



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Keywords: Alu wax, Jet bite, Protrusive interocclusal record (PIR), Horizontal condylar guidance angle (HCGA), Frankfort's horizontal plane (FHP), and Cone Beam Computed Tomography (CBCT).

Introduction:

Prosthetic dentistry intends to regain missing tooth morphology and re-establish an ideal occlusion in accordance with the stomatognathic system of the patient. Among five factors that influence the laws of articulation as given by Hanau, the most important factor in establishing balanced articulation and the most predominant consideration in any patient's oral rehabilitation is condylar guidance. ⁽¹⁾According to GPT9, Condylar guidance is defined as the mandibular guidance generated by the condyle and articular disk traversing the contour of the articular eminence.

Research into the mechanism of condyle and efforts to register movements of the mandible began in the 18th century. ⁽²⁾ The "protrusive wax check bites" described by Christensen in 1905 and the graphic approach introduced by Gysi in 1908 were the first methods used to record the HCG. ⁽¹⁾ Since then, many authors have conducted research to determine the HCG using a variety of approaches and compared the variability between different registration techniques, articulator systems, and recording materials.

There are three basic methods for obtaining HCG: (1) Intraoral methods, (2) Extraoral methods, and (3) Radiographic methods. Intraoral methods such as protrusive wax interocclusal records, leaf gauge, Lucia jig, and intraoral tracers can be utilized to determine the mandible's centric and eccentric relationships. ⁽³⁾ Intraoral record regardless of the type of material employed, represents only one position along the condylar path and horizontal condylar guidance angle (HCGA) varies with the degree of protrusion. ⁽⁴⁾ Even if the registration methods are followed precisely, inaccuracies may occur due to friction interference of smooth movements between the articulator's condylar components. ^(3,5) Extraoral methods include radiographs and pantographs. Boos attempted to record the condylar path using a temporomandibular radiograph in 1951 to identify condylar guidance. During the 1970s to overcome the disadvantages of clinical approaches, authors such as Corbett et al. (1971), Ingervall (1974), Christensen and Slabbert (1978) proposed radiographic methods of recording the HCG.

Radiographic techniques include lateral cephalograms, panoramic radiographs, and computed tomograms. ⁽⁵⁾ They are more accurate as it includes stable bony landmarks and can be easily standardized. ^(2,6,7) Although panoramic radiographs and lateral cephalograms provide a two-dimensional (2D) image of the temporomandibular joint (TMJ), ⁽⁵⁾ CBCT, as a more advanced cutting-edge technology, provides three-dimensional (3D) multiplanar sections without superimposition, allowing the glenoid fossa and articular eminence to be clearly seen from the surrounding structures. ^(5,8) Tomography scans, on the other hand, have been safer, requiring less radiation exposure and becoming more effective since the introduction of CBCT, resulting in its outspread usage in dentistry. ⁽⁹⁾ However, the limitations of CBCT include the high cost of the equipment. ^(10,11)

Most of the studies used panoramic imaging and lateral cephalograms for recording HCGA. ⁽²⁾ There are very few studies comparing clinical and radiographic procedures employing CBCT. So, the goal of this study was to compare HCGA values obtained from protrusive interocclusal records of two different materials (Alu wax and Jet bite – polyvinyl siloxane) with the CBCT (panoramic section) radiography technique.



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Materials and Methods:

The study was conducted in the Department of Prosthodontics, Sibar institute of dental sciences, Guntur, Andhra Pradesh, India. Written consent was obtained from all the patients. Twenty patients aged between 20 and 30 years with a full complement of dentition fulfilling the following criteria were selected.

Inclusion Criteria: Inclusion criteria are Angle's Class I molar and incisal relations, individuals with impacted third molars, absence of crowded or maligned teeth, good oral hygiene, and periodontal status.

Exclusion Criteria: Exclusion criteria are facial or skeletal malformations, Poor neuromuscular coordination, and Individuals with severe Temporomandibular joint disorders (myofascial pain dysfunction syndrome, osteoarthritis, internal derangements, rheumatoid arthritis, ankylosis, tumors, trauma, and developmental disorders), deteriorating general health.

A) Making of impression & obtaining casts:

Diagnostic impressions were made using a stock tray (perforated stainless steel) using irreversible hydrocolloid impression material. The impressions were rinsed, dried, inspected, and disinfected using 2% glutaraldehyde (Cidex) for 10 minutes and the cast was poured with type III dental stone. Six wedge-shaped notches were made at the top of the maxillary cast for the split cast where two notches toward the outer edge of the posterior border, two in the first molar region, and two in the lateral incisor region.

B) Face bow transfer

Facebow transfer was done using a Hanau spring bow and this assembly was transferred to the articulator using indirect mounting transfer and the casts were mounted (Figure 1 and Figure 2).

C) Interocclusal bite registration method:

To obtain records initially, the patient was trained to move the mandible forward till the teeth are in an edge-to-edge relationship. Two bite registration materials were used, i.e., alu wax and jet bite (polyvinyl siloxane). To obtain a wax record a two-sheet thick alu wax rim is adapted to the maxillary arch and the patient was asked to move the teeth into edge-to-edge relation (Figure 3 and Figure 5). This record was transferred to the articulator (HANAU Wide-View Articulator). Protrusive relation was assessed and reconfirmed before setting the horizontal condylar inclinations, and locknuts were tightened. In all the cases, the articulator was programmed by a single operator only.

D) Programming of articulator:

In this study, Hanau articulator was modified to obtain accuracy of condylar guidance. The articulator has a numerical scale condylar track with 5° increments. A protractor with angulations from 0° to 60° was mounted to the condylar shaft for more precise values. The protractor's center was set to align with the condylar shaft's center. While measuring the angle, the protractor's 0° was aligned with the 0° pointer. A 23-gauge orthodontic wire was used to make an L-shaped extender and was attached to the zero-reference line. While interpretation, if the protractor's pointer was at 0.5mm or more, the higher value is considered as the reading, and if the pointer was at less than 0.5mm, the lower value is considered. To determine the horizontal condylar guidance articulator condylar locks were loosened, and the protrusive record was positioned on the mandibular cast. The maxillary cast was placed



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on the imprints of the protrusive record. The upper member of the articulator was adjusted so that the maxillary cast would seat into the indentations of the split cast, and the two parts were held together firmly (Figure 4 and Figure 6). Readings of the condylar guidance angle values on the right and left sides of the articulator were recorded. Same procedure was repeated three times until two similar values are obtained (Figure 7). The condylar screws were tightened, and the right and left condylar readings were recorded and calculated.

E) Radiographic method:

CBCT was obtained with the patient's mandible in a protrusive position along with bite registration material (Figure 8). Articular eminence and mandibular fossa were identified on both the right and left sides (Figure 9). Tracings were done on the CBCT image, i.e., the Tangent of the Posterior slope of the articular eminence was drawn, a line joining the superior most point of the external auditory meatus (Porion) and the Inferior most point of orbital margin (Orbitale) were marked. This represents the radiographic horizontal condylar guidance angle (Figure 10). The angles between these two lines were evaluated by using a protractor.



FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4



FIGURE 5

FIGURE 6

FIGURE 7

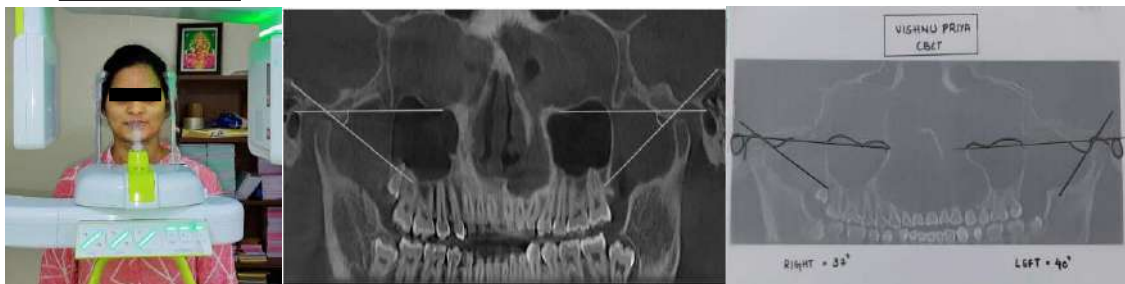


FIGURE 8

FIGURE 9

FIGURE 10


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Statistical Analysis: Statistical analysis was done using SPSS version 20 software (IBM Corp., Armonk, NY, USA). Descriptive statistics, paired sample t-tests, and Pearson's correlation coefficient tests were done to analyze the study data. Bar charts and matrix scatter plots were used for data presentation.

Results Summary:

Table 1 represents the comparison of horizontal condylar guidance angle values between the right and left sides in both CBCT and clinical method using Alu wax protrusive inter-occlusal record. There were no significant differences between the right and left sides in both the clinical and radiographic methods (Figure 1). Significantly higher horizontal condylar guidance angle values were observed on the right side in the clinical method using jet bite protrusive inter-occlusal record when compared to the left side (Table 2). No such differences were observed in the CBCT evaluation (Figure 2). Comparison of horizontal condylar guidance angle values between CBCT and clinical method using Alu wax protrusive inter-occlusal record was done on right and left sides. Significant differences were noted with higher mean horizontal condylar guidance angle values documented using CBCT compared to the clinical method. Similar observations were made with regard to jet bite protrusive inter-occlusal record using CBCT, demonstrating significantly higher mean horizontal condylar guidance angle values on both the right and left sides.

Table 1: Comparison of horizontal condylar guidance angle values between right and left sides in both CBCT and clinical method using Alu wax PIR

Method	Side	Mean	N	Std. Deviation	Std. Error Mean	t value	P value
Clinical	RIGHT	31.90	10	2.767	.875	0.068	0.948
	LEFT	31.80	10	4.917	1.555		
CBCT	RIGHT	37.00	10	2.160	.683	-0.525	0.612
	LEFT	38.00	10	6.146	1.944		

Paired sample t test; $p \leq 0.05$ considered statistically significant; PIR—Protrusive Inter-occlusal Record

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 SIBAR INSTITUTE OF DENTAL SCIENCES
 GUNTUR-522509, A.P., India.

Figure 1: Comparison of horizontal condylar guidance angle values between right and left sides in both CBCT and clinical method using Alu wax PIR

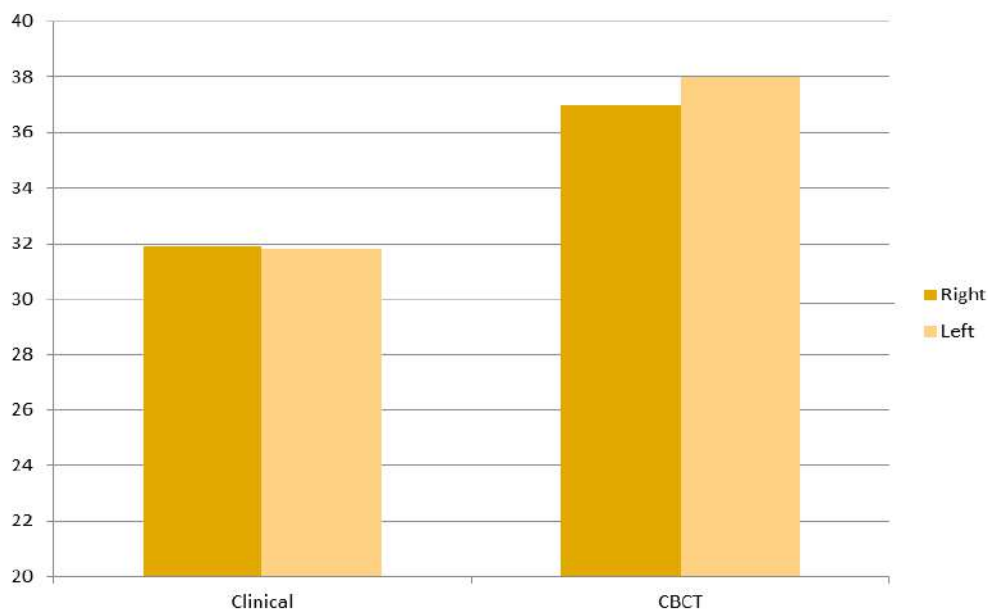


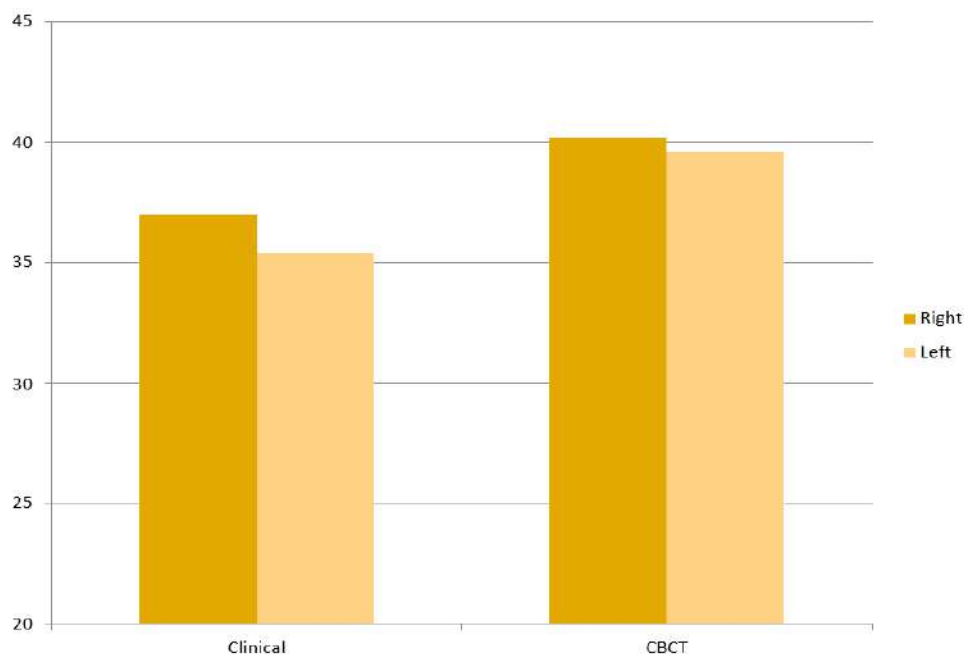
Table 2: Comparison of horizontal condylar guidance angle values between right and left sides in both CBCT and clinical method using Jet bite PIR

Method	Side	Mean	N	Std. Deviation	Std. Error Mean	t value	P value
Clinical	RIGHT	37.00	10	2.357	.745	2.753	0.022*
	LEFT	35.40	10	1.647	.521		
CBCT	RIGHT	40.20	10	2.573	.814	0.874	0.405
	LEFT	39.60	10	1.265	.400		

Paired samples t test; $p \leq 0.05$ considered statistically significant; * denotes statistical significance; PIR – Protrusive Inter-occlusal Record


Dr. L. KRISHNA PRASAD
 DEAN
 SIBAR INSTITUTE OF DENTAL SCIENCES
 GUNTUR-522509, A.P., India.

Figure 2: Comparison of horizontal condylar guidance angle values between right and left sides in both CBCT and clinical method using Jet bite PIR



Discussion:

The relationship between the occlusal surface morphology and the condylar path traced during mandibular movements have been well documented. Gysi (1910), Gilis(1926), and Gysi & Kohler (1929) were the first investigators to recognize the importance of determining the horizontal path of condyles while restoring occlusion in patients.⁽¹⁾ HC Gangles range from 5°-55° in studies conducted by various authors. (Zamacona, Otaduy, Aranda,1992., dosSantos Jr, Nelson, Nowlin, 2003., Lundeen, Wirth, 1973., Woelfel, Winter, Igarashi, 1976., Hobo, Mochizuki, 1982).⁽¹²⁾

The oldest method of recording HCGA is by using an interocclusal record.^(13,14,15) It is a widely practiced and accepted clinical method to record horizontal condylar guidance angle values (Posselt,1968.,Mohl,et.al.1988.,Posselt,1968.,Rosensteil,Land,&Fujimoto, J.2006).⁽¹²⁾ Alu wax and Poly vinyl siloxane were chosen as materials of choice to obtain inter-occlusal protrusive records in this study. In comparison to base plate wax, Millstein et al. discovered that adding 0.05 mm thick aluminum laminate between wax wafers loaded with copper particles enhanced accuracy. In comparison to the wax recording materials, the Coprwax aluminum laminated, metalized wax wafer has shown less distortion.⁽¹⁶⁾Breeding and Dixon investigated the compression resistance of numerous elastomeric interocclusal record materials. He stated that interocclusal

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SIBAR INSTITUTE OF DENTAL SCIENCES
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recording material made of poly vinyl siloxane had the best compression resistance.⁽¹⁷⁾

Another method of recording HCGA used in this study was utilizing radiographs.⁽⁴⁾ Radiographic methods have been demonstrated to record horizontal condylar guidance more precisely than other methods (dosSantos Júnior, Nelson, & Nummikoski,1996., Christensen,& Slabbert, 1978., Gilboa, Cardash, Kaffe, & Gross, 2008). Christensen and slabbert stated that "No radiographically determined sagittal condylar guidance angle corresponded with that acquired using intra-oral records". The angle determined by radiographs had a higher mean value than the clinical data.⁽⁶⁾

The clinical and CBCT radiographic image of the sagittal outline of the articular eminence and glenoid fossae was identified in all 20 subjects. Using Alu wax PIR horizontal condylar guidance angle values between the right and left sides in the clinical method were 31.90° and 31.80°. Using Alu wax PIR horizontal condylar guidance angle values between the right and left sides by CBCT method were 37.00°and 38.00°. Using jet bite PIR horizontal condylar guidance angle values between the right and left sides in the clinical method were 37.00° and 35.40°.Using jet bite PIR horizontal condylar guidance angle values between right and left sides by CBCT method were 40.20°and 39.60°.Comparison of horizontal condylar guidance angle values between the clinical method and CBCT using Alu wax PIR on the right side were 31.90° and 37.00°. Comparison of horizontal condylar guidance angle values between the clinical method and CBCT using Alu wax PIR on the left side were 31.80° and 38.00°. Comparison of horizontal condylar guidance angle values between clinical method and CBCT using jet bite PIR on the right side were 37.00°and 40.20°.Comparison of horizontal condylar guidance angle values between clinical method and CBCT using jet bite PIR on the left side were 35.40° and39.60°.

There was no significant difference between the right and left sides in both the clinical and radiographic methods using Alu wax PIR. In the clinical method utilizing jet bite PIR, the right side had significantly higher horizontal condylar guiding angle values than the left side. On CBCT, no such differences were found. Condylar guidance values recorded using CBCT were higher than those obtained using the clinical method by using both the records.

According to the literature, the right and left eminences rarely have the same slants and contours. Csado et al, Shrestha et al, and Prasad et al found smaller mean differences between the right and left HCG angle in their studies. These values are statistically not significant but clinically and radiographically, the value of HCG angles was higher on the right side as compared to the left side.⁽¹³⁾ Alshali et al. found no statistically significant differences in sagittal condylar inclination values between the male and female groups or between the right and left sides. Sagittal condylar inclination angle decreased with age on both the right and left sides, with statistically significant changes in values for both methods. Godavarthi et al found that the condylar guidance angle for the right and left sides was 38.62°and38.05°respectively, which was statistically insignificant.⁽⁸⁾

Gilboa et al. discovered a positive association between anatomic and radiographic angles for articular eminence inclination, with correlation coefficients of 0.561 and 0.802 for the left and right sides, respectively.⁽¹⁸⁾According to a study by Kaur S et al., the mean difference in the angles of inclination of the inferior border of the zygomatic arch measured by radiographic and anatomic methods was 8 and 8.47 for the left and right



Dr. L. KRISHNA PRASAD
DEAN

sides, respectively.⁽¹⁹⁾

In this study CBCT values are on an average of 6° - 7° higher than the clinical method utilizing alu wax interocclusal record. CBCT values on an average of 3° - 4° higher than the clinical method utilizing Jet bite interocclusal record. Based on this study CBCT values have a moderate correlation with the clinical method utilizing jet bite interocclusal records.

Tannamala et al found a 2° - 4° difference in HCG angle between OPG and the protrusive occlusal record,⁽²⁰⁾ while Shreshta et al found a 9° - 10° difference in HCG angle between CBCT images and the protrusive occlusal record,⁽⁶⁾ and Das et al found no significant difference in HCG angle between CBCT images and the protrusive occlusal record.⁽⁸⁾ Shetty and colleagues also found higher condylar guidance values from the radiograph when evaluating the reliability of programming the articulator using the radiographs and the interocclusal records.⁽²¹⁾ Radiographically determined condylar angles provided greater results than intraoral recording techniques, according to Christensen et al. In a study by Vadodaria, condylar guidance acquired by CBCT was about 10° higher than clinical methods, as evidenced by Jerath⁽¹⁴⁾ et al, Kwon⁽⁹⁾ et al, and Naqash⁽²⁾ et al, who found that HCG angle values acquired from CBCT measurements were 5° - 6° higher than those obtained from protrusive occlusal records.

In general, the results of this investigation show that none of the clinical approaches were found to provide condylar guidance angle values that were comparable to the CBCT. Dimensional changes in materials, in accuracies discovered in the casts, or failure to achieve the desired accuracy of the fit of interocclusal record on the occlusal surfaces are all possible reasons for the drawbacks of the clinical method.⁽²²⁾ According to Muller et al, the removal and placement of the record eventually produce discrepancies. According to Donegan and Christensen the common reason for the inconsistency of intra oral methods is that the horizontal condylar angle changes with the degree of protrusion, regardless of the material selected.^(4,6) According to Ratzmann et al, Intraoral method of recording condylar guidance angle, have inferior levels of reproducibility and are prone to variation in the operator, instrument, and occlusal records.⁽⁶⁾

CBCT scans for condylar measures may be beneficial, especially in the case of complex oral rehabilitations. To corroborate the current findings, substantially higher sample sizes with Condylar Guidance angle values are required to confirm the current findings. This would ease the procedure to establish a predictable occlusal scheme in the prosthetic rehabilitation of patient.

Summary and Conclusion:

Although many clinicians depend on the average condylar guidance angle values ranging from 22° to 65° for the fabrication of the prosthesis, occlusal harmony could not be established since the HCI of the patient did not match with the patient's own values. HCGA values obtained via radiographic techniques can be utilized directly to program the semi-adjustable articulators, eliminating the need for technique-sensitive-dependent clinical approaches which are dependent on the operator or patients' neuromuscular control.

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Electric Dental Anesthesia: A Review

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Abstract

The use of efficient local anesthetic is crucial in contemporary dentistry. The majority of young patients who receive local anaesthetic injections feel scared and anxious. Even more concerning is the possibility that uncomfortable child will put off getting the necessary dental treatment and might put off getting it again as adults. Local anesthetic is the primary method of pain management in the field of dentistry, however research is ongoing to identify more creative and effective approaches. Several pharmacological and alternative delivery techniques have been created to alleviate the discomfort connected with local anaesthetic injections. TENS has been proposed as a more convenient and advantageous substitute for traditional LA methods for decreasing anxiety. TENS is described as the direct stimulation of nerves by electrical impulses of brief duration and small amplitude. To explain the mechanism of action of TENS in regulating pain, a number of theories have been put forth, including the Gate Control Theory, Endorphin Release Theory, and Serotonin Release Theory. Hence, the purpose of present review of literature is to discuss the electronic dental anesthesia (EDA)/transcutaneous electric nerve stimulation (TENS) in detail.

Keywords: Pain, Electronic Dental Anaesthesia, Local Anaesthesia, TENS

Introduction: It is crucial to manage pain during both invasive and non-invasive dental procedures because discomfort may cause patients to refuse care or put off getting it altogether.¹ In dentistry, invasive procedures, such as tooth extractions and surgeries, are more frequently associated with pain than non-invasive ones. The use of local anaesthetics is used to prevent and manage pain, and they are thought to be the safest and most effective medications available for these purposes. But because many people are afraid of the needle used when injecting, the act of administering these medications also causes panic in the patients. Even in preverbal children, fear of pain is not unwarranted because untreated pain has a negative impact on health outcomes and is recalled by preverbal children.²

Several methods have been suggested to reduce pain caused by the administration of local anesthetic agents. These include the following: Application of topical analgesics such as amethocaine³, Distraction techniques^{4,5}, Counter irritation⁶, Warming the anaesthetic agents⁷, Adjusting the rate of infiltration by reducing the speed of the injection⁸, Buffering the local anesthetic agent^{9,10}

TENS, also known as transcutaneous electrical nerve stimulation, is a peripheral nerve stimulation technique for pain relief. It's a technique that provides us a promising way to produce dental anesthesia by using a mild amount of electric current and is based on the much-established Gateway Theory of pain control given by Malzack and Wall in 1965.¹¹ TENS has been given U.S. Food and Drug Administration approval.^{12,13} In this mode of therapy, electrodes are used to transmit pulsed electrical current, which is created in one of two ways - direct current or batteries - along the intact skin's surface in order to stimulate superficial nerves and relieve localised pain. TENS is frequently used in the realm of medicine to treat both acute and persistent pain.¹⁴ Purpose of present review of literature is to discuss the electronic dental anesthesia/transcutaneous electric nerve stimulation in detail.

History of TENS: The idea of relieving pain through the use of electricity is not a new thought. Ancient Egyptians applied electrogenic fish to body part of that hurt. Electrostatic generators rose to prominence in the eighteenth century, but by the end of the nineteenth century, their employment had decreased due to the widespread use of other pharmaceutical techniques. Melzack and Wall came to the conclusion in 1965 that electrical stimulation of the nervous system can help stimulate large diameter afferent nerve fibres, hence blocking the transmission of unpleasant sensations via the brain's central nervous system. This was written about in the book "Pain Mechanisms: A New Theory." In clinical studies, it was found that electrical current stimulation of brain inhibitory pathways helped reduce pain. The effectiveness of implants that activated the dorsal column was first evaluated using TENS therapy. TENS was later discovered to be a stand-alone treatment.^{11,13}

Type of TENS: The most common type of TENS utilised in dental practises is conventional TENS. It can be used continuously throughout the day with periodic breaks to prevent skin irritation. AL-TENS can be used for 30 minutes at a time and employs low frequency with high amplitude. Intense TENS therapy uses high-frequency and high-amplitude pulsed current, which is less pleasant and is not frequently employed.¹⁴⁻¹⁶ (**Table no. 1**)

Conventional TENS	The targeted stimulation of A-beta fibres is the justification for using traditional TENS. These afferent neurons have a wide diameter, are non-noxious, and have a low pain threshold. The central nervous system's nociceptive neurons experience an inhibition of activity as a result of TENS stimulation. Pain relief is attained by raising the pulse amplitude in TENS to create a nonpainful paraesthesia below the electrodes. ¹⁵
Acupuncture-Like TENS	AL-TENS functions by stimulating high-threshold peripheral afferent neurons called A-delta fibres. As a result, descending pain inhibitory circuits are activated. As a result of this therapy's activation of numerous muscle afferent nerve fibres, muscle twitching is observed. For pain relief, electrodes are placed over myotomes or trigger points, or at acupuncture points. ¹⁷
Intense TENS	To limit the spread of painful stimuli in peripheral nerves and activate extrasegmental analgesic mechanisms, high-intensity TENS is used to stimulate high-threshold cutaneous A-delta fibres. ¹³

Parts of TENS: Main parts of TENS systems are:

1. TENS unit.
2. Lead wires.
3. Electrodes

TENS Unit: It is a generator of electric pulses. It comes in two forms: "Clinical" model: This is used by dentists in the clinic and generates electricity by being linked to the building's electrical socket. Model "Patient": This is a compact, transportable device that the patient can carry in their pocket or fasten to their belt or outfit. It has a battery inside as a power supply.¹⁸



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DEAN

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Lead Wires: These establish an electrical connection between the electrodes and the TENS unit.¹⁹

Electrodes: The TENS unit's electric flow is transformed into an ionic current flow in living tissue by the use of electrodes. Both extra- and intra-oral placement of electrodes is possible. There are two types of extraoral electrodes: Flexible electrodes made of silicone rubber impregnated with carbon are attached to the skin's surface with the help of an electrically conductive gel. Surgical tape is used to keep them in position. Tin plate or aluminium electrodes that don't conform to the body are attached to the skin's surface using cotton pads or sponges soaked in tap water. Cotton roll electrodes, clamp electrodes, and sticky electrodes are the intraoral electrodes. Nowadays, adhesive electrodes are the most used kind. Due to their thinness and flexibility, these electrodes can easily acclimate to the oral mucosa.²⁰



Figure no 1: TENS unit A) Adaptor B) TENS unit C) Electrodes D) Lead wire

Procedure: To get rid of any skin oils or other things that can obstruct the flow of electricity, the electrode pad site is gently swabbed with surgical spirit. Before positioning, electrode gel is placed to the electrode pad. The electrode pad is then taped into place to prevent displacement while the patient is advised to open their mouth widely as it is done during therapy. Once the device is turned on, the physician can progressively increase the amplitude dial to the point where the patient feels a noticeable feeling. This amplitude level is kept for the first few seconds to give the patient time to adjust to the novel sensation of electronic anaesthetic. Up until the fasciculation is detected on a neighbouring area, such as the lower lip in the case of a mandibular arch, the amplitude is increased to the next level and the cycle is repeated. Reaching the "minimum therapeutic level," at which the dental procedure can start, is indicated by muscle fasciculation.^{21,22}

Discussion: The primary goal of the paediatric dentist is to gain the child's cooperation during various paediatric procedures in the dental office. The most difficult aspect of the procedure is giving anaesthesia to young individuals. During a paediatric operation, the child's main concern is getting uncomfortable local anaesthetic (LA) injections. A youngster experiences psychological distress at the mere sight of needles and syringes, which

interferes with their ability to control their conduct. Reducing the child's fear of pain during LA injections increases their trust in the dentist, resulting in the child cooperating with the procedure.²³

There are numerous methods available to lessen pain with LA injections. The foundation of transcutaneous electrical nerve stimulation (TENS) is the gate control theory introduced by Melzack and Wall in 1965. The first description of its utilisation in several medicine-related sectors came from Shane and Kessler in 1967. For pain relief during LA injections, transcutaneous electrical nerve stimulation directly stimulates the nerves with electrical impulses of brief duration and small amplitude. During LA injections, transcutaneous electrical nerve stimulation was found to be more comfortable and effective in lowering pain and anxiety.^{12,24}

Analgesic effect of TENS is based on two main theories: Gate control theory of pain and endogenous opioid theory.

Gate control theory; the most widely accepted explanation to describe the mechanism of action of TENS is the gate control theory of pain, which Melzack and Wall developed in 1965. They proposed that the dorsal horn of the spinal cord's substantia gelatinosa serves as a gate control system that modifies afferent patterns from peripheral fibres before they have an impact on the spinal cord's first central transmission [T] cells. Pain is transmitted by tiny, unmyelinated "C" fibres, and their activity retains the gate in a somewhat open posture. To prevent impulses from reaching T cells, activity of big myelinated A fibres closes the gate and pre-synaptically inhibits input from C fibres.¹¹

Endogenous opioid theory, Reynolds (1969) demonstrated in 1969 that electrical stimulation of the periaqueductal grey region of the midbrain causes analgesia comparable to that brought on by morphine. This eventually led to the discovery of a number of morphine-like substances known as endorphins, which exist at different levels of the pain control pathway.²⁵ Therefore, another theory for how TENS works is that it increases the release of endogenous opioids in the spinal cord, which may happen as a result of activating local circuits within the spinal cord or descending pain-inhibitory pathways.²⁶

Choudhari SR et al. (2017) conducted a study on comparison of TENS and 20% benzocaine topical anesthetic gel was done in reducing pain during administration of IANB injection. It was found that TENS can significantly reduce the pain and discomfort when compared to application of 20% benzocaine gel for 2 min.²⁴ In similar study conducted by Siddiqui A et al. (2021) compared efficacy of TENS and lignocaine topical anesthetic gel in reducing pain during administration of IANB injection and found that transcutaneous electronic nerve stimulation can be used as an effective means to reduce the intensity of pain during local anesthetic injection in pediatric dental patients.²⁷

During various dental procedures, electrical stimulation lessens a number of disruptive clinical behaviours, such as weeping and abrupt movement, which aids in the patient's behavioural management.²⁸ A 93% success rate was noted when TENS was used as an analgesic in place of LA during restorative therapies in a research by Bishop.²⁹ From the literature it can be said that EDA is non-invasive, safe and can be used to achieve anesthesia in needle-phobic patients. As compared to local anesthesia there is no postoperative anesthesia after the TENS unit is turned off. Patients are able to self-administer TENS treatment and learn to titrate dosages accordingly to manage their painful condition. This results in positive acceptance by the patients.³⁰

Conclusion: The use of TENS during LA administrations has been found to be much more effective, pleasant, and pain-reducing. Pediatric dentistry has found transcutaneous electrical nerve stimulation to be a safe, dependable, and useful in invasive dental procedures.

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Oral Hygiene Practices and Caries Experience among School Leaving Children in Rural Area

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Abstract

Background: Dental caries is the most common chronic childhood disease and its treatment is the most prevalent unmet health need among school-leaving children. Caries experience among school leaving age group is important as India is a rapidly growing nation in terms of population as today's children are tomorrow's future citizens of the nation. **Methodology:** To know the oral hygiene practices and caries experience among school-leaving children in a rural area, a cross-sectional study was conducted in the Chebrolu Mandal using a multistage random sampling technique to obtain the required sample size. The data were collected using pretested structured questionnaire followed by oral examination using the decayed, missing, and filled teeth (DMFT) index. The descriptive analysis included mean and standard deviations, Chi-square test, unpaired *t*-test, and one-way analysis of variance. **Results:** Only a few children have a habit of brushing their teeth twice daily 137 (27.6%); rinse their mouth after every meal 116 (23.4%); dispose of toothbrush every 3 months (11.7%); spends <1-min brushing (4.8%); and cleans their tongue using tongue cleaner (25.2%). When DMFT scores were compared to the brushing techniques of the children, significantly higher mean DMFT scores were observed in children who brush once a day (1.49 ± 1.133). **Conclusion:** Caries' experience was more in children who brushed their teeth once a day with horizontal brushing technique and this shows that the study subjects do not follow correct brushing techniques and other oral hygiene practices.

Keywords: Dental caries, DMF index, oral hygiene, tooth brushing

INTRODUCTION

Dental caries is viewed as a most extreme public health problem universally because of its high pervasiveness and critical social effect. The World Health Organization proclaims that 60%–90% of school children worldwide have experienced caries, with the disease being most prevalent in Asian and Latin American countries.^[1] Dental caries is the most common chronic childhood disease and its treatment is the most prevalent unmet health need in children.^[2]

Dental caries affects people of all ages with the highest priority risk group being school-going children. More than 50 million h are lost from schools annually due to oral hygiene-related diseases. Studies have shown that school hours are skipped because of high tooth decay in both primary and permanent dentition. According to the National Oral Health Survey 2002–2003, caries prevalence in India was 63.1% (mean

decayed, missing, and filled teeth [DMFT] 2.3) for 15 years of age group children.^[3]

Dental caries causes pain, discomfort in chewing, food lodgment, headache, restlessness, and sleeplessness and thus influence the esthetic, functional, well-being, and quality of life. It additionally causes foul breath and taste. Further progressed the infection can spread from the tooth to the surrounding soft tissues with resultant complications such as cavernous sinus thrombosis and Ludwig's angina (Cellulitis) which can be life-threatening.^[4]

Children who suffer from poor oral health are 12 times more likely to have more confined activity days including missing schools than those who do not. While there has

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been improvement in the oral health of children in the last few decades, tooth decay remains one of the most common childhood diseases, in both industrialized and developing countries. A substantial portion of children in many developing countries are affected by tooth decay and most decay is left untreated due to limited access to oral health services.^[5]

Unfortunately, oral care was more concentrated on the treatment aspect than on preventive care. Oral health is a fundamental part of well-being throughout life, because of the relationship of the oral cavity with the improvement of a personality, discernments, and quality of life. Untreated oral problems among children habitually lead to major general health problems, critical pain, interrupt with eating, and school absence. The earliest sign of a new carious lesion is the appearance of a chalky white spot on the tooth surface, which indicates an area of demineralization of the enamel surface.^[6]

Taking into account that school age is a compelling period during which each child perceives health-related behaviors, convictions, and attitudes and that dental caries is irreversible, endeavors ought to be focused on revealing factors that help in the prevention of dental caries.^[7]

With this background, we have done a study in the Chebrolu Mandal to know the oral hygiene practices and caries experience among school-leaving children in a rural area.

METHODOLOGY

A cross-sectional, multilocality, school children-based study was conducted from December 2019 to January 2020 in Chebrolu Mandal where the list of all the schools, with children of school leaving age, situated in Chebrolu Mandal in Guntur district was obtained from the District Education Officer. A multistage random sampling technique was carried out to obtain the required sample size. For computing sample size, a pilot study was conducted among 20 school children to know the prevalence of dental caries and the feasibility, and validity of the questionnaire. Out of 20 children examined, a prevalence of 80.5% dental caries was observed, while content validity was assessed using the content validity ratio in the target population, and the value was found to be 0.75. The sample size was derived using the formula Z^2PQ/d^2 where Z = standard normal deviate – set at 1.96 at 95% confidence level, P = proportion of the study population ($P = 90.7\%$), $q = 1 - p$, and d = degree of accuracy desired at 0.05; it was determined that 496 school children were included into the study. Ethical clearance was obtained from the institutional ethical committee (37/IRB/SIBAR/2019).

In the first stage, the Mandal was divided into four zones: north, south, east, and west. School children who were present on the day of examination were included in the study. Children with physically or mentally challenged, who were not willing to participate, and were absent on the day of examination were excluded from the study. The structured format consisted of three sections. The first section collected

demographic information of the participants such as age in years, and gender. The second part of the format consisted of questions regarding oral hygiene practices which included the frequency of cleaning and materials used to clean the teeth. The third part consisted of data regarding dental caries experience which was recorded using the DMFT index for permanent dentition. Oral examination was conducted in the school classrooms, under the normal room light with a mouth mirror and CPI probe, and according to the WHO criteria. The collected data were analyzed using the IBM SPSS Statistics for Windows, Version 20.0. IBM Corp, Armonk, New York, USA software package. The descriptive analysis included mean and standard deviations, Chi-square test, unpaired t -test, and one-way analysis of variance. The level of significance was set at $P \leq 0.05$.

RESULTS

A total of 496 children participated in the study, of which 240 (48.4%) are male and 256 (51.6%) are female children with a mean age of 15.23 ± 0.58 years, while 490 (98.8%) of them are using a toothbrush and paste for cleaning their teeth and has adopted horizontal method of brushing (74.2%). Only a few children have a habit of brushing their teeth twice daily 137 (27.6%); rinse their mouth after every meal 116 (23.4%); dispose of toothbrush every 3 months (11.7%); spends <1-min brushing (4.8%); and cleans their tongue using tongue cleaner (25.2%) [Table 1].

The mean DMFT of the study participants was 1.35 ± 1.106 , where males had more mean DMFT score (1.66 ± 1.168) compared to females (1.07 ± 0.964) ($P = 0.001$) while male children reported higher scores of decay component (1.28 ± 1.007), missing component (0.05 ± 0.218), and filled component (0.35 ± 0.655) of the DMFT index [Figure 1].

When DMFT scores were compared to the brushing techniques of the children, significantly higher mean DMFT scores were observed in children who brush once a day (1.49 ± 1.133); who do not rinse their mouth after every meal (1.46 ± 1.018), and in children who spend less than 1 min for brushing (1.79 ± 1.215) [Table 2].

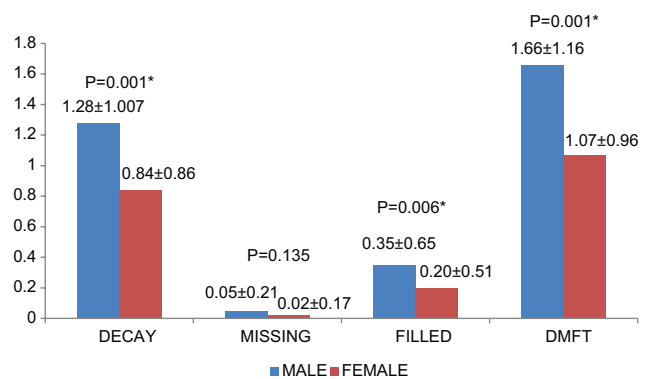


Figure 1: Illustrating the gender-wise comparison of mean DMFT. Chi-square test, *statistically significant. DMFT: Decayed, missing, and filled teeth

Table 1: The demographic details and brushing habits of the study population

Descriptives	Frequency (%)
Gender	
Male	240 (48.4)
Female	256 (51.6)
Method of cleaning	
Toothbrush and paste	490 (98.8)
Brush and powder	4 (0.8)
Finger and powder	2 (0.4)
Frequency of brushing	
Once	359 (72.4)
Twice	137 (27.6)
Method of brushing	
Horizontal	368 (74.2)
Vertical	58 (11.7)
Both	70 (14.1)
Disposal of brush	
3 months	58 (11.7)
6 months	73 (14.7)
Yearly	111 (22.4)
When frayed up	91 (18.3)
Don't know exactly	163 (32.9)
Time spend on brushing (min)	
<1	24 (4.8)
1	44 (8.9)
2	215 (43.3)
>2	213 (42.9)
Rinsing mouth after every meal	
Yes	145 (29.2)
No	116 (23.4)
Sometimes	235 (47.4)
Cleaning of tongue	
Tongue cleaner	125 (25.2)
Finger	13 (2.6)
Toothbrush	273 (55.0)
Don't clean	85 (17.1)

Table 2: Mean decayed, missing, and filled teeth of the participants according to their brushing habits

Independent variable	n	Mean (DMFT) ± SD	Significance
Frequency of brushing			
Once	359	1.49±1.133	0.001*
Twice	137	1.01±0.951	
Rinsing mouth after every meal			
Yes	145	1.16±1.047	0.043*
No	116	1.46±1.018	
Sometimes	235	1.39±1.173	
Time spend on brushing (min)			
<1	24	1.79±1.215	0.045*
1	44	1.57±1.149	
2	215	1.37±1.140	
>2	213	1.24±1.035	

*Statistically significant. Chi-square test. SD: Standard deviation, DMFT: Decayed, missing, and filled teeth

One decayed, two decayed, three decayed, and four decayed teeth were observed to be more in males (40.4%, 23.3%, 10.8%, 2.1%) compared to females (37.9%, 15.6%, 5.1%, 0%) whereas no decayed tooth is observed more in females (41.4%) than males (23.3%) which was statistically significant (0.001) [Table 3].

One decayed, two decayed, three decayed, and four decayed teeth were observed to be more in participants who brushes once a day (40.4%, 21.2%, 8.4%, 1.4%) compared to participants who brushes twice a day (35.8%, 14.6%, 6.6%, 0%) whereas no decayed tooth is observed more in twice a day brushes (43.1%) than once a day brushers (28.7%) which were statistically significant (0.023) [Table 4].

DISCUSSION

Decayed teeth are one of the most common chronic diseases of childhood in the low middle income countries like India where untreated cavities might cause pain and infections that may lead to various problems with eating, speaking, playing, learning, and eventually affects the development of the children.^[8] Children who have poor oral health often miss school and might receive lower grades than the children who do not. Therefore, treating the decay at an early age results in the cessation of further progression of oral diseases thereby lowering the financial burden of dental-care treatment and achieving a better quality of life.^[9]

In the present study, 240 (48.4%) were male and 256 (51.6%) were female children showing a female preponderance which was, in contrast, to the study done by Singh and Sethi^[10] where male children are more than female children. Four hundred and ninety (98.8%) children use a toothbrush and paste which was similar to the study conducted by Bhagat *et al.*^[11] while 2 (0.4%) of them use the finger with tooth powder as oral hygiene practice.

In this study, the overall mean DMFT of school children was 1.35 ± 1.106, and boy's mean DMFT (1.66 ± 1.168) was slightly higher than girls (1.07 ± 0.964), which was, in contrast, to a study done by Pai *et al.*^[12] and Aparna *et al.*^[13] and Al-Rafee *et al.*^[14] where the mean DMFT of study subjects was higher when compared to the present study, whereas the mean DMFT for the present study was higher when compared with the studies done by Goel *et al.*^[15] and Brighton Mafuvadze *et al.*,^[16] Okeigbemen^[17] and Bhagat *et al.*^[11] In the current study, the mean DMFT for male children was 1.28 ± 1.007, 0.05 ± 0.218, 0.35 ± 0.655, respectively, and for females was 0.84 ± 0.867, 0.02 ± 0.176, 0.20 ± 0.514, respectively, which was higher in males than in females, might be due to the reason that the females are concerned with aesthetics and also could be because they maintain their oral hygiene better than males, which was similar to the study conducted by Aparna *et al.*^[13] and it was in contrast to the studies done by Brighton Mafuvadze *et al.*,^[16] Khalid *et al.*^[18] and Arora *et al.*^[19] In the present study, the mean DMFT is less when compared with the retrospective analysis done by Kundu *et al.*^[20]

Table 3: Frequency of gender wise distribution of the decayed tooth

Gender	No decay, n (%)	One decayed tooth, n (%)	Two decayed tooth, n (%)	Three decayed tooth, n (%)	Four decayed tooth, n (%)	P
Male	56 (23.3)	97 (40.4)	56 (23.3)	26 (10.8)	5 (2.1)	0.001*
Female	106 (41.4)	97 (37.9)	40 (15.6)	13 (5.1)	0	

*Statistically significant. Chi-square test

Table 4: Comparison of frequency of tooth brushing with the number of decayed teeth

Frequency of brushing	No decay	One decayed tooth	Two decayed tooth	Three decayed tooth	Four decayed tooth	P
Once	103 (28.7)	145 (40.4)	76 (21.2)	30 (8.4)	5 (1.4)	0.023*
Twice	59 (43.1)	49 (35.8)	20 (14.6)	9 (6.6)	0	

*Statistically significant. Chi-square test

In this study, dental caries was recorded higher in males than females; this might be because females are more concerned with esthetics so they got them treated with tooth-colored restorations. Because girls are fond of esthetics, as a result, girls might brush and floss their teeth more thoroughly every day during this period to avoid gum diseases and has better oral health than their counterparts.^[21]

In the present study, children who brushed twice daily showed 43.1% caries free when compared to children who brushed once daily. Brushing twice results in good oral hygiene, whereas the lower rates in caries-free study subjects might be due to improper brushing techniques and poor oral hygiene practices. Tooth brushing is regarded as an important vehicle for the application of anti-caries agents, such as fluorides into our system. Most patients still cannot accomplish adequate plaque removal by performing oral cleanliness procedures at home. As a result, most dentists recommend brushing teeth twice a day to improve oral hygiene through plaque control. Brushing twice also eliminates food impaction while shortening the impact of sucrose by cleaning teeth after meals seems to be recommendable.^[22]

Limitations

Cross-sectional studies are observational studies that look at data from a group of individuals at one point in time. They are widely used to assess the prevalence of health outcomes, comprehend health determinants, and identify population characteristics. Finally, the detection of dental caries in children was performed without radiographs.

Recommendations

Hierarchical studies have to be done to find out oral hygiene practices and caries experience among school leaving children in rural areas as most of the population in India lives in rural parts than in the urban counterparts where there was fewer establishments of oral health services.

CONCLUSION

Although the overall caries experience in the present study was less and a very less number of children had a habit of

brushing twice daily, caries experience was more in children who brushed their teeth once a day, majority of the children adopted to horizontal brushing technique and this shows that the study subjects do not follow correct brushing techniques and other oral hygiene practices. There is a need to conduct oral health education programs on correct brushing techniques and other oral hygiene aids.

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Conflicts of interest

There are no conflicts of interest.

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Research Article

Evaluation of Malnutrition and Quality of Life in Patients Treated for Oral and Oropharyngeal Cancer

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Background. Oral and oropharyngeal cancer is a debilitating disease with high morbidity and mortality. Depending on the site and extent of the involvement of the cancer and the type of treatment modality, these patients can develop pain, trismus, xerostomia, dysphagia, and taste disturbances, compromising them socially and nutritionally. The aim of the study was to evaluate malnutrition and quality of life in patients treated for oral and oropharyngeal cancer. **Methodology.** A cross-sectional study was conducted which included 97 patients treated for oral and oropharyngeal cancer. The quality of life of the selected patients was assessed by using a validated European Organization for the Research and Treatment of Cancer's Quality of Life Questionnaire, Head and Neck and Mandibular Function Impairment Questionnaire. Pre- and posttreatment weight of the patients were assessed, and weight loss of $\geq 10\%$ of pretreatment weight was considered as malnutrition. The chi-square test was used to correlate the symptoms with the quality of life. A paired *t* test was used to assess the differences in weight before and after treatment, and a *p* value of < 0.005 was considered as significant. **Results.** The most commonly reported symptoms were xerostomia (93.81%), pain (81.44%), and dysphagia (76.3%). A total of 40.2% of the individuals in the study had malnutrition. Malnutrition was comparatively lower in the group who had nutritional supplements. **Conclusion.** The quality of life in patients treated for oral and oropharyngeal cancer deteriorates immediately after the treatment; however, it significantly improves over time.

1. Introduction

In a developing country like India, head and neck cancer is quite common and is ranked as the tenth most common cancer by the International Agency for Research on Cancer [1]. In 2018, global incidence and mortality related to oral and oropharyngeal cancer were estimated to be 447,751 and 228,389, respectively, and in India, incidence and mortality rates were recorded to be 137,895 and 87,569, respectively [2]. The number of oral and oropharyngeal cancer survivors has increased in the recent decade owing to superior diagnostic techniques and advanced treatment modalities [3–5]. Although the number of deaths has decreased, the treatment of oral and oropharyngeal cancer leaves patients compromised physically and mentally [3, 6]. Various

treatment modalities of oral and oropharyngeal cancer include surgery, chemotherapy, radiotherapy (RT), or a combination of them [7, 8]. Depending on the site and extent of the involvement of cancer and the type of treatment modality, these patients develop pain, trismus, xerostomia, dysphagia, and taste disturbances compromising them socially and nutritionally. The present study was undertaken with an aim to assess malnutrition and the quality of life (QOL) in patients treated for oral and oropharyngeal cancer.

2. Materials and Methods

Sample size calculation: Based on the article by Kamstra et al., [9], the correlation coefficient derived/reported is 0.67. With an alpha error of 0.1% and a power of 99.9%, the *Z*

values of the given alpha and beta values are 3.29 and 4.26. With the correlation coefficient and using the above-mentioned formula, the required sample size was 87 in number. A prospective study was conducted on 97 patients treated for oral and oropharyngeal cancer after obtaining clearance from the Institutional Ethical Committee (Ref Protocol No. 15121). Inclusion and exclusion criteria for the study are given in Table 1.

Demographic data, pretreatment weight, details including the site, extent, and staging of the cancer, and treatment details including the mode of treatment and duration were retrieved from hospital records. The study population was divided into two groups, namely, the 3 months group and 6 months group, i.e., individuals who came before 3 months and at 6 months for follow-up after treatment. On the day of the study, informed consent was obtained from the patients and they were then clinically evaluated. As part of the study, body weight was assessed and the patient's oral cavity was thoroughly examined to evaluate their mouth opening, dentition, oral hygiene, and for the presence of any mucosal abnormalities. Patients were then asked about the symptoms related to their treatment and were asked to grade their symptoms by using the European Organization for the Research and Treatment of Cancer's Quality of Life Questionnaire and the Head and Neck35 (EORTC QOL-H&N35) and Mandibular Function Impairment Questionnaire (MFIQ). These questionnaires were modified in few areas to suit the Indian population and validated by our resident dietician and oncologist. The quality of life was assessed, and changes in the body weight were correlated with the scores of the questionnaire. All data were coded and transferred to an Excel spreadsheet (Microsoft Corp., Redmond, WA, USA), and a descriptive statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) with a confidence limit of 80%. Items from both the questionnaires were grouped according to symptoms such as swallowing problems, chewing disabilities, dry mouth, sensory impairment, impaired social activities, and psychological problems. The range of score for each symptom was calculated by adding all the lower and upper limits of questions in that group from both the questionnaires. The range of score obtained for each symptom was divided into 3 groups, namely, mild, moderate, and severe, by extracting an average of the scores of the 2 questionnaires, and the patients with moderate and severe scores were considered to have a significant problem. An example for the averaging of scores is as follows: To address the chewing problem, we have question numbers 4, 5, 6, 10, 12, 13, 14, and 15 from the validated MFIQ and question number 15 from the validated EORTC. Hence, for chewing problems, the minimum and maximum score obtained were 9 and 36, respectively. This range of score is divided into three categories: $9 \leq 18$ which denotes patients having mild problems, $19 \leq 27$ indicating moderate problems, and $28 \leq 36$ denoting severe problems. The categorization of the questions addressing each problem is given in Table 2. The scores and grading for each symptom are given in the Table 3. The chi-square test was used to correlate the symptoms with the quality of life. The paired *t* test was used to assess the

TABLE 1: Inclusion and exclusion criteria of the study.

Inclusion criteria
(i) Patients aged above 18 years who were treated for oral and oropharyngeal cancer
Exclusion criteria
(i) Patients with metastatic disease, i.e., stage IV C
(ii) Patients treated with cancer below the level of the hypopharynx
(iii) Patients with poor performance state, not fit to receive any radical treatment
(iv) Patients not fit for concurrent chemotherapy
(v) Patients not willing to provide the consent for study
(vi) Patients who did not complete the course of treatment

differences in weight before and after treatment, and a *p* value of <0.005 was considered as significant.

3. Results

Among the 97 patients treated for oral and oropharyngeal cancer, 74.2% of the patients were male and 25.8% were females. The mean age of the study population was 55 (SD \pm 10.6) years. 78% of the patients had consumed tobacco either in the smoke or smokeless form, and 60% had consumed alcohol on a regular basis. The site of involvement, staging, and treatment details are summarized in Table 4.

The most commonly reported symptoms were xerostomia (93.81%), pain (81.44%), and dysphagia (76.3%). Chewing problems and psychological scores had significantly reduced in the 6 months group when compared to the 3 months group. The comparison of scores for quality of life in the 3 months and 6 months group is shown in Figure 1.

Comparison of weight changes between the two groups showed statistically nonsignificant results with a *t* value of 1.73 but was statistically nonsignificant with a *p* value of 0.098. Malnutrition between the 2 groups was compared, and it was noted that the prevalence of malnutrition was significantly higher (56.4%) in the 3 months group (*p* value 0.04). Only sensory difficulty for taste sensation was significantly associated with malnutrition (*p* value 0.029). The results for association between other symptoms with malnutrition are shown in Table 5.

The results from the chi-square test showed that malnutrition was significantly lower in the group which had nutritional supplements (*p* value 0.033). Chewing problems, dysphagia, and dry mouth (sticky saliva) were significantly observed to be higher among individuals treated with surgery alone, and trismus was significantly higher in patients who were treated with a combination of surgery and RT. The association between the oral symptoms, psychological burden, and impaired social activities with various modes of treatment is given in Table 6.

4. Discussion

Cancers affecting the oral and oropharyngeal region and their treatment modalities adversely affect the patients' emotional, physical, and functional well-being. These experiences can deeply scar the patients' lives leading to a

TABLE 2: Categorization of the questions.

S. no.	Symptoms	EORTC (question number)	MFIQ (question number)
1.	Chewing problems	15	4, 5, 6, 10, 12, 13, 14, 15
2.	Trismus	9	3, 11
3.	Dysphagia	5, 6, 7	8
4.	Pain	1, 2, 3, 4, 21	Nil
5.	Sensory impairment	11, 12	Nil
6.	Xerostomia	10	Nil
7.	Social activities	16, 18, 19, 20	1, 2, 7, 9
8.	Psychological score	8, 13, 14, 17	Nil

TABLE 3: Scoring and grading of oral symptoms.

Symptoms	Mild	Moderate	Severe
1. Chewing disabilities	9 ≤ 18	19 ≤ 27	28 ≤ 36
2. Trismus	3 ≤ 6	7 ≤ 9	9 ≤ 12
3. Dysphagia	4 ≤ 8	9 ≤ 12	13 ≤ 16
4. Pain	5 ≤ 10	11 ≤ 15	16 ≤ 20
5. Sensory difficulties	2 ≤ 4	5 ≤ 6	7 ≤ 8
6. Sticky saliva	1	2 ≤ 3	3 ≤ 4
7. Social activities	8 ≤ 16	17 ≤ 24	25 ≤ 32
8. Psychological burden	4 ≤ 8	9 ≤ 12	13 ≤ 16

TABLE 4: Summary of the clinical and treatment details of participants.

Category	n (%)
	10 (10.3%)
Buccal mucosa	34 (35%)
Tongue	12 (12.4%)
Oropharynx	18 (18.6%)
Floor of the mouth	3 (3.1%)
Retromolar trigone	2 (2.1%)
Soft palate	12 (12.4%)
Maxilla	6 (6.2%)
Mandible	15 (16%)
Stage 1	33(34%)
Stage 2	35(36%)
Stage 3	14(14%)
Stage 4	3 (3%)
Surgery alone	64 (66%)
Surgery + radiotherapy	30 (31%)
Surgery + radiotherapy + chemotherapy	

dramatic decrease in their QOL and can indirectly cause malnutrition. The QOL assessment is an important tool measuring the outcomes of cancer treatment and has been evaluated in this study.

In our study population, the male-to-female ratio was 2.88:1 which is similar to the results of Nagy et al. [1] (2:1), Gritz et al. [10] (2.5:1), and Hassanein et al. [11] (2.3:1). The mean age of the study group was 55 years which is consistent with the results of Nagy et al. [1] (53.8 years), Gritz et al. [10] (58.4 years), and Hassanein et al. [11] (58 years). The most common site of tumor in our study was the tongue (35%)

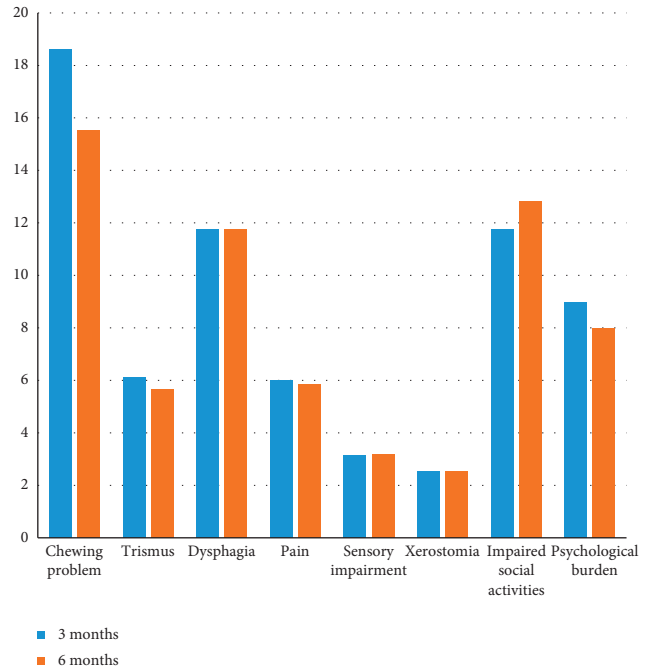


FIGURE 1: Representing comparison of scores between the 3 months and 6 months group.

followed by the floor of the mouth (18.6%), oropharynx (12.4%), maxilla (12.4%), and buccal mucosa (10.3%). Our results were similar to studies conducted by Rinkel et al. [12] who stated that the common sites were the tongue (38%), followed by the floor of the mouth (10%).

The most common treatment modality in the present study was the combination of surgery with RT, followed by the combination of surgery, RT, and chemotherapy (CT). This can be explained by the fact that most of the cancers in our study were in stage II and stage III which usually required combined treatment modalities. Combination of surgery with RT was the common mode of treatment in the studies conducted by Rinkel et al. [12] (50%), Kamstara et al. [9] (51.25%), and Nazar et al. [13] (47.2%). In the study conducted by Scharloo et al. [14], the results showed that RT was the commonly employed treatment modality and accounted for 40.7% of the cases. Thomas et al. [15] found that 88.3% of the patients had undergone primary or adjuvant RT. In the investigation carried out by Vartanian et al. [16] on 301 patients, it was found that 52.5% of the patients were treated only by surgery, 11.3% had undergone only RT,

TABLE 5: Association between various symptoms and malnutrition.

Symptoms	Prevalence	Percentage in which malnutrition is absent	Percentage in which malnutrition is present	<i>p</i> value
Chewing problem	32	56.25	43.75	0.617
Trismus	37	62.16	37.84	0.709
Dysphagia	74	56.76	43.24	0.274
Pain	79	59.49	40.50	0.899
Taste disturbance	37	46	54	0.029
Xerostomia	91	61.53	38.47	0.172
Social activity	72	56.94	43.06	0.331
Psychological	69	62.32	37.68	0.426

TABLE 6: Association between the oral symptoms, psychological burden, and impaired social activities with various modes of treatment.

Symptoms	Surgery + CT + RT (%)	Surgery + RT (%)	Surgery alone (%)	<i>p</i> value
Chewing problems	16.70	39.10	66.70	0.045
Trismus	16.70	48.40	33.30	0.012
Dysphagia	60.00	82.80	100.00	0.033
Pain	93.30	76.60	66.70	0.12
Taste disturbance	33.30	40.60	33.30	0.782
Sticky saliva	80.00	100.00	100.00	0.001
Social activity	80.00	70.30	100.00	0.354
Psychological	83.30	65.60	66.70	0.207

and 32.6% were treated with a combination of surgery and RT.

In the present study, the most commonly reported symptoms were xerostomia (93.81%) followed by pain (81.44%) and dysphagia (76.28%). These findings can be explained by the fact that most of the participants were treated by RT. Xerostomia chiefly occurs due to the severe damage and fibrosis of salivary glands caused by RT. These findings were similar to the results of the study conducted by Kamstara et al. [9] who observed that xerostomia was the most common symptom among patients treated for oral and oropharyngeal carcinoma and the other most commonly reported symptoms were trismus and dysphagia. Rathod et al. [17] in their study noticed that xerostomia was the most common symptom associated with the treatment of HNC. The study also revealed that dysphagia and altered taste sensations in oral cancer patients worsened after treatment. A study conducted by De Graeff et al. [18] revealed that the common symptoms associated with treatment of oral and oropharyngeal cancer were pain, difficulty in eating and speech, and sensory impairment. Pain was the second most commonly reported symptom in our study (81.44%). Most of the studies in the literature have suggested pain as the worst symptom experienced as a consequence of cancer therapy [19]. The experience of pain after surgery is described as a nociceptive pain, lasting for a couple of months with moderate improvement over time [20, 21]. Surgical management can also cause tissue and nerve damage resulting in chronic pain syndromes. Terrel et al. [22] in their study among patients treated by mandibular bone resection found that hyperalgesia and allodynia was experienced by approximately 50% and 90% of the patients, respectively. Pain can also be because of the mucositis since most of the patients had undergone the combination of surgery and RT. The systematic review by Trotti et al. [23] stated that the

incidence of oral mucositis in patients treated with RT and chemoradiation was very high accounting for 80% of the patients.

In the present study, the scores of various symptoms were lower in the 6 months group when compared to scores of individuals in the 3 months group. These findings suggested that the severity of the symptoms decreased as the duration of treatment increased. The scores of chewing disabilities, trismus, dysphagia, pain, taste disturbances, and psychological scores were comparatively lower among individuals who came for follow-up at 6 month interval. However, only the scores of chewing disabilities and psychological burden were significantly reduced in the 6 months group when compared with the 3 months group. This can be attributed to the prophylactic extraction of teeth and the consequent inadequate healing of the surgical site as a part of the treatment protocol or even a lack of proper prosthetic wear at 3 months interval during treatment follow-up. The improvement at 6 months may be due to the healing of the surgical site in such cases. Adverse effects of treatment such as extraoral surgical scars, pigmented skin, and alopecia which can significantly impact the psychological well-being of individuals are severe immediately after treatment but improve subsequently over time. These treatments also contributed to inability in movement of the tongue resulting in difficulty of speech. All these factors inhibit them from routine activities such as social speaking and social eating and eventually affect them psychologically in due course of the treatment. The scores for sensory difficulties, xerostomia, and impaired social activities are higher in 6 months group when compared with the 3 months group; however, the difference was not statistically significant.

Biazevic et al. [24] recorded the immediate effects of tumor resection in oral and oropharyngeal cancer on the

health-related QOL and found reduction in overall rating indicating the improvement of the QOL after 6 months of treatment. It was observed that the commonly impaired functions were chewing difficulties, taste disturbances, pain, and problems associated with swallowing and speech. In the current study, comparison of the scores between 3 months and 6 months showed significantly high scores in chewing problems and psychological burden in the 3-month interval group. Jaw movements for opening chewing and swallowing are correlated with mobility of the tongue and the mandible. Most of our patients were treated for cancer of the tongue and floor of the mouth which have significant effects on chewing and swallowing functions. These are also significantly impaired immediately after the treatment, but improve after a duration of 6 months, which explains the reduction of their scores.

Shepherd et al. [25] conducted a study on oral cancer patients with an aim to examine the impact of surgical, RT, and combination treatment on QOL. In this study, it was found that function reduced immediately after treatment and most functions improved to near baseline levels by 3 months after treatment. Rathod et al. [17] evaluated the outcomes of treatment in HNC patients and noticed that there was a substantial deterioration in the QOL (trismus, xerostomia, pain, and senses) scores 3 months after treatment although the improvement was noted in all the scores by 6 months' time. These findings were very similar to the findings of our present study. Agarwal et al. [26] in a prospective study evaluated changes in the QOL 6 months after the surgical treatment of carcinoma of the tongue and found that there was a significant decrease in scores of the appearance of the patient, dysphagia, chewing, speech, taste, and xerostomia indicating an improvement of the QOL.

Prevalence of malnutrition (weight loss of $\geq 10\%$) in our study was 40.2%, among which 56.4% were in the 3 months group and 43.6% in the 6 months group. Taste disturbances were significantly associated with malnutrition. Altered taste sensation is very distressing for the patients and is associated with decreased appetite and confusion between bitter and sour taste and the inability to discriminate among various tastes. The results of our study were similar to a few other studies in the past. McLaughlin [27] had found that dysgeusia has shown a statistically significant association with malnutrition. Suzuki et al. [28] had revealed that appetite is frequently affected by altered taste sensation along with xerostomia and oral mucositis. Ogama and Suzuki, [29] concluded that patients who were exposed to a cumulative dose of 50 Gy had taste disturbances which severely affected their appetite. However, studies conducted by Jager-Witenaar et al. [30] and Gellrich et al. [31] showed that dysphagia and chewing problems were mainly associated with malnutrition. Kubrak et al. [32] has suggested that malnutrition may be due to dysphagia and mouth sores caused by the treatment of oral and oropharyngeal cancer.

Our research revealed that malnutrition was absent in a significant number of patients (72.5%) who were on nutritional supplements. Ravasco et al. [33] in a prospective study on HNC patients stated that weight loss is more prevalent in patients who were not receiving dietary

counselling when compared to patients who received dietary counselling. van den Berg et al. [34] stated that nutritional interventions during treatment had a positive influence on the outcomes of treatment and resulted in considerably lower malnutrition status in patients treated for oral and oropharyngeal cancer.

5. Conclusions

In the present prospective study, we observed that the QOL in patients treated for oral and oropharyngeal cancer deteriorates immediately after treatment but significantly improves over time. Our study also highlights the importance of nutritional supplements, their positive influence on the outcomes of treatment, and their beneficial effects on patients with malnutrition.

Abbreviations

QOL:	Quality of life
MFIQ:	Mandibular Function Impairment Questionnaire
EORTC QOL-H&N35:	European Organization for the Research and Treatment of Cancer's Quality of Life Questionnaire and the Head and Neck35.

Data Availability

Supporting data are available from the corresponding author on request.

Conflicts of Interest

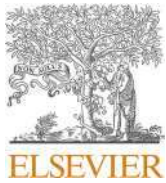
The authors declare no conflicts of interest.

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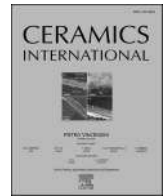
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Rapid synthesis of eggshell derived hydroxyapatite with nanoscale characteristics for biomedical applications

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ABSTRACT

Utilizing eggshells to synthesize a value added product like nanocrystalline hydroxyapatite (HA) has received a lot of attention from researchers since they are composed of CaCO₃ with biologically essential trace elements such as Mg, Si, etc. Different biomedical applications need HA with appropriate nanoscale characteristics like crystallinity, particle size, morphology, surface area, mesoporous nature, etc. The same can be achieved by tuning the reaction parameters, choosing a suitable mode of preparation and utilizing organic modifiers. Here, we report the rapid synthesis of eggshell derived HA in the presence different organic modifiers using a custom built microwave reactor employing the previously optimized parameters. The synthesis process is relatively rapid and gets completed in 5 min. The absence of an organic modifier yielded inhomogeneous size nanorods in the range of 40 – 600 nm. Ethylenediaminetetraacetic acid (EDTA) assisted synthesis resulted in a flower like 1.67 ± 0.12 μm sized HA. Whereas polyethylene glycol 6000 (PEG) and cetyltrimethylammonium bromide (CTAB) assisted synthesis produced aggregated nanorods of length 31 ± 8 and 68 ± 20 nm, respectively. While the synthesis with trisodium citrate dihydrate (TSC) resulted in needles of HA with typical length of 32 ± 8 nm. Presence of Na, Mg and Si trace elements are confirmed from the composition analysis. All the samples are found to be mesoporous in nature. The *in vitro* cell culture experiment carried out using fibroblast NIH 3T3 cell line clearly revealed equal or higher cell viability for the samples synthesized in presence of organic modifiers as compared to sample produced without organic modifier. Thus, from the present study we find that the synthesis of eggshell derived HA using different organic modifiers via a custom built microwave reactor can be a potential approach for the rapid preparation of precursor materials with suitable nanoscale characteristics for developing bone fillers, drug/protein delivery carriers, tissue engineering scaffolds, etc.

1. Introduction

Hydroxyapatite [HA, Ca₁₀(PO₄)₆(OH)₂] is the dominant mineral phase in the human skeletal system [1,2]. Synthetic HA has been widely used in bone tissue engineering and other biomedical applications in dentistry and orthopaedics owing to its compositional and structural similarity with the mineral phase of bone and teeth [3,4], consequently its excellent bioactive and cytocompatible nature [5]. In addition, the usage of HA is not only limited to the biomedical field, but also used as an adsorbent for organic dyes and heavy metal ions, catalyst, electret

and proton conductor [6–8]. Current scenario is the development of HA based materials with enhanced properties through nanotechnology by utilizing novel physical/chemical processes [6,9]. Nanoscale characteristics of HA should be optimized to achieve better performance for different biomedical applications [10]. Particularly, the physicochemical and biological properties of mesoporous HA nanostructure is different from bulk materials [9,11]. Moreover, factors such as crystallinity, particle size, morphology and surface area of mesoporous HA nanostructures play a vital role in biomedical applications [12]. Hence, significant efforts are being made to control the size and shape of HA

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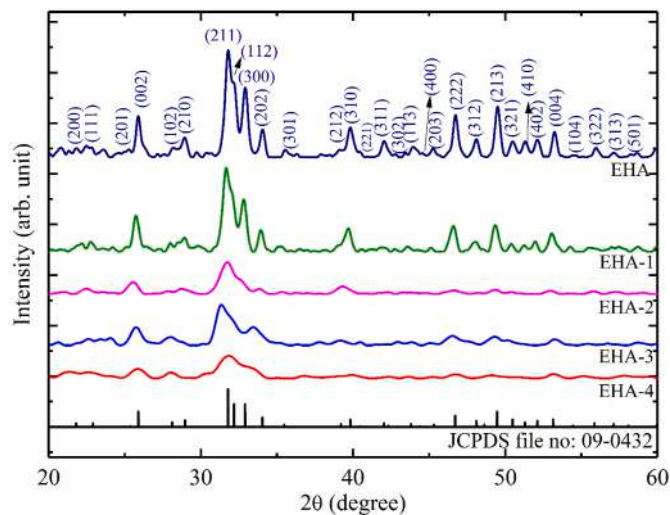


Fig. 1. XRD patterns of the samples prepared without organic modifier (EHA), with organic modifiers (EHA-1, EHA-2, EHA-3 and EHA-4) and simulated data for HA (standard JCPDS no: 09-0432)

Table 1

Lattice parameters, crystallite size, crystallinity and c/a values of prepared samples.

Sample code	Lattice parameters (Å)		Average crystallite size (nm)	Average crystallinity (X _c)	c/a ratio
	$a=b=9.4180$	$c=6.8840$			
EHA	9.4160	6.8769	54 ± 3	3.61 ± 0.6	0.7304
EHA-1	9.4389	6.9147	42 ± 2	1.73 ± 0.5	0.7325
EHA-2	9.3961	6.9748	23 ± 5	0.29 ± 0.1	0.7423
EHA-3	9.4301	6.9137	28 ± 2	0.31 ± 0.1	0.7331
EHA-4	9.4074	6.8865	21 ± 3	0.20 ± 0.1	0.7320

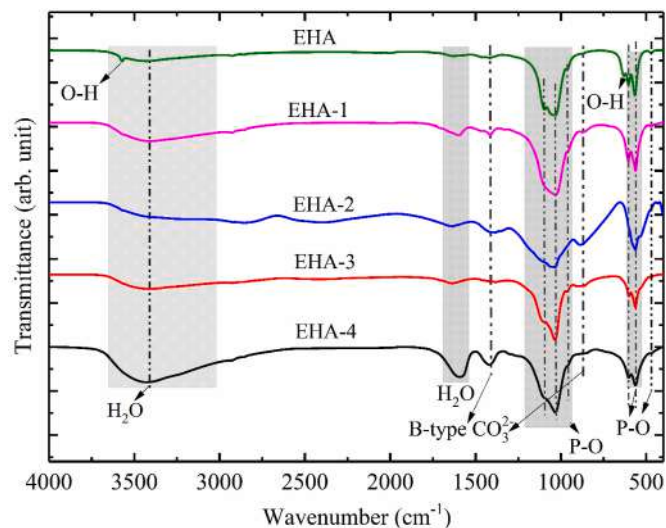


Fig. 2. FTIR spectra of samples prepared without the organic modifier (EHA) and with organic modifiers (EHA-1, EHA-2, EHA-3 and EHA-4)

nanoparticles.

It was found that the preparation method dramatically influences the characteristics of the HA produced. The reaction parameters such as temperature, pH, ageing time, etc. are also important to synthesize HA with superior nanoscale characteristics [13]. Moreover, different organic modifiers and surfactants were also effectively used to control the characteristics of nano HA since calcium and phosphate ions can

easily interact with organic species during the synthesis [14]. Several methods such as chemical precipitation [15], sol-gel [16], micro-emulsion [17], hydrothermal [11], electrochemical [18], microwave [19] and solid state method [20] were reported for the synthesis of nano HA. Among the different synthesis methods, the microwave technique is an efficient and straightforward method to prepare HA nanoparticles rapidly. In the past few decades, many research papers have been published on the synthesis of HA using the conventional domestic microwave oven [19].

Several researchers have utilized various biogenic sources such as corals, eggshells, fish scales, seashells, animal bones, etc., as a calcium source to synthesize nanocrystalline HA [15]. Among them, eggshells are a highly preferred calcium source since they are mainly composed of 94% calcium carbonate (CaCO_3) with trace elements such as magnesium (Mg), silica (Si), etc., [21]. Annually worldwide large quantities of eggshells are discarded as landfills from restaurants, hatcheries, etc., [22]. Moreover, when compared to HA derived from commercial reagents, eggshell derived HA exhibited superior bioactivity, excellent osteoconductivity, osteoinductivity and cell proliferation and is often attributed to the presence of trace elements that originated from the eggshell source [23]. Egg white and eggshell based hydrogel composite scaffold fabricated by Huang et al. exhibited better bioactivity [24]. Bone cement and 3D scaffold prepared from eggshell derived calcium phosphates were reported to be suitable for hard tissue regeneration, as it has a good cement setting behaviour, compressive strength, biocompatibility and better osteogenic differentiation as compared to synthetic bone cement/scaffold [25,26]. Also, eggshell derived nano HA reinforced with the polymer to fabricate composite membrane or scaffolds revealed enhanced thermal, mechanical and biological properties compared to pure polymer membrane/scaffold [27-29]. Protein delivery, drug delivery and waste water remediation have also been studied using eggshell derived HA [7,30,31].

Although several reports are available on the synthesis of nano HA from eggshell through different techniques, microwave assisted chemical precipitation is highly preferred by several researchers due to its advantages like simplicity, economical, rapid process, reproducibility, high yield, etc., [19]. Also, recent research suggested that microwave technique directly results in mesoporous HA nanostructures even without employing hard templates or surfactants [32,33]. Nanoscale HA with mesoporous characteristics have received significant attention in developing drug/protein carriers, implants and tissue engineering scaffolds. Even though mesoporous HA can be obtained via microwave technique, we can control the size, morphology and mesoporous characteristics of HA using different organic reactive molecules. Recently, we reported the impact of various reaction parameters for HA synthesis from eggshell waste using lab scale and a custom built pilot scale microwave reactor made using four magnetrons (1.1 kW per magnetron) [34]. The custom built pilot scale microwave reactor significantly reduced the microwave irradiation time as compared to the domestic microwave oven used as a lab scale reactor. The objective of the present work is to study the formation of eggshell derived HA using the above mentioned custom built pilot scale microwave reactor in the presence of reactive organic compounds such as EDTA, PEG, TSC and CTAB employing the necessary parameters optimized in the previous work.

2. Experimental

All the chemicals, namely nitric acid (HNO_3), disodium hydrogen phosphate (Na_2HPO_4), sodium hydroxide (NaOH), ethylenediaminetetraacetic acid ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$), polyethylene glycol 6000 ($\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$), trisodium citrate dihydrate ($\text{C}_6\text{H}_9\text{Na}_3\text{O}_9$) and cetyltrimethylammonium bromide ($\text{C}_{19}\text{H}_{42}\text{BrN}$) were analytical reagent grade acquired from Merck, India. All reagents were used without further purification and double distilled water was employed as the solvent. Broiler eggshells were utilized as the calcium source in the present study due to its abundant availability in India.

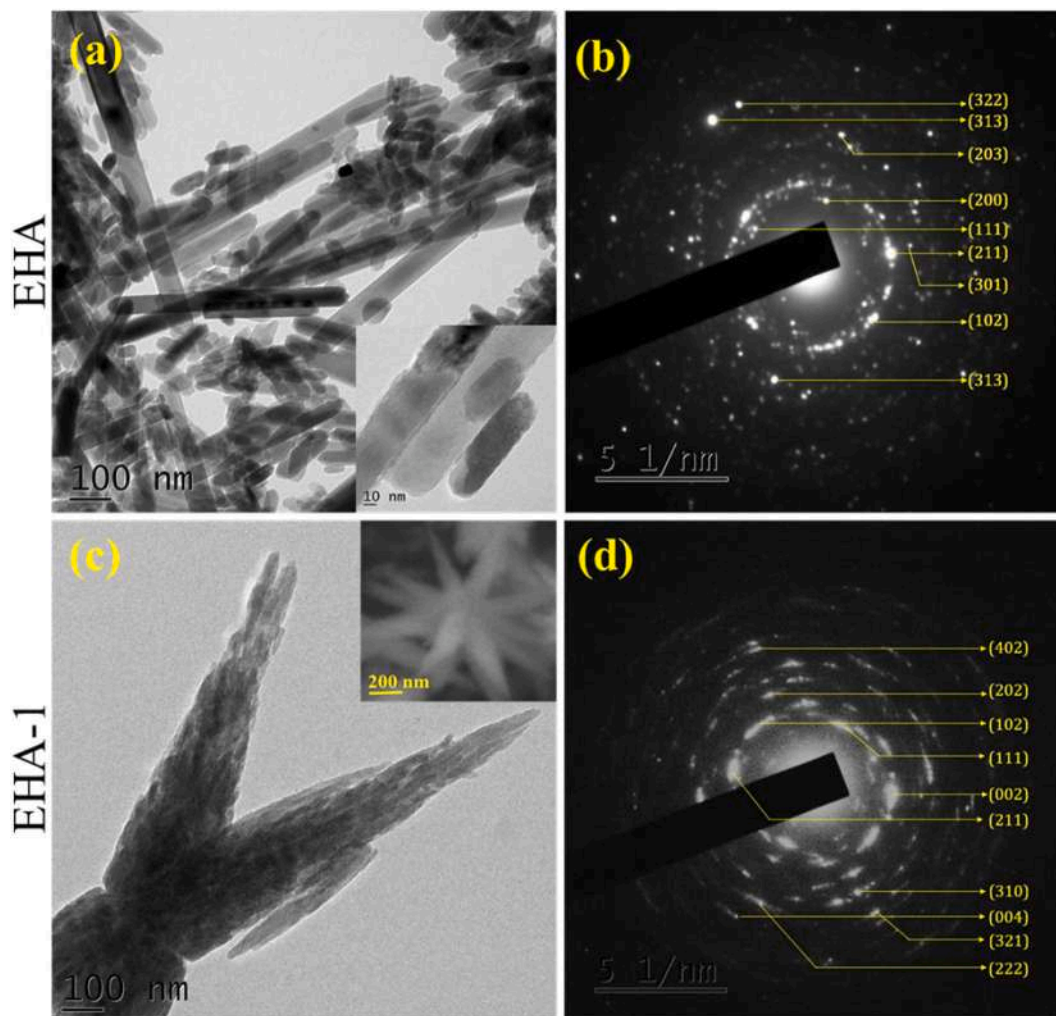


Fig. 3. HRTEM images and SAED pattern of EHA and EHA-1. Inset in Fig. 3(c) is the SEM image of EHA-1.

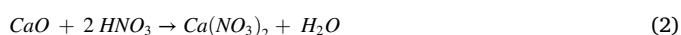
2.1. Custom built pilot scale microwave reactor

The pilot scale microwave reactor used in this study was developed by modifying conventional microwave furnace (supplied by VB ceramic consultants, Chennai, India). The reaction vessel used for the reactor replaced the susceptor of the microwave furnace. The reactions were conducted in water as the dielectric medium. The reaction vessel was designed in such a way with the lid having the provisions for inlet and outlets. A suction pump is connected to the reaction vessel for releasing the pressure. There are four magnetrons (1.1 kW/magnetron, 2.45 GHz) of which two operate at a time, one on both side to allow homogeneous radiation inside the reactor. Hence, microwave power during the operation is 2.2 kW. After completing the reaction, the instrument is manually switched off as the sensing unit of microwave furnace that allows automatic power cut-off cannot be used when the instrument is operating in reactor mode.

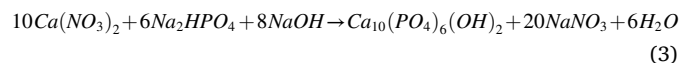
Eggshells were collected, cleaned manually and then placed in a furnace at 900 °C for 1 h. At this temperature, eggshells transform into calcium oxide (CaO) by evolving carbon dioxide (CO₂) according to the following equation (1).



The eggshell derived CaO (0.1 M) was dissolved in nitric acid (0.2 M) to form calcium nitrate solution (eqn. (2))



0.06 M disodium hydrogen phosphate solution was prepared by dissolving an appropriate amount of Na₂HPO₄ in double distilled water. Obtained calcium nitrate solution was stirred vigorously at room temperature to which Na₂HPO₄ solution was added dropwise while maintaining the pH of the reaction mixture above 10 using NaOH and was stirred for 30 min at room temperature. The expected chemical reaction is given in equation (3),



The obtained reaction mixture (100 ml) was transferred into the microwave reactor chamber followed by microwave irradiation at 2.2 kW for 5 min. The resulting white precipitate acquired was centrifuged (6000 rpm) and washed 6 times with double distilled water to remove the by-products, followed by drying at 110 °C for 5 h in a hot air oven. Finally, dried cakes were crushed using mortar and pestle to get white powder which is named as EHA. Experiments were also carried out in presence of EDTA (0.2 M) [34], PEG (1 wt.%) [35], TSC (0.0333 M) [36] and CTAB (0.06 M) [37] in which the former three organic modifiers were added along with the calcium reagent while the latter one was mixed with phosphate reagent. The individual organic modifier concentration mentioned above was chosen based on the optimized concentration available in the literature. The samples prepared using EDTA, PEG, TSC and CTAB were named EHA-1, EHA-2, EHA-3 and EHA-4 respectively.

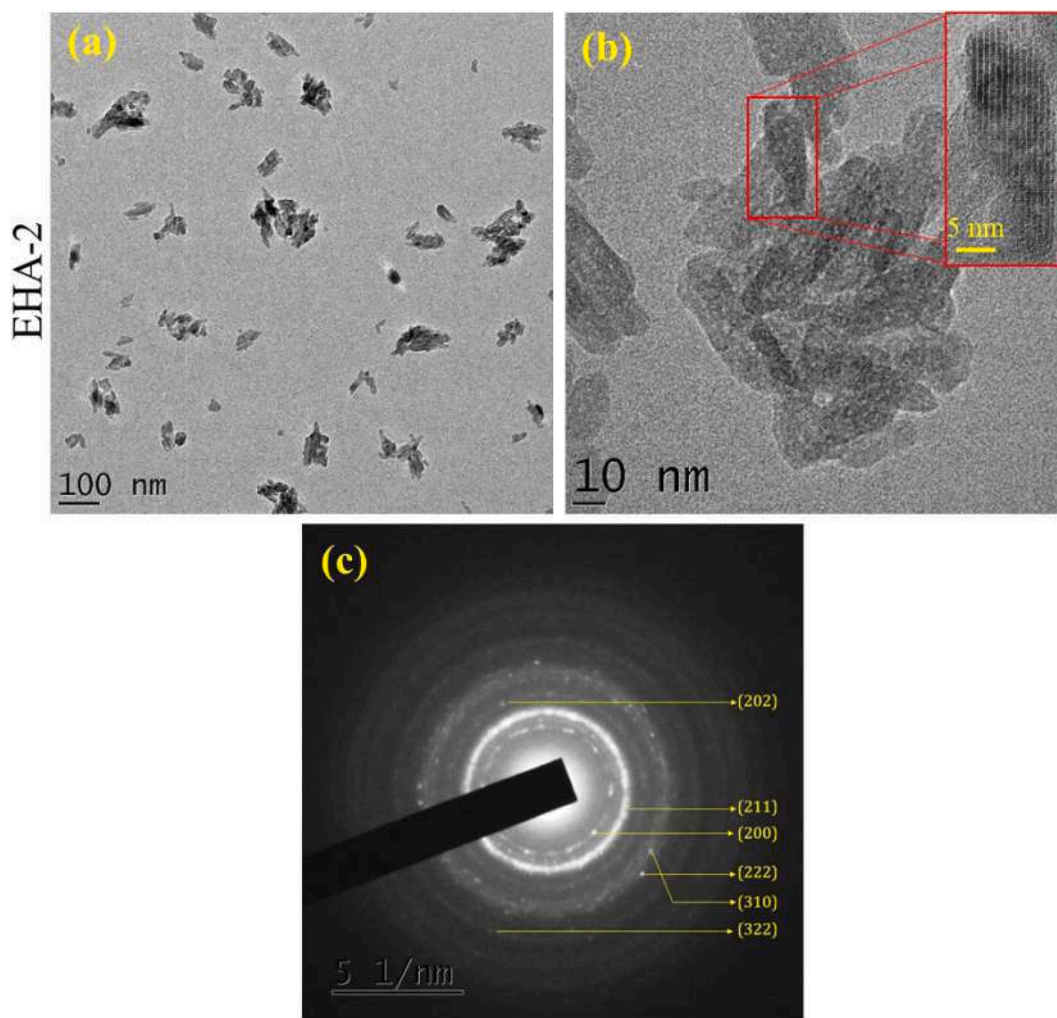


Fig. 4. (a, b) HRTEM images (c) SAED pattern of EHA-2 sample

2.2. Characterization

Phase purity, crystalline nature and lattice parameters of the synthesized samples were analysed by powder X-ray diffraction (XRD) technique (Rigaku MiniFlex-II) using monochromatic Cu- α radiation (1.5406 Å). The powder XRD patterns were recorded in the range between $20^\circ \leq 2\theta \leq 60^\circ$ with a scan speed of $10^\circ/\text{minute}$ and step size of 0.02° . In addition, the full width at half maximum (FWHM) was calculated from the XRD pattern to measure the average crystallite size by the Scherrer method [38].

The functional groups present in the prepared samples were examined using Fourier transform infrared spectroscopy (FTIR). Powder samples were ground with KBr and pressed into a disc using a pelletizer. The FTIR spectra were recorded from 4000 to 400 cm^{-1} using a Perkin Elmer RX1. Particle size, morphology and crystalline nature of the prepared samples were analysed using high resolution transmission electron microscopy (HRTEM- JEOL/JEM 2100) and selected area electron diffraction (SAED). For HRTEM analysis, small amount of sample was dispersed in ethanol under sonication for 30 min and $1\ \mu\text{l}$ of the dispersed sample was placed on the carbon coated copper grid. The elemental composition of the samples was analysed using energy dispersive X-ray analyser (EDAX) by INCA, Oxford instruments. Scanning electron microscopy (SEM, Carl Zeiss - EVO 18) was used to analyse the surface morphology of the prepared samples.

The pore size, pore volume and surface area of the samples were determined using the N_2 adsorption-desorption isotherm method

(Micromeritics ASAP 2020). In addition, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of synthesized products was carried out from 34 to 1200°C with heating rate of $10^\circ\text{C}/\text{minute}$ using Jupiter (NETZSCH STA F3) instrument.

2.3. Cytocompatibility assay

The cytocompatibility of samples was tested with fibroblast NIH 3T3 cell lines (purchased from National Centre for Cell Science (NCCS), Pune, India) by MTT assay. The fibroblast NIH 3T3 cells were cultured with 10% of fetal bovine serum (FBS) and 1% of penicillin-streptomycin followed by incubation at 37°C with the humidified atmosphere in 5% of CO_2 . The cultured fibroblast cells were sowed at a density of 1×10^5 cells/ml in 96 well tissue culture plate and incubated at standard culture settings (37°C , 5% of CO_2 and 95% air) for 24 h. After incubation, synthesized powder samples were added into tissue culture plate at various concentrations such as 50, 100, 250 and $500\ \mu\text{g}/\text{ml}$ and then incubated at 37°C for 24 h in CO_2 incubator. Thereafter, $15\ \mu\text{l}$ of MTT and phosphate buffered saline (PBS) was added to 96 well plate and then incubated at 37°C for 4 h in a CO_2 incubator. After this, the supernatant of MTT solutions was discarded and the obtained formazan crystals were dissolved in $100\ \mu\text{l}$ of dimethyl sulfoxide (DMSO) and the optical density (OD) was measured at $570\ \text{nm}$ using a microplate reader. The percentage of viable cells was calculated from the following equation (4)

$$\text{Percentage of cell viability} = \left(\frac{OD_{\text{sample}}}{OD_{\text{control}}} \right) \times 100 \quad (4)$$

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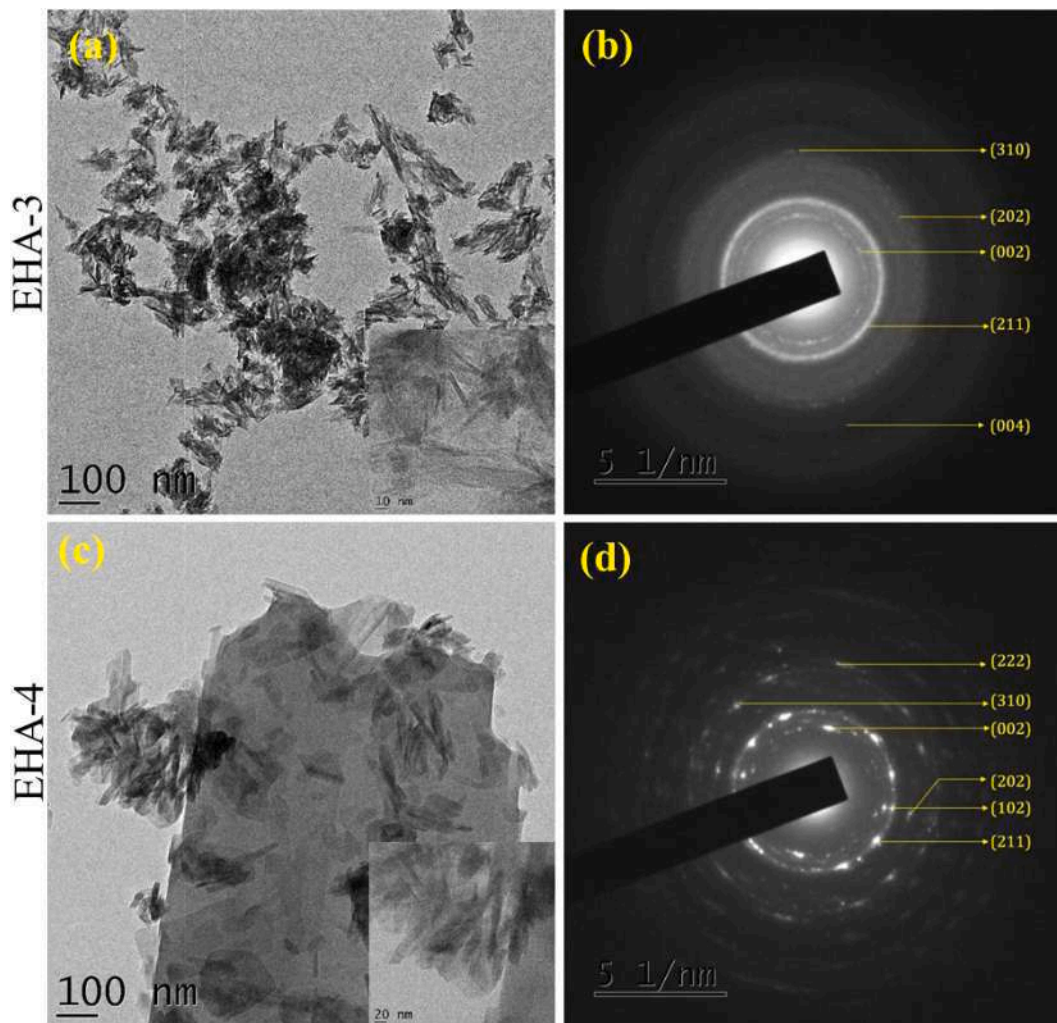


Fig. 5. HRTEM images (a & c) SAED pattern (b & d) of EHA-3 and EHA-4 sample

Table 2

Elemental composition of the prepared samples.

Sample code	Elements (at.%)					Ca/P
	Ca	P	Na	Mg	Si	
EHA	11.50	07.64	0.41	0.23	0.16	1.51
EHA-1	10.61	07.48	0.74	0.18	0.18	1.42
EHA-2	15.03	10.29	2.81	0.57	0.27	1.46
EHA-3	12.47	08.37	0.23	0.03	0.18	1.49
EHA-4	06.47	04.72	0.22	0.05	0.13	1.37

where, OD_{sample} is the optical density of cells with powder sample and OD_{control} is the optical density of cells without sample.

2.4. In vitro cell attachment study

300 mg of prepared powder sample was made into a disc of about 13 mm diameter and 1 mm height using pelletizer by applying pressure at 20 MPa. The prepared disc samples were transferred to 6 well tissue culture plate. Fibroblast NIH 3T3 cells were cultured in 96 well plate at a density of 1×10^5 cells per well in Dulbecco's modified eagle medium (10% of FBS and 1% of penicillin-streptomycin) under incubation at 37 °C for 24 h in CO₂ incubator. 200 μ l of the above cultured cells were seeded on the surface of the disc and incubated at 37 °C for 24 h in a CO₂ incubator. Then, the culture media was removed and the samples were

washed with PBS and the discs were fixed with 4% of glutaraldehyde solution for 30 min followed by slow dehydration in ethanol of concentration 70%, 80%, 90% and 100% and dried at room temperature. Then, the dried samples were sputter coated with gold for 10 min to observe cell attachment on the surface using field emission scanning electron microscopy (FESEM, JEOL JSM-6390L).

3. Results and discussion

3.1. XRD

Fig. 1 shows the powder XRD patterns of samples prepared without and with the organic modifier. From the obtained XRD patterns we have seen that all five samples exhibited the characteristic diffraction peaks of HA (JCPDS file no: 09-0432 with hexagonal structure) without any other calcium phosphate phases. Pristine EHA (sample prepared without organic modifier) sample exhibit resolved peaks in between 30 and 35°. However, a significant decrease in the intensity of the diffraction peaks was observed for samples prepared with organic modifier EHA-1, EHA-2, EHA-3 and EHA-4. Moreover, the broadness of the diffraction peaks was found to increase for EHA-1, EHA-2, EHA-3 and EHA-4. These results clearly indicate that the presence of an organic modifier during the sample preparation plays a crucial role in modulating crystallization and control the growth of HA under microwave irradiation. The calculated average crystallite size and crystallinity of EHA, EHA-1, EHA-2, EHA-3 and EHA-4 are given in Table 1. It is interesting to note that PEG, TSC

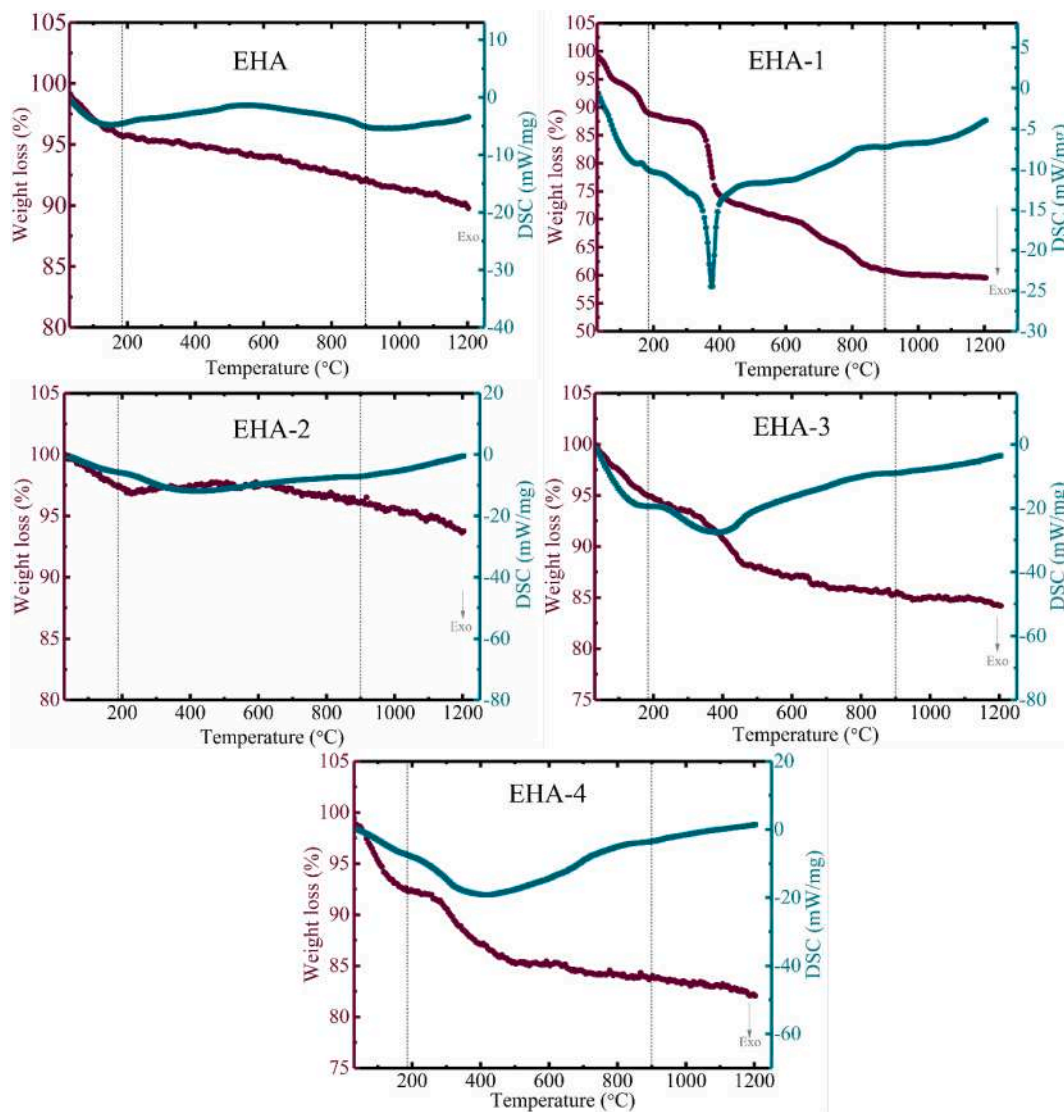


Fig. 6. TGA/DSC curves of the prepared samples

Table 3

Weight loss percentage of the prepared samples.

Sample code	Weight loss (%)			Total weight loss (%) (34–1200 °C)
	Step-I (34–180 °C)	Step-II (180–900 °C)	Step-III (900–1200 °C)	
EHA	4.26	3.58	2.38	10.22
EHA-1	11.03	28.07	1.36	40.46
EHA-2	2.51	1.33	2.41	6.25
EHA-3	5.04	9.42	1.36	15.82
EHA-4	7.43	8.56	1.93	17.92

and CTAB highly inhibited the crystallization of HA when compared with EDTA. Lattice parameters and lattice distortion of prepared samples were calculated and listed in Table 1. Significant difference in the crystallite size and crystallinity were found in between the prepared samples, which also confirm that organic modifiers can play a vital role in crystallization of HA by making interaction with calcium/phosphate precursor [13].

3.2. FTIR

FTIR spectra of samples prepared without and with organic modifiers

are shown in Fig. 2. In the FTIR spectra the doublet peak observed at 604 and 566 cm^{-1} corresponds to the characteristic bending mode of phosphate (PO_4^{3-}) group of HA. Also, other phosphate peaks of symmetric and antisymmetric stretching vibrations appeared at 962 and 1030–1120 cm^{-1} respectively. The small peak detected for all the samples at 475 cm^{-1} is assigned to the bending mode of PO_4^{3-} group. The peaks that are present at 631 and 3572 cm^{-1} are ascribed to the characteristic OH^- group of HA and they are very sensitive to the crystallinity of HA [38]. Importantly, these peaks are not clearly visible in the FTIR spectrum of EHA-1, EHA-2, EHA-3 and EHA-4 due to their relatively poor crystalline nature which agrees with XRD results. Broad absorption band centred at 3420 cm^{-1} and a small band in between 1600 and 1645 cm^{-1} are ascribed to the stretching and flexural modes of adsorbed H_2O molecules and they are observed for all samples. The band at 1420 cm^{-1} and a small peak at 879 cm^{-1} are assigned to carbonate (CO_3^{2-}) group, which is acquired from the atmosphere during sample preparation (alkaline condition). The observed positions of carbonate peaks indicate that it is substituted for phosphate group in HA and formed as B-type carbonated HA [39].

3.3. HRTEM

Fig. 3(a) and (b) shows the HRTEM images and selected area electron

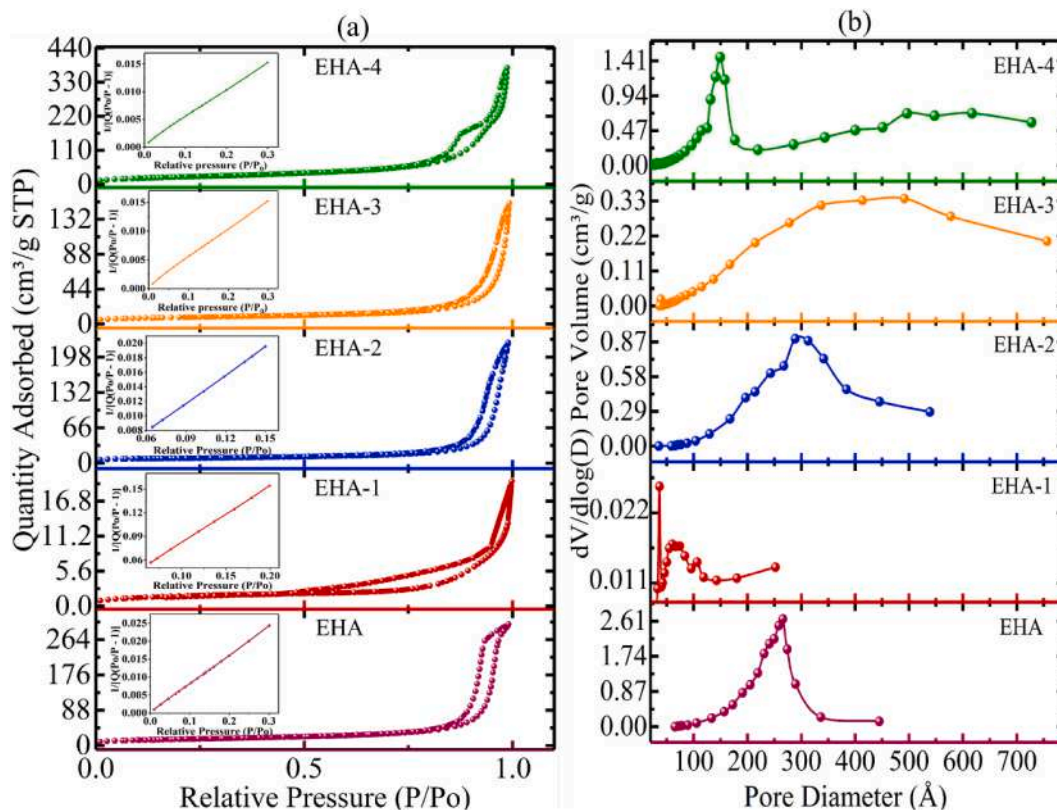


Fig. 7. (a) N_2 adsorption/desorption isotherm (inset BET surface area plot), (b) BJH pore size distribution of prepared samples

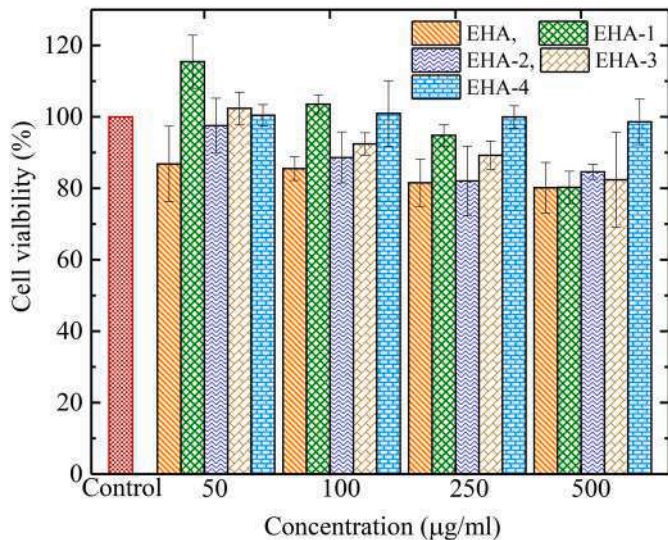


Fig. 8. Cell viability percentage of prepared samples with fibroblast NIH 3T3 cells

diffraction (SAED) pattern of EHA. HRTEM images of EHA clearly indicate that it consists of inhomogeneous rod like particles with clear boundaries. The average length and width of particles was calculated as 40 – 600 nm and 34 ± 10 nm respectively (Image J). SAED pattern of the EHA sample (Fig. 3(b)) reveals bright spots arranged in continuous ring which is owing to its polycrystalline nature and it is indexed with Miller's planes observed in XRD.

HRTEM, SEM images and SAED pattern of EHA-1 are shown in Fig. 3 (c) and (d). The SEM (inset in Fig. 3(c)) morphology of obtained EHA-1 sample shows the flower like structure with size of about 1.67 ± 0.12

μm . SEM observation of the sample synthesized using EDTA exhibited a flower like structure as reported in our previous work [34] which is shown in the inset of Fig. 3(c). A more close observation of an individual flake of the flower using HRTEM is shown in Fig. 3(c). Fig. 3(d) shows the SAED pattern of EHA-1 sample which is exhibiting continuous ring of diffused spots that represents the polycrystalline nature of EHA-1. Moreover it shows clear reflections from (402), (202), (102), (111), (002), (211), (310), (004), (321) and (222) Miller's planes of HA.

HRTEM pictures in Fig. 4(a) and (b) display the PEG assisted synthesized EHA sample (EHA-2). It exhibited rod shape with aggregates containing less number of nanoparticles of length and width 31 ± 8 nm and 8 ± 1 nm respectively. Remarkably, individual particles of EHA-2 sample contained pores of ~ 5 nm as can be seen from Fig. 4(b) (inset at top right corner). A magnified image of EHA-2 in Fig. 4(b) shows that the obtained nanoparticles are almost homogeneously sized. The SAED pattern of EHA-2 is made up of continuous rings with lucid spots revealing the formation of polycrystalline material.

From the HRTEM images of EHA-3 it is observed that the sample consists of highly agglomerated needle like nanoparticles having a length of about 32 ± 8 nm and width 3 ± 1 nm (Fig. 5(a)). EHA-3 exhibits polycrystalline nature which was confirmed from the lucid ring observed in the SAED pattern (Fig. 5(b)). HRTEM image (Fig. 5(c)) of EHA-4 exhibits highly aggregated nanorods of 68 ± 20 nm length and 10 ± 3 nm width. Fig. 5(d) shows the SAED pattern of EHA-4 containing bright spots arranged in ring pattern indicating the polycrystalline nature of the sample.

3.4. EDAX

The compositional analysis on the as synthesized powders was studied using EDAX. The prepared samples are composed of elements such as calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg), silicon (Si), carbon (C) and oxygen (O). The elemental composition of the synthesized samples examined by EDAX is given in Table 2. The Ca/

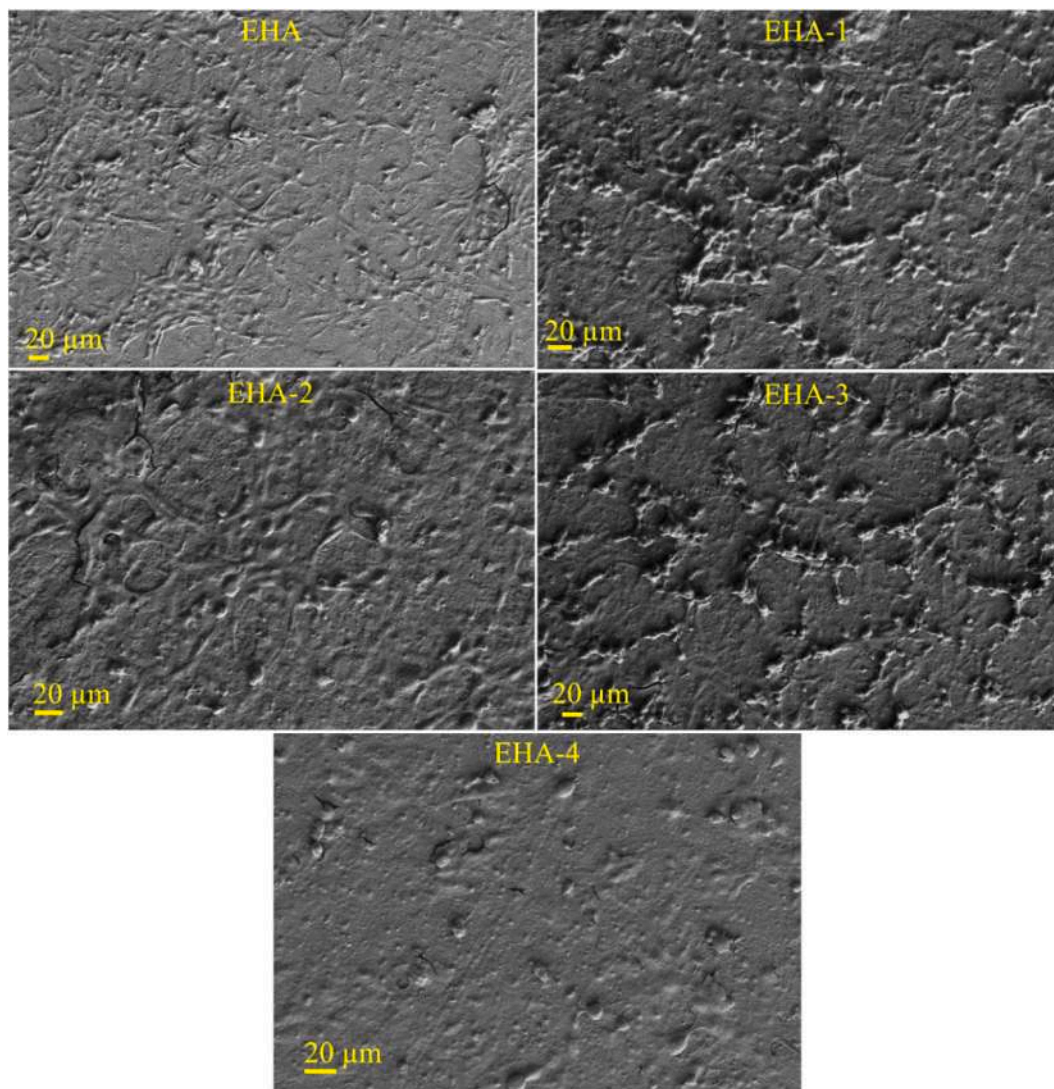


Fig. 9. FESEM micrographs of fibroblast NIH 3T3 cell adhered on the prepared samples disc surface.

P ratio obtained for the samples is 1.51, 1.42, 1.46, 1.49 and 1.37 respectively for EHA, EHA-1, EHA-2, EHA-3 and EHA-4. The obtained Ca/P ratio of the powder samples are less than 1.67 confirming that the synthesized samples are indeed non-stoichiometric HA.

Analysis of the elemental composition of chemically untreated eggshell was done by EDAX. The eggshell studied was found to be composed of elements such as calcium (13.8 at.%), phosphorus (0.3 at.%), sodium (0.9 at.%), magnesium (0.7 at.%), silicon (0.2 at.%), carbon (27.2 at.%), oxygen (55.9 at.%), fluorine (0.8 at.%) and iron (0.2 at.%). The eggshell was chemically processed to obtain HA, which contains trace elements such as magnesium, silicon and sodium. Trace elements in the prepared HA nanoparticles might be mostly contributed by the eggshell.

3.5. TG/DSC

TGA and DSC plots of the EHA, EHA-1, EHA-2, EHA-3 and EHA-4 samples are presented in Fig. 6. TGA plots of all the prepared samples revealed gradual weight loss due to the liberation of surface adsorbed water and carbonate molecules. All the samples showed three steps of weight loss, first exothermic weight loss observed in between ~ 34 and 180 °C for all samples is ascribed to the dehydration of the prepared samples and evaporation of physically/surface adsorbed water molecules. The second weight loss observed between ~ 180 and 900 °C is due

to the removal of chemisorbed water and gradual elimination of carbonate molecules. The final weight loss of all the samples observed in the temperature range of ~ 900 – 1200 °C corresponds to the decomposition of the HA phase into β – tricalcium phosphate (β -TCP) due to removal of the structural OH^- group. Table 3 shows the temperature and its corresponding weight loss percentage of the prepared samples. It is noted that EHA-1 showed high weight loss in first and second steps which clearly indicates it contains a large amount of water molecules and carbonate content.

3.6. BET

Fig. 7 shows the plots of N_2 adsorption-desorption isotherm, Brunauer–Emmett–Teller (BET) surface area (inset in Fig. 7(a)) and pore size distribution of the prepared samples. Herein, the BET surface area plot of all the samples showed a linear variation which pointed out the accuracy in the quantitative measurement of surface area. The BET surface area of the EHA, EHA-1, EHA-2, EHA-3 and EHA-4 samples was observed as 54.74, 5.97, 33.57, 87.96 and 30.38 m^2/g , respectively. In general, the surface area of a material mainly depends upon the particle shape and size [40]. The obtained results clearly state that HA prepared in the presence of organic modifiers can change the surface area by controlling the shape and size of HA nanoparticles. Herein, Fig. 7(a) shows adsorption isotherm of all the samples which revealed type IV

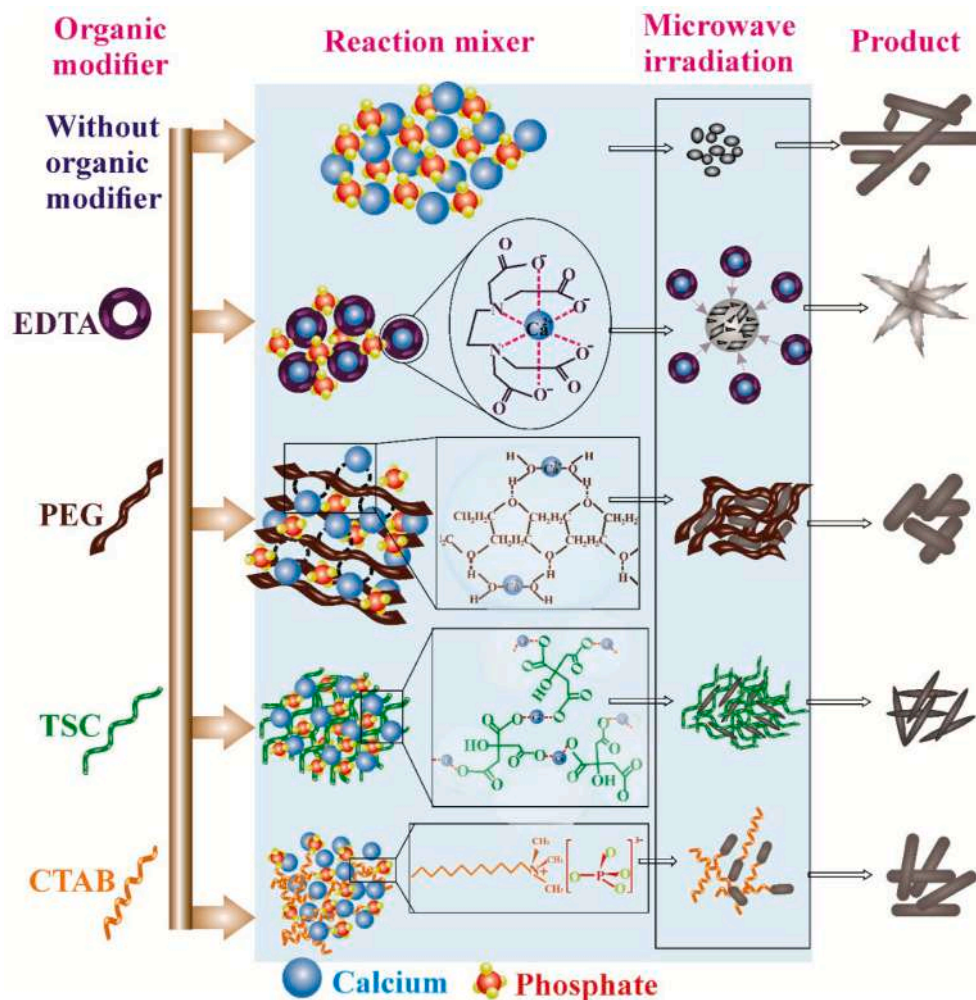


Fig. 10. Schematic representation of the formation of HA under microwave irradiation (2.2 kW) in the presence of different organic modifiers

physorption isotherm according to the IUPAC classification [41]. This result clearly represents that the prepared samples are mesoporous materials. Fig. 7(b) shows the pore volume and pore diameter of the prepared samples. The Barrett-Joyner-Halenda (BJH) method was used to calculate the pore volume of samples. The pore volume of EHA, EHA-1, EHA-2, EHA-3 and EHA-4 were found to be 0.47, 0.03, 0.35, 0.58 and 0.24 respectively. Average pore diameter was found to be 221.04, 079.87, 262.69, 279.83 and 181.36 Å for EHA, EHA-1, EHA-2, EHA-3 and EHA-4 samples, respectively. According to IUPAC classification, the observed hysteresis loop clearly shows that the EHA contains cylindrical pore channels whereas other samples have slit shaped pore channels [41,42].

3.7. Cytocompatibility

Fig. 8 displays the cell viability of fibroblast NIH 3T3 cells cultured with different dosages of prepared samples. It is obvious that the prepared samples exhibited good cell viability for the different dosages of the samples. All the examined samples exhibited cell viability of above 82%. Cell viability of the samples (EHA to EHA-3) decreased with increasing concentration of the samples but EHA-4 sample maintained cell viability above 98% for all concentrations. However, slight variation in the cell viability is observed between the samples which may be attributed to their internal characteristics.

3.8. Cell adhesion

Cell attachment on the disc surface of EHA is a vital prerequisite stage for the growth of cells, migration and other functions. Fig. 9 shows the FESEM micrographs of the cells cultured on prepared disc samples for 24 h. All the samples exhibit suitable cell attachment on the disc surface. However, the EHA-4 sample showed less cell attachment than the other four samples. The presence of trace ions (Mg, Si, Na and CO_3^{2-}) inherited from the eggshell is believed to significantly enhance the biomineralization, cell proliferation and osteogenic properties of HA [43,44].

In recent years, material scientists are interested in the microwave method of material synthesis since it is an efficient and quick way to synthesize inorganic nanoparticles. Recently, our research group has designed and fabricated a microwave reactor capable of operating at 2.2 kW. In the present study, we have conducted the synthesis of mesoporous HA nanoparticles with different sizes and shape using some organic modifiers (Fig. 10) employing the above mentioned custom built microwave reactor in 5 min.

During synthesis size and shape of the HA, particles can be controlled using organic modifiers. The organic modifiers such as EDTA, PEG and TSC interact with calcium ions to make complex forms. The polyamino carboxylic acid of EDTA (hexadentate unit) reacts with Ca^{2+} to form a Ca-EDTA complex [45], PEG is a weak chelating and non-ionic organic modifier and chelate the Ca^{2+} ions in the form of PEG-O- Ca^{2+} -O-PEG [46], while citrate ion reacts with Ca^{2+} ions to form calcium citrate complex [36]. These complexes reduce the availability of free Ca^{2+} ions

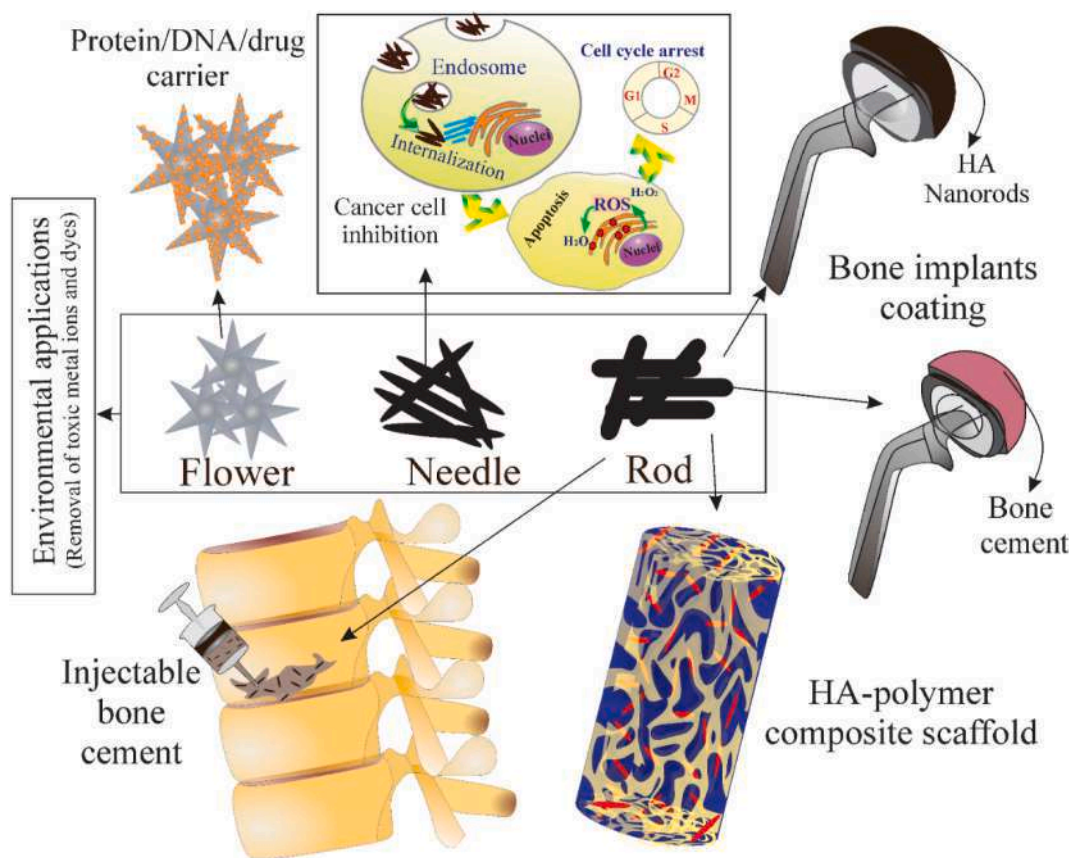


Fig. 11. Schematic diagram represents the different applications of various size and shape of HA nanoparticle

and inhibit the formation of HA at ambient temperature. The anionic organic modifier CTAB belongs to the soft template category; it, interacts with water to form positive charge micelles which react with the negatively charged PO_4^{3-} ions to form $\text{CTA}^+\text{-PO}_4^{3-}$ due to strong electrostatic interaction [14,47]. Under microwave irradiation or thermal impact, the reaction mixture containing calcium complex releases Ca^{2+} ions which immediately react with phosphate and phosphate complex releases PO_4^{3-} ions which abruptly react with calcium to form HA crystal with controlled size and shape. The mechanism of formation of flower like HA in presence of EDTA was previously reported by several researchers [45,48,49]. Jung Sang Cho et al., reported the spherical, rod and fibre like HA nanoparticles formed in presence of different concentration of PEG organic modifiers [50]. Xiaoying Jin et al., studied the formation of HA nanorods with various aspect ratio through the hydrothermal method in the presence of sodium citrate organic modifier [36]. YingJun Wang et al., explained the formation mechanism of HA nanorods in the presence of CTAB surfactant [51].

HA nanoparticles with different sizes and shapes find wide application in hard tissue replacement, drug/protein/DNA carrier, etc. Xin-Yu Zhao and co-workers reported that flower like nanostructured HA has high adsorption capacity of proteins like bovine serum albumin-165 mg/g, hemoglobin-164 mg/g and DNA (fish sperm DNA-112 mg/g) [52]. Further, it has exhibited pH dependent protein release behaviour, revealing that the flower like morphology is suitable for physio-pathological pH responsive drug carrier application. Rod like HA nanoparticles are reported to enhance the cell attachment and migration leading to osteogenic efficacy. For example, osteogenic bioactivity of the rod shaped HA nanoparticle studied by Yulin Li et al. showed that stem cells could efficiently adsorb on less crystalline nanorods that mimic the natural HA. Ca^{2+} and PO_4^{3-} ions released by the partial dissolution of the HA nanorods at an acidic lysosomal condition regulate the osteogenic activity of stem cells [53]. HA nanorods are reinforced as an

inorganic material in the composite scaffold to make bone substitutes for bone regeneration. In fact, HA nanorod addition in a polymeric material can increase mechanical properties and stimulate new bone formation [12]. Zandi et al. studied the biological and mechanical properties of HA nanorod reinforced gelatin composite scaffold fabricated using the freeze drying method. The obtained HA-gelatin composite scaffold increased the cell attachment and migration as compared to a pure gelatin scaffold [54].

Yubei Qiu et al. prepared bone morphogenetic protein-2 (BMP-2) loaded mesoporous HA nanorods mixed with silk fibroin/chitosan suspension and it was used to fabricate composite scaffold by freeze drying method. The presence of mesoporous HA nanorods induced *in vitro* osteogenic differentiation and promoted *in vivo* osteogenic efficacy. Furthermore, the mesoporous HA composite scaffold revealed the sustained release of BMP-2 and the concentration of BMP-2 release contributed to the desired bioactivity and improved osteogenic efficacy [55]. Li et al. reported the PEG surfactant assisted synthesis of HA nanorods where they introduced HA nanorods into polyurethane based injectable bone cement which showed enhanced bioactivity and mechanical properties towards osteoporosis management [56]. Also, Che et al. prepared composite bone cement using poly methyl methacrylate and rod like HA nanoparticles, which revealed an improved degree of bone mineralization and proliferation of bone progenitor cells [57].

On the other hand, nano HA is widely used in environmental applications because it reveals excellent adsorption, surface reactive and ionic exchange properties. For example, removal of Pb(II) ions from waste water by the sphere and rod shaped HA nanoparticles reported by Bharath et al. clearly indicated that HA nanoparticle of rod shape adsorbed more Pb(II) ions from waste water as compared to spherical one [58]. Varaprasad and co-workers studied the acidic blue 113 dye removal from waste water using rod like HA based hydrogel nanocomposite [59]. Inhibition ability of breast cancer cell growth by HA

Table 4

Size and shape of HA particles synthesized in presence of various organic modifiers by different methods.

Starting materials		Method of synthesis and conditions			Particle shape & size		Ref.
Chemical formula	Organic modifier	Method	pH	Time	Shape	Size	
Ca(NO ₃) ₂ ·4H ₂ O Na ₂ HPO ₄	EDTA	Microwave 700 W	13	30 min	leaf-like flake	L: 1–2 μm W: 150–200 nm	[62]
Ca(NO ₃) ₂ ·4H ₂ O Na ₂ HPO ₄	EDTA	Microwave 600 W	10	19 min	Rod	D: ~5 nm L: ~15 nm	[49]
Ca(NO ₃) ₂ ·4H ₂ O Na ₂ HPO ₄	EDTA	Microwave 750 W	between 9 and 14	30 min	Elliptical Rod	15–17 nm × 25–28 nm D: ~200 nm	[63]
Eggshell Na ₂ HPO ₄	EDTA	Microwave 600 W	13	10 min	Flower	L: 0.5–1 μm W: 100–200 nm	[48]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄ NH ₄ F	TSC	Microwave 600 W	10	25 min	Rod	L: 26 ± 3 nm W: 9 ± 2 nm	[64]
Eggshell Na ₂ HPO ₄ (Previous work)	EDTA	Microwave reactor 2.2 kW	13	5 min	Flower	2 μm–3 μm	[34]
Ca(NO ₃) ₂ ·4H ₂ O Na ₂ HPO ₄	EDTA	Hydrothermal 120 °C	5 and 12	12 h	Needle and rod	~50–200 nm	[65]
Ca(NO ₃) ₂ (NH ₄) ₂ HPO ₄	PEG-400 PEG-6000 PEG-20000	Hydrothermal 150 °C	11	40 h	Rod (PEG-400) Rod (PEG-6000) Rod (PEG-20000)	L: ~30–130 nm D: ~15–35 nm L: ~30–120 nm D: ~20–30 nm L: ~30–170 nm D: ~15–35 nm	[66]
Ca(NO ₃) ₂ ·4H ₂ O Na ₃ PO ₄ ·12H ₂ O	TSC	Hydrothermal 150 °C	Not stated	24 h	Rod (Ca/Ca:1/3) Rod (Ca/Ca:4/3)	L: 49 nm D: 13 nm L: 44 nm D: 9 nm	[36]
Ca(NO ₃) ₂ Na ₃ PO ₄	CTAB	Hydrothermal 150 °C	Not stated	10 h	Rod	L: 150 nm	[46]
CaCl ₂ ·2H ₂ O (NH ₄) ₂ HPO ₄	CTAB and PEG-6000	Hydrothermal 180 °C	10.5	24 h	Rod (CTAB) Chain (PEG-6000)	L: 250 nm D: 30 nm L: 180 nm D: 20 nm 5–50 nm	[58]
CaCl ₂ ·2H ₂ O (NH ₄) ₂ HPO ₄	CTAB	Hydrothermal 180 °C	12	12 h	Rod	5–50 nm	[67]
CaCl ₂ H ₃ PO ₄	CTAB	Hydrothermal 150 °C	9	12 h	Rod	L: 1125 nm D: 60 nm	[51]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄	CTAB	Hydrothermal 150 °C	8	12 h	Nanowire	L: 600 nm D: 33 nm	[68]
Eggshell HCl Na ₂ HPO ₄	CTAB	Hydrothermal 180 °C	Between 9 and 12	12 h	Rod	L: 161 ± 44 nm D: 52 ± 8 nm	[43]
Na ₂ HPO ₄ ·2H ₂ O CaCl ₂	CTAB	Hydrothermal 180 °C	Between 9.5 and 11	18 h	Rod	L: 136 nm D: 29 nm	[69]
Eggshell H ₃ PO ₄	CTAB	Hydrothermal 160 °C	12	10 h	Rod	100–300 nm	[37]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄	CTAB/PEG 600	Hydrothermal 120 °C	11	22 h	Rod (CTAB) Dandelion (CTAB & PEG 600)	D: 50–120 nm D: 80–150 nm	[70]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄	EDTA/monoethanolamine	Hydrothermal 200 °C	10	7 h	Bundles of rod	L: ~10 μm W: ~1 μm ~ 2 μm	[71]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄	Trisodium citrate dihydrate (Ct)/CTAB	Hydrothermal 180 °C	5	24 h	Micro flake [Ct/CTAB (1/1)]	~ 2 μm	[72]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄	CTAB	Microwave hydrothermal 200 °C	10	30 min,	Rod	L: 136.52 nm D: 43.17 nm	[73]
Ca(NO ₃) ₂ ·4H ₂ O Na ₂ HPO ₄	CTAB/Sodium salicylate	Reflux 100 °C	11.5	2 h	Rod	L: 100–500 nm D: ~ 50 nm	[74]
CaCl ₂ K ₂ HPO ₄ ·3H ₂ O	CTAB	Reflux 120 °C	12	24 h	Rod	L: 22 nm D: 68.9 nm	[14]
CaCl ₂ ·2H ₂ O K ₂ HPO ₄	EDTA	Reflux 140 °C	9	120 h	Elongated crystal	D: 0.1 m L: 1.5 μm	[75]
CaCl ₂ ·2H ₂ O K ₂ HPO ₄	CTAB	Reflux 83 ± 2 °C	12	24 h	Rod	L: 500 nm–2 μm D: 20–50 nm	[76]
CaCl ₂ K ₂ HPO ₄ ·3H ₂ O	CTAB	Reflux 120 °C	12	24 h	Rod	L: 500–1000 nm T: 50–100 nm	[77]
CaCl ₂ ·2H ₂ O Na ₂ HPO ₄	TSC	Precipitation 80 °C	8.5	96 h	Platy crystal	L: 38.9 ± 8.0 nm W: 28.7 ± 4.2 nm T: 6.1 ± 2.2 nm 30 nm	[78]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄ NH ₄ F, Fe ₃ O ₄	TSC	Precipitation Room temperature	10	24 h	Rod	30 nm	[79]
Ca(NO ₃) ₂ ·4H ₂ O (NH ₄) ₂ HPO ₄	CTAB	Precipitation 80 °C	12	24 h	Rod	L: 75–150 nm D: 10–25 nm	[80]

(continued on next page)

Table 4 (continued)

Starting materials		Method of synthesis and conditions			Particle shape & size		Ref.
Chemical formula	Organic modifier	Method	pH	Time	Shape	Size	
Ca(NO ₃) ₂ (NH ₄) ₃ PO ₄	TSC	Precipitation	Between 7 and 8.5	54 min	Needle	L: 100–150 nm D: 10–20 nm	[81]
CaCl ₂ Na ₂ HPO ₄	CTAB	Wet precipitation	9.5 ± 0.5	24 h	Spherical	D: 80 ± 12 nm	[82]
Ca(H ₂ PO ₄) ₂ ·H ₂ O Ca(OH) ₂	PEG-20000	One step calcination	Not stated	30 min	Not stated	50–80 nm	[83]
Eggshell derived CaO HNO ₃ Na ₂ HPO ₄	EDTA (*) PEG (#) TSC (@) CTAB (⊗)	Microwave reactor	<10	5 min	Rod (Without organic modifier) Flower (*) Rod (#) Needle (@) Rod (⊗)	L: 40 - 600 nm W: 34 ± 10 nm 1.67 ± 0.12 μm L: 31 ± 8 nm W: 8 ± 1 nm L: 32 ± 8 nm W: 3 ± 1 nm L: 68 ± 20 nm W: 10 ± 3 nm	Present work

L: Length, D: Diameter, W: Width, T: Thickness

nanoparticles of three different morphologies under *in vitro* conditions revealed that needle HA nanoparticles reduced the growth and proliferation of breast cancer cell lines by more than 73% [60]. Moreover, needle like HA nanoparticles has high surface charge, roughness and adhesion strength which significantly enable the growth of osteoblast (*in vitro*) and *in vivo* osteointegration efficacy of scaffold in rabbit model [61]. Thus, research community is showing more focus on obtaining HA nanoparticles with various size and shape to prepare bone substitutes with suitable properties for biomedical applications. The schematic shown in Fig. 11 clearly reveal the significant role of size and shape of HA nanoparticles for various applications.


The size and shape of HA particles synthesized in the presence of the organic modifiers employed in the present study by different methods reported in the literature is listed in Table 4. As observed from the table, the time consumed for the synthesis is from few hours to several hours when the method adopted was hydrothermal, reflux, precipitation, etc. The microwave reactor we utilized consumed just 5 min for the synthesis process. Therefore, eggshell derived HA nanoparticles with suitable characteristics for various biomedical applications can be rapidly synthesized by choosing appropriate organic modifier and our custom built microwave reactor.

4. Conclusion

In summary, we have synthesized mesoporous carbonated HA using eggshell biowaste as the calcium source via a custom built pilot scale microwave reactor in 5 min in the presence and absence of organic modifiers, employing the optimized parameters from our previous work. HA synthesized without organic modifier resulted in inhomogeneous sized rods of HA. While the presence of organic modifier controlled the physicochemical properties like particle size, surface area, morphology, mesoporous nature, etc. Micron sized flower like HA formed with EDTA as the modifier. However, the other organic modifiers produced rods or needles of typical size less than 100 nm. The *in vitro* cytotoxicity experiments of the prepared HA samples on fibroblast NIH 3T3 cell line clearly evidenced their excellent biocompatibility and revealed good cell attachment. Thus the custom built pilot scale microwave reactor is capable of producing eggshell derived HA rapidly with favourable characteristics in the presence of a suitable organic modifier for developing bone fillers, drug/protein delivery carriers, and tissue engineering scaffolds.

Declaration of competing interest

None.



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Case Report

Pliable Dentures- An Alternate Denture Base Material- Case Reports

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ABSTRACT:

The use of thermoplastic resins for the denture base began around 1950s. Flexible removable denture prosthesis are also known as non-clasp, metal-free, or clasp free dentures. Flexible denture base material is a viable option to treat various partial and completely edentulous conditions. The current article describes the use of thermoplastic resin material as a treatment option for different clinical situations.

Keywords: - flexible dentures, Valplast, thermoplastic resin

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INTRODUCTION

Dentures are a mode of treatment for replacing missing teeth since 700 BC. Improvement in the quality of materials used for fabricating dentures was a continuous process to meet the patient's demands and apply them for different clinical situations. Implants and fixed partial dentures are

more preferred and advantages over removable partial dentures but, in some clinical conditions, removable partial dentures may be the only treatment option. Other than a conventional removable dental prosthesis, flexible partial dentures are superior in terms of esthetics and comfort.

COMPOSITION

It was first introduced as Valplast and Flexiplast to dentistry in 1956 ^(1, 2)

Component	Role
Polyamide nylon thermoplastic material	Resin derived from dicarboxylic acid, diamine, amino acid and lactams transparency, high impact resistance,
Elastomeric resins	Flexibility
Glass fibers	Strength

ADVANTAGES

1. Soft and inherent flexibility.
2. Clinically unbreakable.
3. The translucency of the material picks up the tissue tone, making it almost impossible to detect in the mouth
4. Good biocompatibility: Because it is, free of monomer and metal.
5. Absorb small amounts of water to make the denture more soft and tissue compatible.
6. High impact resistance
7. Color stability
8. High creep resistance



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9. High fatigue endurance
10. Excellent wear characteristics
11. Good solvent resistance
12. No porosity, so bacteria cannot build up within it.
13. No biological material build up or odors.
14. Low water sorption & good dimensional stability
15. The microcrystalline structure is easy to finish and polish.
16. Will not cause any sore spots as seen with rigid acrylic dentures.
17. Flexible dentures may be used as an alternative treatment plan in rehabilitating the anomalies such as Ectodermal dysplasia.
18. Free movement is allowed by the overall flexibility and can, therefore, be referred to as “a built in stress breaker”.

DISADVANTAGES

1. Flexible prosthesis is difficult to relin and rebase
2. Prone to staining by various ingredients of food, tea and coffee if it is not appropriately polished or cleaned by the patient regularly.
3. Acrylic teeth do not bond chemically with flexible denture base material. They are mechanically retained by making T shape holes- diatorics into

which denture base resin flows to retain teeth mechanically. This is known as Retento-Grip tissue bearing technique. ⁽³⁾

COMMERCIALY AVAILABLE PRODUCTS: - Valplast, Duraflex, Flexite, Proflex, Lucitone, Impak.

INDICATIONS: ⁽⁴⁾

1. Complete dentures, partial dentures, and in cases with bilateral in-operable undercuts when pre-prosthetic surgery is contraindicated.
2. Patients allergic to acrylic monomers
3. Used as an alternative treatment plan in rehabilitating the anomalies like Ectodermal dysplasia.
- a. Special applications-
4. TMJ splints
5. As cosmetic veneers/gum, veneers to mask gingival recession in periodontally involved teeth,
6. Mouth guards in sports,
7. Bruxism splints/ Night guards
8. Obturators
9. Orthodontic retainers.

COMPARISON WITH CONVENTIONAL ACRYLIC

Rigid denture base	Flexible denture base
Brittleness of PMMA- so frequent fracture occurs	Not brittle- Clinically unbreakable
Monomer allergy	Good biocompatibility: because it is free of monomer and metal
Irritation to mucosa	More comfortable, not irritant to mucosa
Rigid Difficult to insert in undercut areas	Soft and inherent flexibility therefore engages undercuts for retention
Less aesthetics, metal clasp	Better aesthetics, aesthetic clasps
Longer fabrication time	Shorter fabrication time
Chemical bond between acrylic teeth and acrylic denture	Mechanical retention between acrylic teeth and denture. De-bonding is a major disadvantage
Can be relined and repaired	Usually cannot be relined and repaired

CASE REPORT 1

A 58 years old male patient reported to the Department of Prosthodontics with a chief complaint of multiple missing teeth in the upper and lower arches. Intraoral examination revealed missing 17, 16, 26 in maxilla and missing 37,34,35,43,44,45,46 in the mandible (fig 1a, 1b, 1c). The denture bearing area was irregular in the mandible with bony undercuts hence, a flexible removable partial denture was advised and cast partial denture was advised for maxilla (fig 2a 2b). The dentures were fabricated and the patient was satisfied with the aesthetics and comfort of the dentures (fig 3, 4).



1. a) intra oral frontal view b) intra oral right lateral view c) intra oral left lateral view



2.a) cast partial denture -maxilla b) flexible removable partial denture -mandible



3.a, b) intra oral views after insertion of prosthesis



4. a, b) intra oral lateral views after insertion of prosthesis

CASE REPORT -2

A 16-year-old female patient reported to the Department of Prosthodontics with a chief complaint of multiple missing teeth. On intra oral examination 17,14,13,12,22,23,24,25,27,37,31,41,47 were missing (fig 5a,5b,5c). Radiographic examination revealed the absence of teeth buds of missing teeth and the patient was susceptible of Ectodermal dysplasia. To enhance comfort to the patient, as it can be built quite thin, a flexible removable partial denture (fig 6a, 6b, 6c) was suggested for maxilla until the patient achieved skeletal maturity after which the patient is suggested for implant therapy for missing teeth.



5a) intra oral frontal view b) intra oral right lateral view c) intra oral left lateral view



6 a, b) flexible denture c) intra oral view of denture

CONCLUSION

Selection of an optimum prosthesis suitable for a clinical situation depends on the skill of the clinician. Partially edentulous condition can be rehabilitated using many advanced treatment options including implants. In challenging situations where there are tilted teeth and deranged occlusion, bony undercuts may complicate the treatment plan. Flexible dentures will fulfil the clinical demand of rehabilitation in such conditions. Flexible dentures were rarely opted by few patients and the clinician but nowadays it has become an elective treatment option.

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Case Report

Customized Lubricating Eye Prosthesis - A Case Report

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ABSTRACT:

Loss of an eye is an emotional and psychological problem to the human being. An ocular prosthesis was fabricated to restore the lost anatomical structure and to correct the defect of the anophthalmic sockets. The tear reflexes do not function at optimal levels in anophthalmic sockets which make prosthesis wear uncomfortable. In this case report presents an innovative hollow ocular prosthesis with functional lubricant reservoir which upon normal blinking would draw the lubricant from the reservoir through an exit hole by the blinking action creates capillary pressure which spreads over the prosthesis allowing a comfortable and long duration of prosthesis wear.

Keywords: Anophthalmic sockets, lubrication, capillary pressure, ocular prosthesis

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INTRODUCTION:

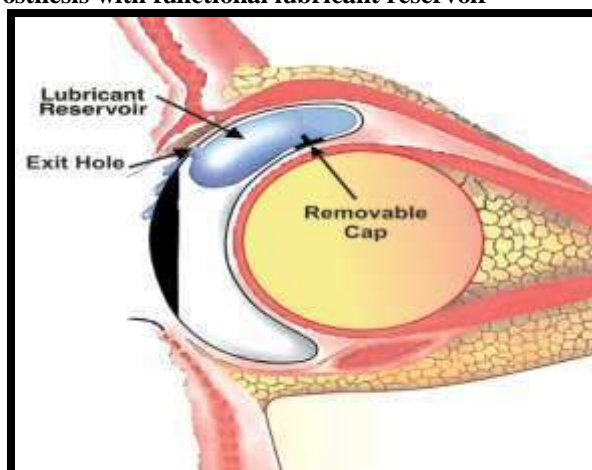
Physical defects that compromise appearance or function which prevents an individual from leading a normal life usually prompt the individual to seek treatment that will reinstate acceptable normally.

The loss of an eye impairs the patient's visual function, yet also results in noticeable deformity. A prosthesis should be provided as soon as possible to raise the spirit and ease the mind of the afflicted.

The fundamental objective in restoring the eye with a cosmetically acceptable prosthesis enables to cope better to face the outside world.

In addition to appearance, comfort is also a prime consideration to most wearers. Due to lack of lubrication in patients with anophthalmic sockets¹ blinking and tear reflexes are at optimal levels which lead to dryness, discomfort, irritation, bacterial infections².

Design of hollow ocular prosthesis with functional lubricant reservoir



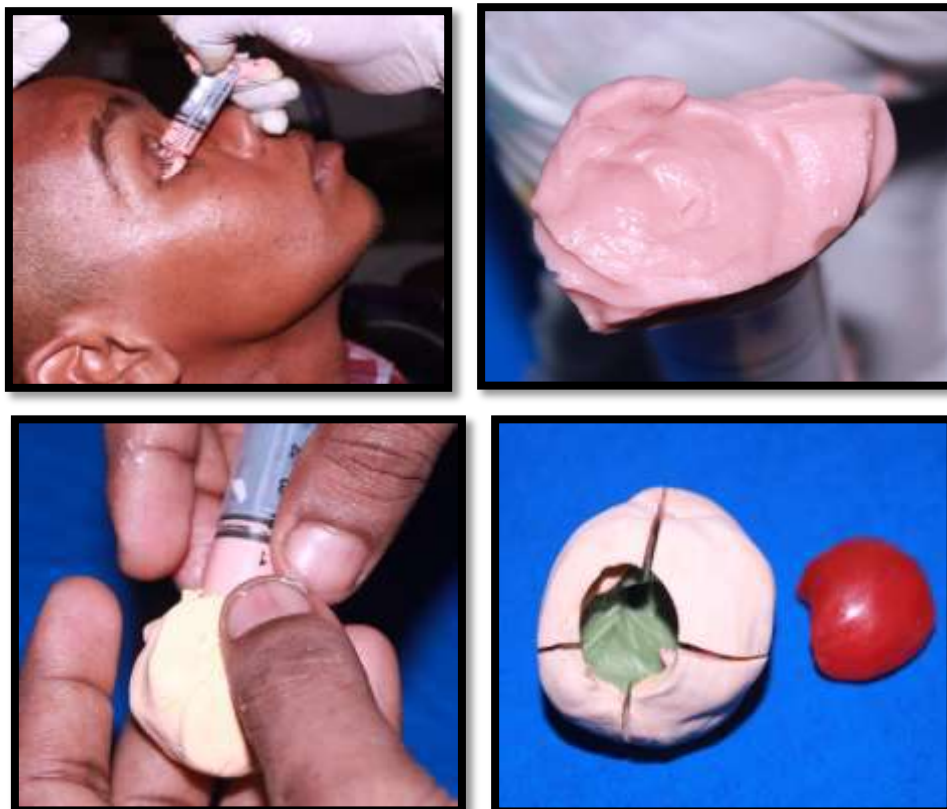
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CASE REPORT

A 28-year-old male patient reported to the Department of Prosthodontics for prosthetic rehabilitation of his lost right eye. On eliciting history, it was found that the patient had accident to his right eye, which necessitated a surgical evisceration of the eye. Clinical examination revealed a completely healed right eye socket. His left eye had normal vision. The patient had no other relevant medical history.

Procedure

The impression was made by injecting alginate impression material into the defected eye by using 5ml syringe. The patient was asked to move his eyes in all directions to facilitate flow of impression material into all aspects of the socket. Then, the patient was asked to look directly at a fixed point six feet away at the level of eye allowing impression of the site with the muscles in neutral gaze position.



- Impression is enclosed with light body, putty then it was indexed for exact location of borders and easy removal of wax pattern.



Wax try in



Flasking procedure

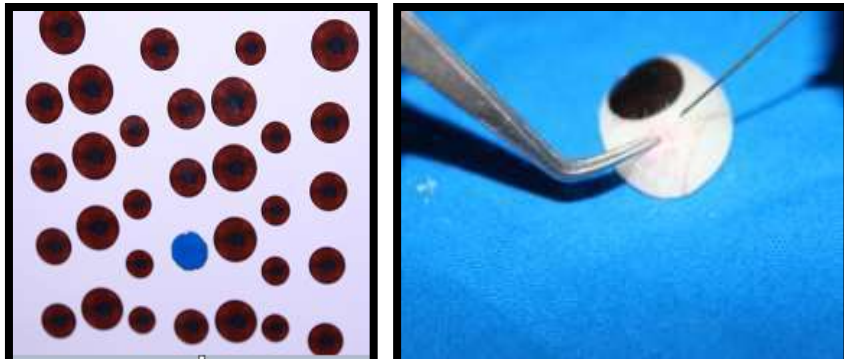

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Packing (done with mixture of heat cure clear acrylic resin and zinc phosphate³) is done by lost salt technique^{4,6}.



- Insertion was done and contra lateral eye life size photograph was taken for iris colour and position.

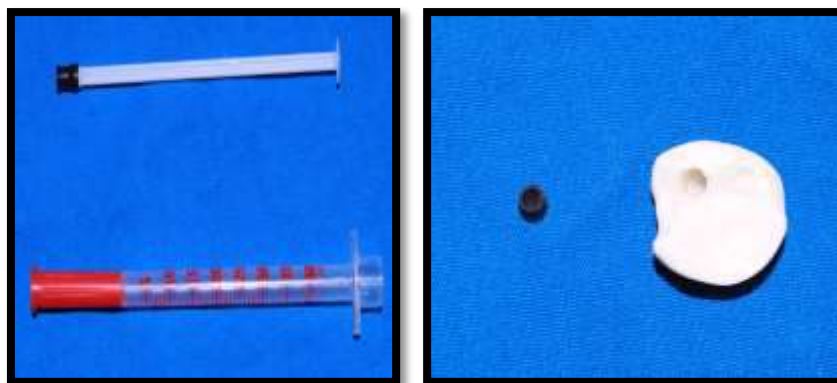


Iris placement in exact position and characterization with veined acrylic was done.



Iris and veins incorporated and exit hole was prepared


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Hollowing of prosthesis by removing salt and placing rubber cup in postero superior aspect of prosthesis.



Placing artificial lubricant and capping the prosthesis was done



Preoperative and postoperative pictures of the patient

DISCUSSION:

Anophthalmic sockets does not had blinking and tearing mechanisms of eye socket because they do not operate at all or are insufficient to allow the patient comfortable prosthesis use. In addition to the problem of dryness, continuing problems of bacterial infections are suffered by many prosthesis users.

This article is mainly to fabricate hollow ocular prosthesis with functional lubricant reservoir in an attempt to solve problems of inadequate lubrication such as dryness, discomfort, irritation, bacterial infections, and mucus deposition over prosthesis

thereby allowing long duration of the use of the prosthesis. The patient is instructed to load the lubricant into the reservoir via the removable cap. During the normal blinking action, the upper eyelid creates a negative pressure which draws the lubricating fluid from the reservoir to the anterior surface of the prosthesis through the exit hole which spreads over the prosthesis by blinking action. The hollow ocular prosthesis also improves comfort by reducing the weight of the prosthesis. The lubrication also gives a wet looking anterior surface which gives a life-like appearance. In cases of bacterial infections

instead of lubricant, antibacterial eye drops can be loaded in the reservoir.

Lost wax technique is used in fabrication of hollow ocular prosthesis to reduce the weight of the prosthesis. In this case report, the technique presented to fabricate the lubricant reservoir by lost salt technique is simple, easy to construct, affordable, uses readily available materials, is functional, and cleansable. However, it has certain drawbacks such as the design might not be feasible in patients with shallow sockets, difficulty in gauging resin thickness, and it depends on patient compliance for loading of lubricant reservoir.

This case report presents a simple technique to place and to match the exact position of the iris by using the life size photograph of the patients left eye to ensure that the same position of iris is achieved in the final prosthesis as it was during try-in thus avoiding squinted eye appearance. It can also be used to assess the orientation of visual axis during try-in.

A modified curing cycle called as reverse curing cycle was followed ensuring elimination of residual monomer by curing at an increased temperature. Veined acrylic is used for characterization and to match the natural eye color. Hollowing of prosthesis by removing salt and placing rubber cup in posterior superior aspect of prosthesis. Placing artificial lubricant⁴ and capping the prosthesis was done. Then exit hole was created on the anterior superior aspect of the prosthesis. During the normal blinking action, the upper eyelid creates a negative pressure which draws the lubricating fluid from the reservoir to the anterior surface of the prosthesis through the exit hole which

spreads over the prosthesis by blinking action. The patient is instructed to load the lubricant into the reservoir via the removable cap.

CONCLUSION:

Success of the ocular prosthesis largely depends on the artistic skills of the operator and maintenance and wearing of the ocular prosthesis by the patient. Through this technique, hollow ocular prosthesis with functional lubricant reservoir presented in this case report can help solve the multiple problems associated with dry eye socket, as a result provide, comfort for the longer duration of wear of the ocular prosthesis.


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Original Research

Effect of salbutamol-based nebulizer on surface roughness and color stability of different dental resin materials – An invitro study.

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ABSTRACT:

Introduction: Several studies have shown that chronic treatment with salbutamol sulfate and inhaled corticosteroids in asthma patients has increased the risk of dental caries and bacterial plaque accumulation, which calls for these patients' special attention by medical and dental health professionals^{1,2,3,4}. **Materials and methods:** In this prospective, an invitro study was done to evaluate the surface roughness changes and color stability of three different dental resins i.e., heat cure acrylic resin I (**ACRYLYN – H, PINK**), cross linked tooth colored acrylic resin (**BRULON INTERNATIONAL, shade A1**) and bis acrylic temporary resin (**PROTEMP 4, shade A1**). up on exposure to salbutamol-based nebulizer. The outcome parameters assessed at the end of 30days, 60days, 90days. Statistical analysis was performed using SPSS version 20 software. **Results & Conclusion:** The study revealed that the inhalation of salbutamol sulfate significantly ($p < 0.01$) affected the surface roughness and color stability of all three resins.

Keywords: Bis acrylic resin, Cross-linked acrylic resin, Nebulizer, Polymethyl methacrylate (PMMA), R_a value, Salbutamol sulfate, Spectrophotometer, Surface roughness tester, ΔE value.

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INTRODUCTION

Salbutamol sulfate is a selective β_2 agonist which is administered in inhaler form, that elicit fast onset bronchodilation in reversible airway obstruction¹. Polymethyl methacrylate (PMMA) is most commonly used denture base resin as well as denture teeth because they are economical, easy to manipulate, fabricate, and repair compared to other materials available for fabrication of denture². Studies by Quirynen et al. showed that an increase in the surface roughness of resin strips above the roughness average (R_a) value of $2\mu\text{m}$ resulted in the bacterial colonization³. The discoloration of treatment restorations when used for longer duration can result in an esthetic problem. Minimizing change of color is a factor that should be used in the selection of materials and techniques⁴. Studies revealed that the antiasthmatic medication exerts effects on dental caries and periodontal disease⁵. However, there were no such studies which evaluated the effect of antiasthmatic drugs on different resin materials. This study aimed to investigate the effect of salbutamol sulfate inhalation in a mist form on surface roughness and color stability of dental resins.

The null hypothesis was that no significant changes in the surface roughness and color of resin materials would occur after salbutamol sulfate exposure.

MATERIALS AND METHODS

Round metal molds of dimensions 10×2 mm were taken to fabricate specimens. Sixty wax patterns for groups I and II were fabricated by pouring molten modeling wax into the customized mold space of dimensions 10×2 mm and was allowed to solidify, then retrieved from the mold for investment. Dental stone was mixed according to the manufacturer's instructions and poured into the dental flasks' base. The wax patterns were invested into the dental stone to half of their height. After the dental stone is set, cold mold seal was applied and is allowed to dry. Dental stone is then mixed and poured into the flask until it is completely filled to form the second pour. After the complete set of the stone, dewaxing was done by immersing the flasks in the dewaxing unit at 100°C for 4 minutes. The flasks were then removed from the dewaxing unit, and residual wax was flushed out. After the mold is dried, cold mold seal was applied, and the heat polymerized resin (group I) and cross-linked acrylic resin (group II) materials were mixed in a porcelain jar separately according to the manufacturer's instructions (21 g of polymer to 8 ml monomer). The heat-cured acrylic resin and cross-linked acrylic resin were packed into the stone mold in the dough stage in both the compartments, and the flasks were placed under hydraulic pressure upto 1000 psi. The resin was allowed to bench cure for 30 minutes. The flasks were removed from the hydraulic press and were attached to the clamps and allowed to undergo short- time polymerization in a water bath

at 72°C for 1.5 h, followed by 30 min boiling in 100°C water in a dental acrylizer. Flasks were allowed to cool down to room temperature and then removed from the water bath and deflasked. The samples were then retrieved carefully, trimmed by using the acrylic trimmer, and all samples (group I and II) were finished with no. 220, 600, 800 silicone carbide grinding papers and polished with 1000 grade abrasive waterproof paper with intermittent movements lasting 10 s each, rinsed with tap water, and air dried. Specimens were polished using a slurry of water and pumice with a brush wheel.

Group III (Protemp 4) Sample fabrication:

The Protemp 4 (3M Deutschland GmbH, Germany. Lot NO: 3384978) provisional material, which is supplied in cartridge form as base and catalyst pastes were dispensed into the metal mold space. A glass coverslip was placed immediately above the template in order to achieve a uniform thickness (2mm) of the sample. After the final set (5 minutes), the glass coverslip and mold were separated, and the samples were retrieved. Later the samples were polished and finished with pumice.

A total of 30 samples were prepared in the same method and were stored in artificial saliva, same as the above two groups.

The specimens of all (group I, II, III) were then randomly divided into two groups ($n = 15$), i.e., the control group and the test group, and were named as group IA, IB, IIA, IIB, IIIA, IIIB. All the samples were stored in containers with a lid filled with artificial saliva (**Fig no:1**). The test specimens were stored using a supporting device.



Fig no :1 control group samples in artificial saliva

Salbutamol sulfate application in a mist form

During the salbutamol sulfate application, the test specimens were placed into a chamber using a supporting device that would allow the samples to remain in a vertical position so that the greater part of their surface would be exposed (**fig no:2**). After exposure to each respule, the test specimens were immersed in artificial saliva, brushed with toothpaste (Colgate Total) and washed under running water to simulate clinical conditions; they were then exposed to the next respule. Each sample was treated with 20 mg respule each day as it is the average daily dose for asthma patients. Readings were noted at the end of 30,

60, 90 days' time intervals.



Fig no 2 Exposure to salbutamol sulfate

Method of evaluating surface roughness:fig no 3

Before each reading, the samples were rinsed under running water for 1 minute and dried with a blotting paper. The surface roughness values (Ra) of the specimens were measured with Talysurf SurfTester (SJ-210P, Mitutoyo Corp, ISO 1997,0.02 inches/sec, Kawasaki, Japan). Three readings were taken from each sample at the center of the specimen, 1mm to the right and 1mm to the left, and the mean Ra value was calculated for each sample. Readings were noted on the control and test group specimens of all groups I, II, III at the end of the 30th day, 60th day, 90th day



Fig no 3 Measuring surface roughness with surface roughness tester (Mitutoyo)

Method of evaluating color stability: (fig no:4)

Color data was recorded using a spectrophotometer (Datacolor spectrum 600TM) by calibrating with a standard calibrator before recording the color measurements. The diameter of the measurement aperture was 6.6mm; the illumination and light beam angle was 90⁰. Color changes were examined for each specimen based on color specifications using the CIE L*a*b* color space system. The CIE L*a*b* system represents a three-dimensional color space with components of lightness (L), red-green (a), and yellow- blue (b). In terms of ΔE values, color data measurements were noted at the end of 30, 60, 90-day time intervals. All color measurements were observed from the central part of each specimen. Values of ΔE ≥3.3 were considered to be clinically unacceptable. To relate the color differences (ΔE) to the clinical

environment, the color data was quantified by the National Bureau of Standards (NBS), United States units, with the following formula: NBS units = ΔE ×0.92. Critical marks of color differences according to the NBS are classified as trace (T): 0.0–0.5; slight (S): 0.5–1.5; noticeable (N): 1.5–3.0; appreciable (A): 3.0– 6.0; much (M): 6.0–12.0 and very much (V): >12.0.

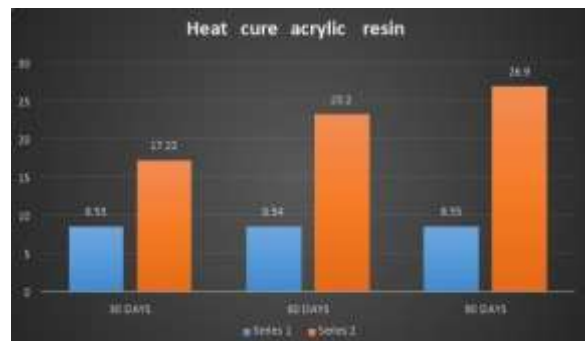


STATISTICAL ANALYSIS

The statistical analysis was performed using software SPSS version 20.0 (I.B.M., NY, U.S.A.). Paired t-test was carried to know the mean comparisons of surface roughness between the test and the control groups of three materials at three different time intervals. One way ANOVA was performed to know the between group comparisons of surface roughness, which was followed by post hoc tests for multi pair wise comparisons with the statistical significance value set as p <0.05. One way ANOVA was done to compare the means of color stability between the three groups at three different intervals followed by Tukey post hoc test with the statistical significance level set as p<0.05.

RESULTS

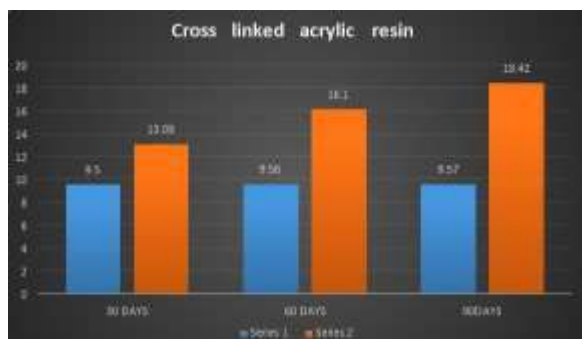
GRAPH 1: Ra Mean values comparison of Group 1 test and control samples (HEAT CURE ACRYLIC RESIN) during different time intervals.



Graph 1 represents the comparison of mean values of Group 1(heat cure acrylic resin) control and test samples, which were obtained during different time intervals. The bar graph represents that there were significant differences observed in test group samples from 30 to 90 days. The lowest mean value was obtained in the control group samples at all time intervals, and the highest mean

value was obtained in the test group samples.

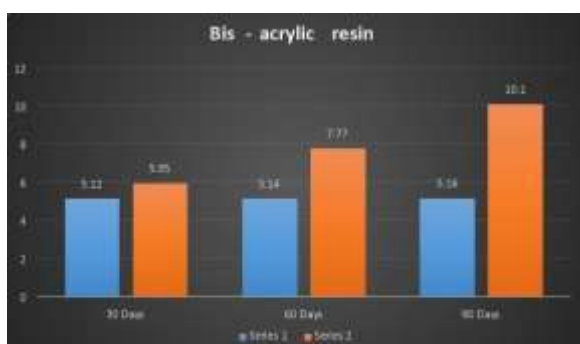
GRAPH 2: R_a Mean values comparison of Group 2 test and control samples (CROSS LINKED ACRYLIC RESIN) during different time intervals.



Graph 2 represents the comparison of mean values of Group 2 (cross linked acrylic resin) control and test samples, which were obtained during different time intervals. The bar graph represents that there were significant differences observed in test group samples from 30 to 90 days. The lowest mean value was obtained in the control group samples at all time intervals, and the highest mean value was obtained in the test group samples.

GRAPH 3: R_a Mean values comparison of Group 3 test and control samples (BIS -ACRYLIC RESIN) during different time intervals.

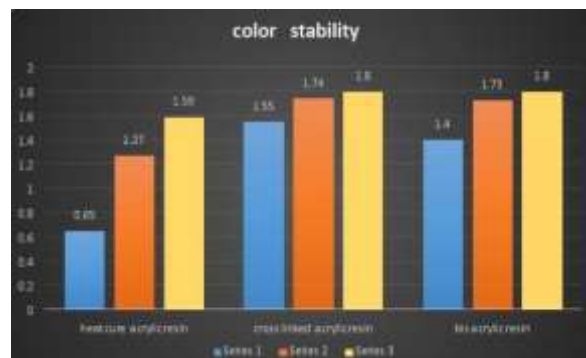
Graph 3 represents the comparison of mean values of Group 3 (Bis- acrylic resin) control and test samples, which were obtained during different time intervals. The bar graph represents that there were significant differences observed in test group samples from 30 to 90 days. The lowest mean value was obtained in the control group samples at all time intervals, and the highest mean value was obtained in the test group samples.



GRAPH 4: ΔE Mean values comparison of Group 1,2,3 during different time intervals.

Graph 4 represents the comparison of ΔE mean values of groups 1,2,3 at three different time intervals. The bar graph represents that there were significant differences

observed in group 3 samples from 30 to 90 days. The lowest mean value was obtained in the group 1 samples at all time intervals, and the highest mean value was obtained in the group 3 samples



DISCUSSION

Bronchial asthma is a globally significant disease. This chronic lung disease has a detrimental effect on the oral cavity, like the reduction of salivary secretion, change in salivary composition and pH. Inhaled β_2 agonists provide a favorable environment for the growth and multiplication of microorganisms causing dental caries (Streptococcus mutans and Lactobacilli) by decreasing salivary secretion^{6,7}. However, the effect of these antiasthmatic drugs on different dental resins has not been studied.

This study aimed at evaluating the effect of antiasthmatic medication in a mist form on change in surface roughness and color stability of heat cure acrylic resin, cross-linked acrylic resin, and bis acrylic resin materials. In this study, both the test and control group samples of all three materials were stored in artificial saliva and were brushed in order to equalize the effect of absorption as well as roughness. This study evaluated the effect on three resins by antiasthmatic medication through a nebulizer for a period of 90 days, and tests were performed at the end of 30, 60, 90 days intervals.

At the end of 30 days of exposure to the mist form of antiasthmatic medication, it was observed that the roughness has significantly increased for heat cure acrylic resin followed by cross-linked acrylic resin and least for bis acrylic resin.

At the end of 60 days, by observing the trend of R_a values it was noted that the change of roughness is more for heat cure acrylic resin when compared to the other two groups of resins.

At the end of 90 days, the R_a values of test group samples have increased. The roughness difference from initial period to final period is comparatively more for heat cure acrylic resin and cross-linked acrylic resin than bis acrylic temporization resin. Results of the present study showed that the inhalation of salbutamol sulfate significantly ($p < 0.05$) affected the surface roughness of all three resins.

Hence the structural properties and finishing procedures of restorative materials affected the surface roughness characteristic⁸. The difference could be the chemical characteristics of the material itself.

This less roughness change of bis acrylic resins can be credited to the fact stated by Balkenhol et al. that bis-acryl composites contain multifunctional monomers, which increase strength due to cross linking. According to Rawls HR, the 3-D network of cross-linked polymer chains forms a rigid structure for the composite resins as the entire network acts as one unit. This phenomenon enhances the surface characteristics of bis-acryl composite resins. Surface hardness is one of the good indicators of resistance to wear and surface deterioration⁹. Diaz-Arnold found that all bis-acrylic resin composite materials exhibit superior microhardness over the traditional methyl methacrylate resins throughout a 14-day interval of investigation¹⁰. It should also be pointed out that in addition to monomers such as bisphenol-A-diglycidyl dimethacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA), the bis-acrylic resins have an organic matrix and inorganic filler particles. Monomers and their derivatives are tend to provide better mechanical properties, reduction in polymerization shrinkage, and excellent polishing properties^{11,12}.

Antiasthmatic inhalers may produce some side effects. The manufacturers report that mouth or throat irritation is a common oral side effect of the drug¹³. Surface roughness is an important clinical criterion of restorative materials. Increased roughness may lead to more plaque retention, bacterial adherence, and gingival irritation¹⁴. The results of the present study may indicate the higher caries susceptibility of asthma patients undergoing treatment with inhalers and nebulizers. During the use of this medication, the drug is applied as a pressurized metered-dose aerosol unit for oral inhalation, which contains a microcrystalline suspension of albuterol sulfate ethanol and oleic acid. These substances cover the teeth and periodontal tissues during inhalation and may remain as a residue after inhalation. Thus, patients using inhalers and nebulizers should be advised to implement more precautionary oral hygiene measures and have their caries activity and periodontal health status regularly examined. Generally, it is advised to rinse the mouth immediately after using the inhaler¹⁵. Temporary restorations are intended for the period between tooth preparation until insertion of the final prosthesis. Even during the time when interim restorations are being present in the mouth, esthetics are important¹⁶. Color stability is important for the esthetics of long-term provisional restorations and has been previously studied in vitro for a variety of interim materials. Proprietary variations in the chemistry, such as size distribution of the PMMA particles, the polarity of

the monomers, color stability, and efficiency of the initiator system for provisional resins, may lead to different degrees of polymerization, water sorption, and consequently, color stability¹⁷ as highlighted by Mazaro et al., the mixture of monomers may affect color stability, considering that most of the bis-acrylic resin polymers are more polar than acrylic resin (PMMA) polymers. These chemical characteristics increase the affinity of bis-acrylic resins for polar liquid molecules, and consequently, facilitates greater sorption of substances that interfere in the color stability of the materials¹¹.

In the current study, inhalation influenced the color of all three resins. This discoloration may possibly be due to the active ingredient of the inhaler nebulizer, which contains a $(C_{13}H_{21}NO_3)_2H_2SO_4$ sulfate group. The ingredients of this drug may affect the sulfate group. The ingredients of this drug may affect the surfaces of the dental materials by forming a pellicle matrix that provides an acidic environment, thus promoting demineralization and increasing surface roughness and discoloration¹⁸.

To relate the color differences (ΔE) to the clinical environment, the color data were quantified by the National Bureau of Standards (NBS)⁴¹, United States units, with the following formula: NBS units = $\Delta E \times 0.92$. Critical marks of color differences according to the NBS are classified as trace (T): 0.0–0.5; slight (S): 0.5–1.5; noticeable (N): 1.5–3.0; appreciable (A): 3.0–6.0; much (M): 6.0–12.0 and very much (V): >12.0. When the mean ΔE values of the specimens were converted to NBS units, in the present study tooth colored cross-linked acrylic resins and bis acrylic resins exhibited a noticeable (N) difference after inhalation, while heat cure acrylic resins were classified as trace (T) to slight (S) at the end of 90 days. Moreover, studies conducted by Gujjari et al., and Mazaro et al.,¹² found that PMMA is more color stable than bis-acryl composite resin, as PMMA showed lower color change values as compared to bis-acrylic resin for cola and coffee solutions. This happens because the PMMA-based materials have a more homogeneous composition, and consequently, the capacity to absorb and adsorb solutions, which may have a direct influence on color stability. Because of the heterogeneity of bis-acrylic resins, the pigmented solution is capable of infiltrating into the midst of small particles of material, thus causing a greater level of pigmentation^{20,12,21}.

Thus, the results of this study indicate that the color stability and surface roughness of all the three types of resins were influenced by asthmatic medication in a mist form and also the exposure interval.

However, the present study has certain limitations as it could not completely simulate the oral environment since the results may vary due to the neutralizing effect of saliva, thermal changes of oral cavity and food, food colorants, microbes present in the oral cavity, etc.,

Furthermore, the dentures have biplanar surfaces which attract more stains rather than flat surface specimens altering the observed results.

To overcome the limitations of this study, dentures of asthmatic patients should be evaluated.

CONCLUSION

The study revealed that the inhalation of salbutamol sulfate significantly ($p < 0.01$) affected the surface roughness and color stability of all three resins.

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Original Research

Clinical study on effect of implant design on primary stability, hard and soft tissue changes

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ABSTRACT:

Introduction: The optimal achievement of osseointegration is multi-factorial which depends on the density and quality of bone, surgical techniques employed, and the design of the implants. Primary stability is one of the fundamental parameters that determine the success of osseointegration. A multitude of new macro and micro-designs have evolved over the years. However, the true influence of shape on primary stability is still surrounded by controversies. **Materials and methods:** This prospective, double-blind, randomized clinical trial was done among 20 subjects, aged between 25 and 60 years, demonstrating a motivation to receive an implant for replacement of the missing teeth. Systemically healthy subjects demonstrating adequate height and width of D2 type of bone were included in the study. The study subjects were then randomly allocated to tapered and cylindrical implant groups. Primary stability, crestal bone loss, bleeding on probing, and mucosal thickness were the outcome parameters assessed at baseline, 3 months, and 6 months. Statistical analysis was performed using SPSS version 20 software. **Results:** There was a statistically significant difference in the primary stability between the study groups with higher mean values observed in the tapered implants group (76.2 ± 1.39 vs 61 ± 3.12 ; $p=0.001$). While differences in crestal bone loss were observed between the groups at 3 months and 6 months follow-up visits, no differences were noted in the mucosal thickness and bleeding on probing. **Conclusion:** The study results demonstrate that tapered implants exhibit higher primary stability and lesser crestal bone loss compared to cylindrical implants.

Keywords: Cylindrical implants, osseointegration

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INTRODUCTION:

Oral rehabilitation with dental implants has gained prominence over the years for both partially and completely edentulous in light of the improved success rates with these treatment options. The optimal achievement of osseointegration is multi-factorial which depends on the density and quality of bone, surgical techniques employed, and the design of the implants.¹ It is the combination of these factors which influences the primary stability of an implant after implant placement.² It has been well established in the

literature that primary stability is one of the fundamental parameters that determine the success of osseointegration.³ Attempts have been made to modify the implant designs in such a manner that contributes towards an improvement in the primary stability by maximizing the contact area of the implant with the bone.⁴ The frequently used designs are the cylindrical and tapered implants. Unlike cylindrical implants, the tapered implants demonstrate a consistent reduction in diameter as we proceed towards the apex. When this narrowing starts from the shoulder of the implant and



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53

continues till the apex, the implants are categorized as fully-tapered differentiating them from those implants where the taper is restricted to cervical, middle, or apical parts.⁵

Primary stability, length of the implant, diameter, shape, thread design, etc all these factors contribute to osseointegration which promotes implant success.⁶ However, the true influence of shape on primary stability is still surrounded by controversies. With this background, the aim of this study was to evaluate the influence of implant design on primary stability and hard, soft tissue changes following implant placement between tapered and cylindrical implants.

MATERIALS AND METHODS:

This prospective, double-blind, randomized clinical trial was done among 20 subjects, aged between 25 and 60 years, demonstrating a motivation to receive an implant for replacement of the missing teeth. The sample size was calculated using G*power 3.1.9.4 software to detect an effect size of 1.4 using the Mann-Whitney U test at 80% power and an alpha error of 5%. The ethical approval for this study was obtained from the institutional ethical committee (Pr.134/IEC/SIBAR/2018). The study was conducted between May 2019 and July 2020. Informed consent was obtained from those participants before conducting the study. Systemically healthy subjects demonstrating adequate height and width of D2 type of bone were included in the study. Pregnant women, lactating mothers, smokers, subjects with uncontrolled diabetes or bleeding disorders, and severe bruxism were excluded. Oral prophylaxis was done for all the study subjects. Clinical evaluation of edentulous sites receiving implants was followed by a radiographic examination by using intraoral periapical radiographs, cone-beam computed tomography (CBCT). Diagnostic casts were made to evaluate the intra-arch space. In order to obtain an ideal position for implant placement, surgical guides were fabricated using acrylic resin. Bleeding on probing was assessed using a pressure-sensitive probe (0.5, 5.5, 8.5, 10.5mm) at baseline, 3rd, and 6th month postoperatively. The probe is passed along the gingival sulcus with the force of 0.25N/cm wait for 30 seconds to score the bleeding index. Mucosal thickness was assessed by using endodontic file no 20 with a rubber stopper. The file is inserted at the midpoint of the attached gingiva between the mucogingival junction and an imaginary line from adjacent tooth CEJ.

Prior to implant placement, subjects were advised to perform mouth rinsing with 0.12% chlorhexidine. After administration of 2% lignocaine with 1:80,000 adrenaline, mid crestal and reliving incisions were given and a full-thickness mucoperiosteal flap was

reflected and Point of entry was gained through a guiding hole made in the surgical guide using a precision drill. The preparation was done with the conventional drilling method in a sequential manner as instructed by the manufacturer (2.0, 2.4/2.8, 3.2/3.65) with the help of a physio dispenser at a speed of 800-2000 rpm. Preselected implants of 10mm length and 3.75 mm width were threaded into the prepared site at a low speed of 25 rpm at the crestal bone level using a handpiece/ratchet with an insertion tool. After placement, the primary stability of the implant was assessed with an osstell peg using a resonance frequency analyzer (Osstell) by attaching a transducer probe to the implant. In both the buccolingual and mesiodistal directions, ISQ measurements were obtained. The mean of the highest measurements in each of the two directions was considered as the ISQ measure for the implant. Soft tissue flaps were approximated using 3-0 silk. The marginal bone level was radiographically assessed at this time. The antibiotic and anti-inflammatory medication was advised for all the study participants along with chlorhexidine mouth rinse for one month. Sutures were removed after 7 days and all the subjects were recalled after 3 months for the prosthesis. During the three-month follow-up visit for prosthesis placement, radiographic assessment of marginal bone loss, evaluation of the mucosal thickness, and bleeding on probing were done. Patients were again recalled three months after the placement of the prosthesis, and crestal bone loss, mucosal thickness, bleeding on probing were assessed. Figure 1 shows the study flow chart.

Statistical analysis was done using IBM SPSS version 20 software (IBM SPSS, IBM, Armonk, NY, USA). The normality of the data was assessed using Kolmogorov Smirnov test and the choice of statistical tests to analyze the study data was made accordingly. Mann-Whitney U tests were used to check the differences in the study parameters between the tapered and cylindrical implant groups at various time points. Repeated Measures Analysis of Variance (ANOVA) tests were used to check the changes in crestal bone loss, bleeding on probing, and mucosal thickness scores within each of the study groups with a change in time.



Figure 1: Primary stability measured with osstell



Figure 2: Measuring bleeding on probing by using pressure sensitive probe at 3 months

Figure 3: Measuring bleeding on probing by using pressure sensitive probe at 6 months



Figure 4: Assessment of mucosal thickness with endodontic k-file at 3 months

Figure 5: Assessment of mucosal thickness with endodontic k-file at 6 months

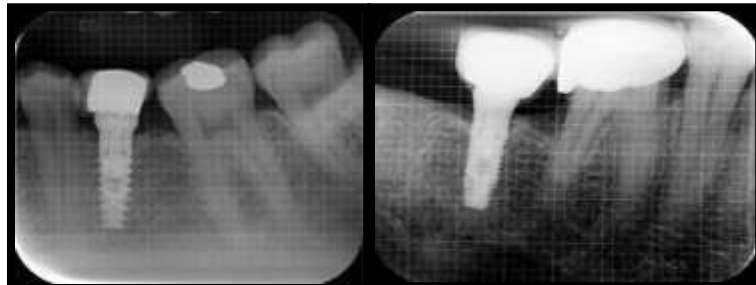


Figure 6: Evaluation of crestal bone loss at 3 months for tapered implant

Figure 7: Evaluation of crestal bone loss at 3 months for cylindrical implant

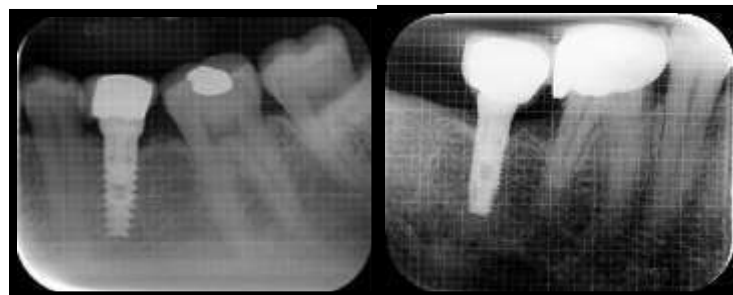


Figure 8: Evaluation of crestal bone loss at 6 months for tapered implant

Figure 7: Evaluation of crestal bone loss at 6 months for cylindrical implant

RESULTS:

The mean age of the study participants was 48.34±9.41 years. There was a statistically significant difference in the primary stability between the study groups with higher mean values observed in the tapered implants group (Table 1). At baseline, there was no crestal bone loss and bleeding on probing in both the study groups. The mean mucosal thickness at baseline in the tapered implants group was 0.92±0.13 mm, while it was 1.03±0.16 mm in the cylindrical implants group (Table 1).

Table 2 gives a comparative account of the crestal bone loss, mucosal thickness, and bleeding on probing at 3 months follow-up between tapered and cylindrical implants groups. Crestal bone loss on the mesial side, distal side, and the overall scores were compared separately between the study groups. None of the study parameters demonstrated significant differences between the study groups at 3 months follow-up. The magnitude of crestal bone loss on the mesial, distal sides and the overall bone loss values were significantly higher in the cylindrical implants group at the 6 months follow-up visit. At 6 months follow-up, though higher mean scores were obtained for bleeding on probing, mucosal thickness in the cylindrical implants group, these differences were not statistically significant (Table 3). Repeated measures ANOVA revealed that there had been significant changes in the parameters of crestal bone loss, bleeding on probing, mucosal thickness during the study period in both the tapered and cylindrical implant groups (Table 4 & Figure 2).

Table 1: Comparison of primary stability between the study groups

Variable	Group	N	Mean±SD	Mean Rank	P Value
Primary Stability (ISQ)	Tapered	10	76.2±1.39	15.5	0.001*
	Cylindrical	10	61±3.12	5.5	
Mucosal thickness	Tapered	10	.92±0.13	8.65	0.15
	Cylindrical	10	1.03±0.16	12.35	

Mann Whitney U test; p≤0.05 considered statistically significant; * denotes statistical significance

Table 2: Comparison of study parameters between the groups at 3 months

Variable	Group	N	Mean±SD	Mean Rank	P Value	
Crestal bone loss	Mesial	Tapered	10	0.2±0.42	9	0.17
		Cylindrical	10	0.5±0.52	12	
	Distal	Tapered	10	0.2±0.42	8.5	0.07
		Cylindrical	10	0.6±0.52	12.5	
	Overall	Tapered	10	0.4±0.69	9	0.03*
		Cylindrical	10	1.1±0.98	12	
Bleeding on probing	Tapered	10	0.5±0.52	9	0.2	
	Cylindrical	10	0.9±0.73	12		
Mucosal thickness	Tapered	10	0.82±0.16	9.6	0.48	
	Cylindrical	10	0.87±0.13	11.4		

Mann Whitney U test; p≤0.05 considered statistically significant; * denotes statistical significance

Table 3: Comparison of study parameters between the groups at 6 months

Variable	Group	N	Mean±SD	Mean Rank	P Value	
Crestal bone loss	Mesial	Tapered	10	0.5±0.52	7.75	0.002*
		Cylindrical	10	1.2±0.63	13.25	
	Distal	Tapered	10	0.3±0.48	7.05	0.005*
		Cylindrical	10	1.2±0.63	13.95	
	Overall	Tapered	10	0.8±0.91	7.75	0.021*
		Cylindrical	10	2.4±1.26	13.25	
Bleeding on probing	Tapered	10	0.5±0.52	9.75	0.52	
	Cylindrical	10	0.7±0.67	11.25		
Mucosal thickness	Tapered	10	0.88±0.16	10.45	0.96	
	Cylindrical	10	0.88±0.13	10.55		

Mann Whitney U test; p≤0.05 considered statistically significant; * denotes statistical significance



Table 4: Changes in study parameters with time in each of the study groups

Variable	Group	Time	Mean±SD	Type III sum of squares	F Value	P Value
Overall crestal bone loss	Tapered	Baseline	0	1.267	5.51	0.014*
		3 Months	0.4±0.69			
		6 Months	0.8±0.91			
	Cylindrical	Baseline	0	7.2	23.14	0.001*
		3 Months	1.1±0.98			
		6 Months	2.4±1.26			
Bleeding on probing	Tapered	Baseline	0	1.67	6.42	0.008*
		3 Months	0.5±0.16			
		6 Months	0.5±0.16			
	Cylindrical	Baseline	0	4.46	8.26	0.003*
		3 Months	0.9±0.23			
		6 Months	0.7±0.21			
Mucosal thickness	Tapered	Baseline	0.92±0.13	0.051	6	0.01*
		3 Months	0.82±0.16			
		6 Months	0.88±0.16			
	Cylindrical	Baseline	1.03±0.16	0.161	74.79	0.001*
		3 Months	0.87±0.13			
		6 Months	0.88±0.13			

Repeated Measures ANOVA; $p \leq 0.05$ considered statistically significant; * denotes statistical significance

DISCUSSION:

The study results demonstrate that tapered implants had better primary stability and less crestal bone loss as compared to cylindrical implants. While there were no significant differences between these two groups with regard to bleeding on probing and mucosal thickness, crestal bone loss absolute values were found to be slightly inclined in favor of tapered implants. Primary stability is considered as one of the fundamental parameters in the determination of osseointegration. It has been established in the literature that the incidence of implant failure was higher with lesser primary stability values.⁷⁻¹⁰ The rationale for this observation could be found in the reduced micromotion and lesser possibility for fibrous tissue formation at the bone and implant junction among implants with higher primary stability.¹¹ While primary stability does depend on factors outside implant design such as type of bone and the surgical technique used etc.,¹² the methodology adopted in this study with robust exclusion criteria and randomization ensures that the differences in primary stability between the study groups could be attributed to the differences in implant design. The difference in design between the tapered and cylindrical implants which influences the magnitude of surface area available to be in contact with the bone could be one of the primary reasons for increased ISQ values in the tapered implants group. This double-threaded design also allows for smoother penetration and attributes high bone condensing properties. Furthermore, tapered implants exert an increased compressive force on the surrounding bone that allows for thorough clamping of

the bone axially between the threads and the collar. These observations are in congruence with the results from previous literature.¹³⁻¹⁶ However, Waechter J et al.¹⁷ and Sakoh J et al.¹⁸ reported comparable ISQ measurements for tapered and cylindrical implants. The evidence on the influence of implant macro-design on the marginal bone loss around implants is equivocal. Lee et al.¹⁹ reported comparatively lesser marginal bone loss values with tapered implants compared. In the study by Lee et al., the mean difference in the increment of marginal bone loss from baseline to one year between tapered and cylindrical implants was 0.14, with higher increments observed in the cylindrical implants group.¹⁹ Similar observations were made in the present study, with the mean increment in crestal bone loss from baseline to 6 months in the cylindrical implant group being higher compared to the tapered implants group, with a mean difference of 1.6 mm. It could be inferred from these findings that tapered implants fare well in comparison to cylindrical implants demonstrate better integration with the proximal bone. These findings are in accordance with those reported by Kadkhodazadeh et al.²⁰ where the marginal bone loss values at 1-year follow-up were 0.88 ± 0.43 mm and 0.61 ± 0.34 mm for cylindrical and tapered implants, respectively. However, Sargolzaie N et al.²¹ reported comparable crestal bone loss for tapered and cylindrical implants at 6-month follow-up, which is in contrast with the findings of the present study. The present study showed no significant differences in the bleeding on probing and mucosal thickness between the tapered and cylindrical implants, which was in accordance with the

findings reported by Sargolzaie N et al.²¹ and Zafiroopoulos GG et al.²²

CONCLUSION:

Within the limits of this study, it was found that the tapered implants demonstrate better primary stability compared to cylindrical implants. Tapered implants also showed reduced crestal bone loss as compared to cylindrical implants at the follow-up evaluations. However, no significant differences were noted between the two implant designs with regard to soft tissue changes.

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Original Research

A comparative evaluation of mandibular anterior ridge resorption in conventional complete dentures and four implants supported over dentures - An *in vivo* study

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ABSTRACT:

Aim: The main goal and purpose of this study was to compare and evaluate the mandibular anterior residual ridge resorption in four implant-supported overdentures and conventional complete dentures. **Methods and Material:** This study involved two groups of 20 patients with different prosthetic constructions. Group (1) mandibular complete dentures (CDs) as a control (10 patients). Group (2) mandibular overdentures supported by four implants placed in the interforaminal region and the first molar region following the two-stage surgical procedure and early loading protocol as a test group (10 patients). Evaluation of anterior residual ridge resorption (RRR) was carried out at baseline, at the time of surgery, and 06 months using CBCT and mucosal thickness was assessed at baseline, three months, and six months. **Statistical analysis:** The data was statistically analysed by using the Mann-Whitney u test. **Results:** The mean change in ridge resorption from baseline to 6 months in the control group is 0.38 ± 0.01 (p-value 0.006). Whereas the mean shift in ridge resorption from baseline to 6 months in the test group was 0.15 ± 0.10 (p-value 0.006). The mean change in mucosal thickness from baseline to 6 months in the control group is 0.54 ± 0.04 (p-value 0.796). Where 0.44 ± 0.05 (p-value 0.796). **Conclusion:** Within the limitations of the study, it was concluded that implant-supported overdentures (ISODs) are an effective treatment modality in the rehabilitation of completely edentulous mandibular arches with improved patient satisfaction, chewing ability and in terms of ridge resorption, mucosal thickness and retention when compared to conventional complete dentures (CDs).

Key-words: Conventional complete dentures (CDs), Implant-supported overdentures (ISODs), Residual ridge resorption (RRR), Cone-beam computed tomography (CBCT).

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INTRODUCTION:

The most common treatment for the edentulous patient is conventional dentures. However, such prostheses have well-documented problems such as lack of stability and retention. Continued loss of alveolar bone can occur over time, and cause previously stable dentures to become ill-fitting. It reported that more than 50% of those with complete mandibular dentures might have problems with stability and retention. They are often concerned about denture moving when eating, speaking, or laughing and report fears about the negative effect of dentures on social conditions. In some cases, people avoid social situations altogether¹.

Implant-supported overdentures (ISOD's) offer better stability, retention, and mastication. Patients report

greater satisfaction with aesthetics because the denture is invisible. Implants reduce bone resorption, and the long-term success rate of implants in the lower mandible is 95%, and there are a few complications¹.

The advantage of the overdenture is the increased chewing ability and improved patient confidence. When compared with an implant-supported fixed prosthesis, the overdenture has the advantage of allowing more natural cleaning as they are removable and anchored by a fewer number of implants².

Factors that are affecting the planning of the overdenture treatment are:

- The number and length of the implants
- Quality and quantity of the anchoring bone
- Economic constraints³.


Dr. L. KRISHNA PRASAD
DEAN

In this study, mandibular anterior residual ridge resorption between the conventional complete denture and four implants supported mandibular overdenture was evaluated, and mucosal thickness was also evaluated.

SUBJECTS AND METHODS:

Methodology:

Twenty completely edentulous patients attending the Department of Prosthodontics. All the patients were grouped into two groups.

GROUP 1 (Control Group): 10 completely edentulous patients to be rehabilitated with conventional complete dentures (CDs).

GROUP 2 (Test Group): 10 completely edentulous patients to be rehabilitated with mandibular four implant-supported overdentures (MIODs) placed in the interforaminal region and first molar region.

Subject selection:

Inclusion criteria:

1. Patients (male or female) within the age group of 40-65 years.
2. Absence of any systemic diseases.
3. Completely edentulous maxillary and mandibular arches.
4. Edentulous period of at least three months.
5. Patients should be the first set of denture wearers.
6. Patients with resorbed mandibular ridges, where the amount of resorption occurred, was estimated on OPG by Wical and Swoope method.
7. Adequate interarch space of 16 - 22 mm.
8. Type 1 & 2 bone density
9. Adequate bone height of 15 - 20 mm anteriorly.

Bone width not less than 5 mm, both buccolingually and mesiodistally in the anterior mandible. Posteriorly bone height not less than 12-18 mm and width not less than 8 mm.

Exclusion criteria:

1. A medical and personal history that would complicate the outcome of the study, such as alcohol or drug dependency, poor health, or any other medical, physical, or psychological reason that might affect the surgical procedure, the subsequent prosthodontic treatment and required follow-up.
2. Heavy smokers.
3. Who received bone grafting in the anterior mandible
4. Patients on radiotherapy.
5. Type 3 & 4 bone density.

Two-piece titanium implant system:

ALPHA BIO implants of size, Anterior- 3.3mm diameter, 13mm length Posterior- 4.3mm diameter,

and 8mm length two-piece implants were placed using implant drivers.

Informed consent was taken from all the patients those who were willingly ready for participation.

CONTROL GROUP PATIENTS

In the control group, preoperative CBCT scans were taken for all the patients for evaluation of available length and width of the bone. Initially, primary impressions of patients were brought in stock edentulous using Type I impression compound (figure 1) by muco-compressive technique into which Type II Gypsum product was poured to obtain primary casts (figure 2). Later custom trays were fabricated. An active border moulding technique was carried out in both maxillary & mandibular arches using a low fusing impression compound (Greenstick compound). Later final impressions (figure 3) by mucostatic technique were made using zinc oxide eugenol impression material. These Impressions were filled with type III Gypsum product to obtain master casts (figure 4). Self-cure acrylic denture bases were fabricated using the sprinkle-on method, and occlusal rims were prepared using modelling wax. Orientation jaw relations were performed using earpiece arbitrary type of face bow (Hanau spring bow) and transferred was to a semi-adjustable articulator (Hanau wide-View). Vertical jaw relations were measured using a combination of anatomic, phonetic, and swallowing methods. Bite registration was done by nick & notch method & mounting was done.

The Gothic arch tracings were done to record centric and protrusive movements of the patients (Fig 5). Inter occlusal records (i.e., Centric record-CR, Protrusive record-PR) were made using jet bite occlusal registration Material (Figure 6). These interocclusal records were used for the programming of the articulator (Figure 7). Programming of the articulator was carried out individually for all the patients using the CR and PRs obtained from them. Lateral condylar guidance was calculated using Hanau's formula $L = H/8 + 12$.

Now the tracers were detached, and maxillary & mandibular occlusal rims were reconstructed to their previous original form. Later, teeth arrangements were carried out. To obtain balancing the functional maxillary palatal cusps of posterior teeth were set in the central groove of the mandibular teeth and the maxillary buccal cusps were kept in contact with mandibular buccal cusps. The buccal cusps and palatal cusps were in articulation and functional in the bilateral and protrusive excursions (Figure 8). The try-in of the trial dentures were done and were evaluated for occlusion, aesthetics, and phonetics. After the patient's written approval of the try-in procedure, denture processing was carried out following the conventional method and were lab remounted. Later occlusal corrections were done and were trimmed, finished & polished; followed by denture insertion (Figure 8).



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Surgical and prosthodontic protocols:

All the surgical procedures were carried under strict aseptic conditions. Surgery was performed under local anaesthesia. The duplicated denture was used as a radiographic stent (fig 9). Surgical access to the mandible was gained through a mid-crestal incision over the keratinized gingiva with a No.15 B.P. blade. Full-thickness flaps were elevated using a periosteal elevator (fig 10). The osteotomy was carried out following the manufacturer's instructions (fig 11). Starting with a pilot drill of 2mm and sequential drilling under copious irrigation was carried out till the desired dimensions were achieved. Once the osteotomy site was prepared, implants of the selected size were placed using implant drivers and torque wrench (fig 12). The full-thickness flap was closed with 3 -0 silk sutures to achieve primary closure (fig 13). CBCT was taken immediately after surgery to evaluate the placement of implants radiographically and for future reference.

Post surgically, patients have advised medication. After one week, sutures were removed. The patient did not wear the previously constructed, the conventional mandibular denture for the first two weeks. Two weeks after the placement of implants, the impression surface of the denture was relieved at sites corresponding to the implant. The O-ring attachment assembly was placed over the two implants, undercuts blocked out, and auto polymerizing resin was used for direct pickup. The denture was finished, polished, and occlusion was adjusted accordingly.

POSTOPERATIVE RADIOGRAPHIC EVALUATION FOR BOTH

CONTROL AND TEST GROUP PATIENTS FOR BONE LOSS:

Postoperatively, all the patients in the control and test group were radiographically evaluated at baseline, at the time of surgery and six months by CBCT scans. The CBCT scans were taken for each patient at baseline (fig 18), at the time of surgery (fig 19) and six months (fig 20) are evaluated for the amount of anterior mandibular RRR. The method consisted of measurements taken from the upper border of the

anterior crest of the mandible to the lower border of the peak of the mandible regions.

MEASUREMENT OF MUCOSAL THICKNESS:

- Mucosal thickness is assessed using 20 No Endodontic file with a rubber stopper (fig 17). The file was inserted at the predetermined reference point to evaluate the mucosal thickness. The reference points will be taken at the midcrestal point (fig 14) and 5mm below from that point labially (fig 15) and lingually (fig 16) in the mandibular anterior region. The mucosa will be pierced at a 90-degree angle with slight pressure until hard tissue is felt. The distance from the tip of the file and rubber stopper will be recorded using an endo scale at various time intervals, i.e., at baseline, 3 months, 6 months.

CONTROL GROUP



Figure 1: Primary impression



Figure 2: Primary cast

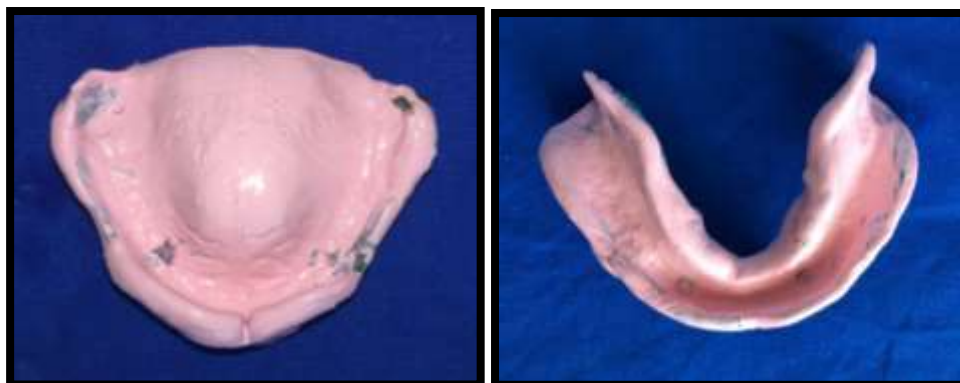


Figure 3: Secondary Impressions

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Figure 4: Secondary casts



Figure 5: Extra oral tracings



Figure 6: Inter occlusal records



Figure 7: Programming of Articulator



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Figure 8: Balancing and CD insertion

Test Group



Figure 9: Surgical stent



Figure 12: Implants placed on left side




Figure 10: Incision and Flap elevation left side



Figure 13: suturing done on left side



Figure 11: Osteotomy site


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Evaluation of mucosal thickness



Figure 14: At mid crestal region.



Figure 16: At lingual side



Figure 15: At labial side

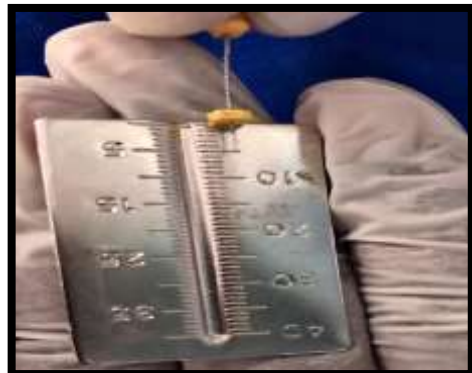


Figure 17: Thickness measurement by endo scale and 20 No endo file

Radiographic analysis

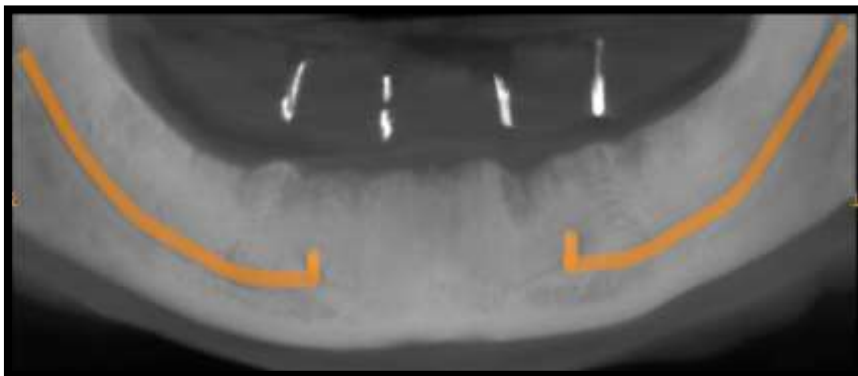


Figure 18: Preoperative CBCT image

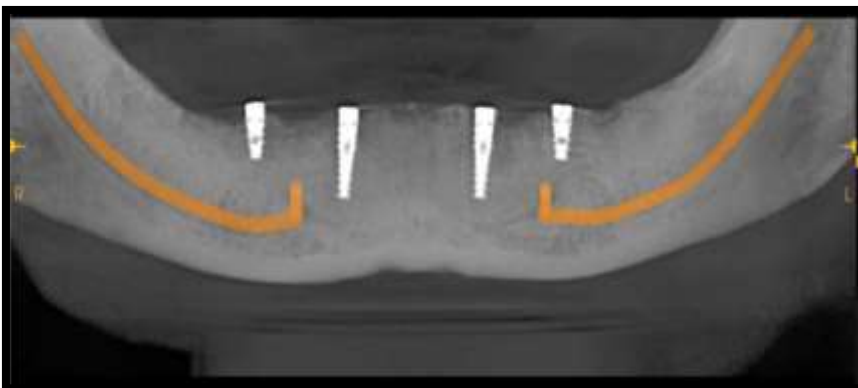



Figure 19: At the time of surgery


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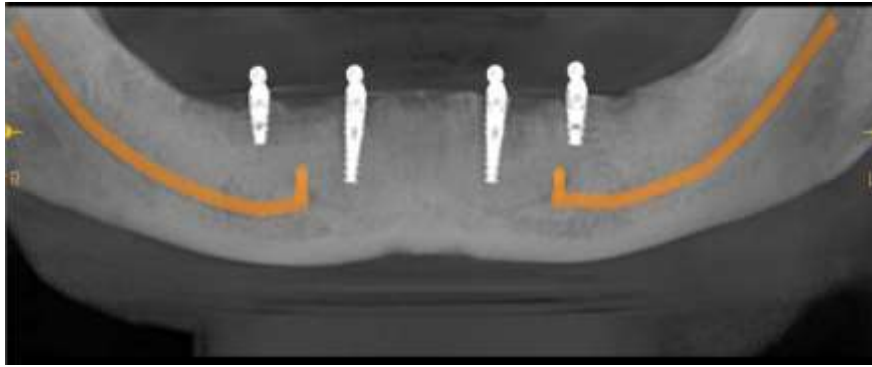


Figure 20: After 6 months

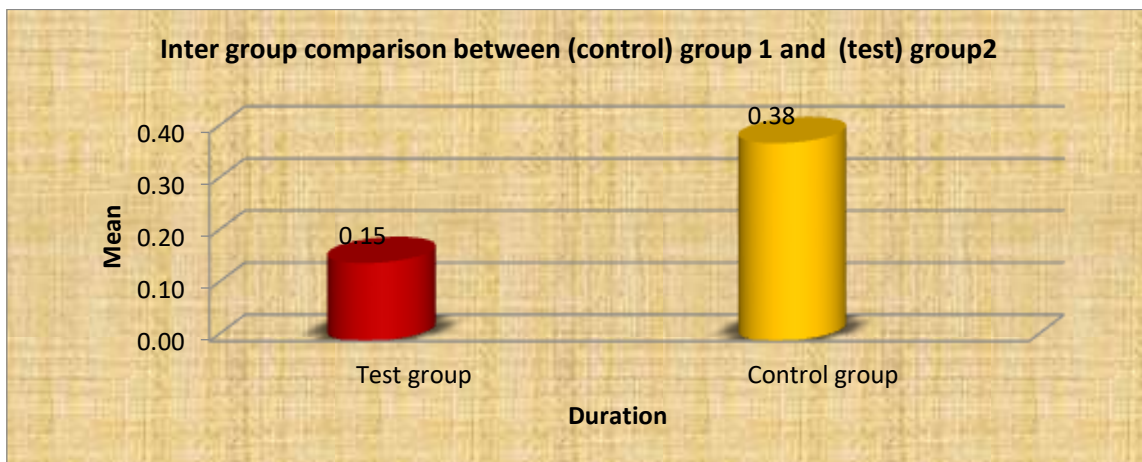
RESULTS:

The mean change in ridge resorption from baseline to 6 months in the control group is 0.38 ± 0.01 (p-value 0.006). Whereas the mean shift in ridge resorption from baseline to 6 months in the test group was 0.15 ± 0.10 (p-value 0.006). The mean change in mucosal thickness from baseline to 6 months in the control group is 0.54 ± 0.04 (p-value 0.796). Whereas the mean change in mucosal thickness from baseline to 6 months in the test group is 0.44 ± 0.05 (p-value 0.796).

(Control) GROUP1 Vs (test) GROUP 2
Table 1: Inter group comparison between group 2(Test group) and group 1(control group)

Region	Time interval	Group 2		Group1		P value
		Mean±SD difference	% of change	Mean±SD difference	% of change	
33	At baseline to at six months	0.15 ± 0.10	-0.59	0.38 ± 0.01	-1.44	0.006 S

Statistical Analysis: Mann-Whitney U test. Statistically significant if P<0.05



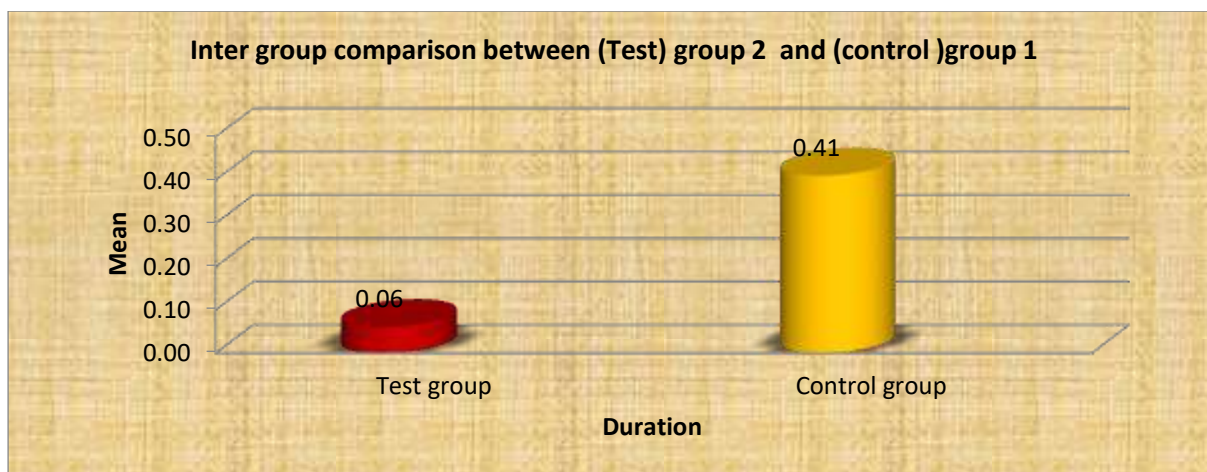
GRAPH 1: Intergroup comparison between (control) group 1 and (test)group2 for ridge resorption

Table 2: Inter group comparison between (Test) group 2 and (control) group 1

Region	Time interval	Test group 2		Control group1		P value
		Mean±SD difference	% of change	Mean±SD difference	% of change	
43	At baseline to at six months	0.06 ± 0.01	-0.23	0.41 ± 0.03	-1.54	0.000 S

Statistical Analysis: Mann-Whitney U test. Statistically significant if P<0.05


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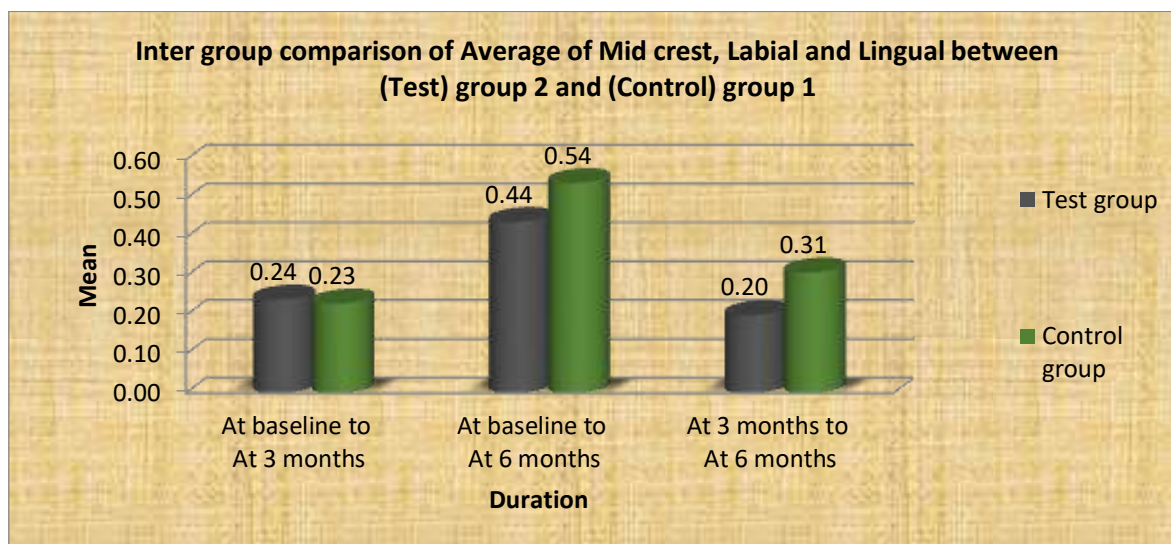
GRAPH 2: Intergroup comparison between (Test) group 2 and (control) group 1 for ridge resorption

EVALUATION OF MUCOSAL THICKNESS (TEST) GROUP 2 Vs. (CONTROL) GROUP 1

Table 3: Intergroup comparison of Average of Mid crest, Labial and Lingual between (test) group 2 and (control) group 1

Duration	(Test) group 2		(Control) group 1		P value
	Mean±SD difference	% of change	Mean±SD difference	% of change	
At baseline to Three months	0.24±0.03	-8.11	0.23±0.01	-6.22	0.579 NS
At baseline to At six months	0.44±0.05	-14.86	0.54±0.04	-14.59	0.796 NS
Three months to at six months	0.20±0.02	7.35	0.31±0.05	-8.93	0.684 NS

Statistical Analysis: Mann-Whitney U test. Statistically significant if P<0.05



GRAPH 3: Intergroup comparison of Average of Mid crest, Labial and Lingual between (Test) group 2 and (Control) group 1 for mucosal thickness

DISCUSSION:

Sadowsky (2001)³ stated that bone in the anterior region, i.e., between two mental foramina, was maintained in implant-supported overdenture. The average annual bony residual ridge height physiological shrinkage was about 0.4mm in the

edentulous anterior mandible. Studies had revealed better patient-based results when two implants supported mandibular overdentures have been used compared with conventional lower dentures.

Atwood et al⁴ and Tallgren⁵ showed an average annual alveolar ridge height reduction of

approximately 0.4 mm in the edentulous anterior mandible resulting from physiologic changes. The anterior mandibular ridge under an implant overdenture may resorb as little as 0.5 mm over five years, and long-term resorption may remain at 0.1 mm annually. Following the above study, the present study also evaluated that the mean bone loss at the mandibular anterior region at baseline, at the time of surgery, and six months in four implants supported mandibular overdenture. Mericske-Stern et al⁶ reported 97% implant survival with two implants (splinted or solitary), irrespective of keratinized tissue, or duration of edentulism. In this study also ball type of attachments has been used due to above-said advantages than bar type of attachments. Roynesdal et al. used two titanium-sprayed stable screw implants in the inter foraminal region in a prospective, 24-month study. After three weeks, placement of the overdenture prosthesis to the ball attachments. The implant survival rate was 100%. So, in this present study also, an early loading protocol was followed. Baten burg et al⁷ evaluated mandibular overdenture patients treated with two implants and with four implants. They found no significant differences in the peri-implant health. Following the above study, in this study, four implants supported overdenture had given. In vitro and in vivo studies by Menicucci et al^{8,9} compared the stresses on the bone surrounding two implants with either a bar clip or ball attachments for overdentures. They found higher stresses on the peri-implant bone with a bar clip attachment. It has been shown that solitary ball attachments are less costly; less technique was sensitive and more comfortable to clean than bars. In this study also solitary ball attachments have been used by the advantages showed by the above studies. Gupta A, Rathee S, Agarwal J, Pachar R¹⁰, conducted a study for the presurgical measurement of crestal bone thickness at various implant sites using CBCT images. So, in this study, also cone-beam computed tomography was used to evaluate the preoperative and postoperative bone heights.

PROSTHODONTIC PROTOCOLS

The patient was suggested not to use the conventional dentures for the first two weeks. Two weeks after the placement of implants, the impression surface of the denture was relieved at sites corresponding to the implant. Impression and transfer of the exact positions of the implants to the working cast should be accurate.

Two techniques are commonly used to incorporate the attachment into the denture base. The direct method allows the housings to be inserted intraorally. The indirect technique accomplishes laboratory processing. Common problems with indirect technique may be possible movement and damage to the attachment during packing procedures. Direct pickup technique for the incorporation of housings for ball attachments was used in this study,

as described by Dominici JT et al¹¹. The denture was finished, polished, and inserted.

In an in vivo study, Fontijn-Tekamp et al¹² compared a trans mandibular design of four implants and two anteriorly placed endosseous implants. They found that masticatory forces did not differ between the mainly implant-borne and mucosa-implant-borne treatments.

According to the above study, masticatory forces affecting the mucosal thickness in implant-supported overdenture and conventional complete denture has been assessed. There is no significant statistical difference observed in mucosal thickness assessment.

LIMITATIONS:

The present study was conducted on a sample size of twenty patients within the time-lapse of just six months.

- Evaluation of crestal bone loss with only one of the available systems was considered in the present study.
- The results might vary with other implant systems
- Prospective studies on a more extensive group of patients and long-term evaluation required.

CONCLUSION:

- A decreased ridge resorption in the mandibular anterior region in implant-supported overdenture wearers than conventional complete denture wearers over a while.
- So, it can be advised that implant-supported overdenture is an effective treatment modality in the rehabilitation of completely edentulous mandibular arches with improved patient satisfaction, masticatory capacity, and in terms of ridge resorption and retention.
- Whereas, mucosal thickness did not show much significant difference between both the groups.

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Assessment of Disinfectants on Adherence of *Candida Albicans* to Soft Denture Liner

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ABSTRACT

Aim: The aim of the study is to assess and compare the efficacy of four different disinfectants on adherence of *Candida albicans* to soft denture liner.

Materials and Methods: A total of 55 samples of soft lined acrylic blocks are fabricated and subjected to adherence testing for *Candida albicans*. The samples were then immersed in disinfectant solutions such as Distilled water, 100% Tea tree oil, 100% Neem extract, 2 % Chlorhexidine and 0.5% Sodium hypochlorite. Using the digital colony counter the number of cfu/ml of *Candida albicans* were determined on each sample before and after disinfection.

Statistical Analysis Used: Kruskal Wallis ANOVA and Post hoc tests were performed to analyze pairwise differences between the study groups at each of the dilutions used in this study.

Results: Tea tree oil demonstrate-d highest inhibitory effect on the growth of *Candida albicans* at each of the three dilutions tested. At dilutions 10 and 100, neem extract was observed to be having the next highest inhibitory effect on *Candida albicans* after tea tree oil; however, the efficacy of neem extract reduced with an increase in dilution with chlorhexidine demonstrating the second highest inhibitory effect on *Candida albicans* adherence at 10³ dilution. Posthoc tests were performed to analyze pairwise differences between the study groups at each of the dilutions used in this study. At a dilution of 10, tea tree oil demonstrated significant difference with all other disinfectants except neem extract. At a dilution of 100, similar observations were made with no significant difference in the *Candida* adherence between tea tree oil and neem extract. However, at a dilution of 1000, tea tree oil showed significant difference only with sodium hypochlorite.

Conclusion: It was observed that there exists a significant difference between the disinfectants used in this study on adherence of *Candida albicans* to the soft denture liner.

Keywords: *Candida Albicans*, Soft denture liner, Acrylic blocks, Tea tree oil, Neem extract, Chlorhexidine, Sodium hypochlorite, Colony Counter

Introduction

Denture soft liners distribute the functional load evenly on the denture-bearing area to avoid local stress concentration and improve denture retention by engaging undercuts.

They are primarily used for patients with atrophic ridge, bony undercuts, bruxism, congenital or acquired oral defects, xerostomia, dentures opposing natural teeth, traumatic ulceration.

Soft liners made from silicone or acrylic-based material, both of which may be heat cure or self-cure.^{1,2,3} The favourable properties of them include high bond strength to the denture base, dimensional stability, colour stability, easy application, biocompatibility, and low cost and their disadvantages are loss of softness^{2,3}

Candida albicans is an opportunistic pathogenic yeast. It is usually a commensal organism but can become pathogenic due to local and systemic predisposition caused by chronic irritation or immunocompromised patients. Some natural alternatives have received increased popularity in recent decades.⁴ Efforts to validate their use as therapeutics have come for increasing scrutiny in-vitro and in-vivo. Tea tree oil (TTO) is a volatile essential oil derived mainly from the Australian native plant, *Melaleuca alternifolia*. Tea tree oil is the most active ingredient in many topical formulations used to treat cutaneous infections as it has antimicrobial activity.

The literature documents several reports conducted to evaluate and compare the effectiveness of Chlorhexidine, Sodium hypochlorite, Tea tree oil and Neem extract on adherence of *Candida albicans* to the soft liners. However, till recent day no studies have been conducted to evaluate and compare the effectiveness of Chlorhexidine, Sodium hypochlorite, Tea tree oil and Neem extract on adherence of *Candida albicans* to the soft liners. The present in-vitro study was undertaken to evaluate and compare the antifungal effectiveness of 2% Chlorhexidine, 0.5% Sodium hypochlorite, 100% Tea tree oil and 100% Neem extract on the adherence of *Candida albicans* to soft denture liner.

Materials and methods

An in-vitro study was done in the Department of Prosthodontics and Crown & Bridge, Sibar Institute of Dental Sciences, Takellapadu, Guntur. Metal dies and molds fabricated at Solidworks, Patancheru, Telangana. Adherence testing and colony counting were done in the Department of Microbiology, Vignana University, Guntur.

Standardized aluminium mold preparation: (Figure -1)

Two aluminium blocks of 100 × 70 × 12 mm taken and marked for cutting a mold space of 4×4×2mm and 4×4×4mm. After the markings are done on the block, it is cut using a computerized milling machine into the predetermined dimensions.

Preparation Of Samples:

Total of 55 samples prepared following the below steps.

- Preparation of wax patterns
- Preparation of Poly Methyl Meth Acrylate (PMMA) resin samples
- Packing of the soft liner on resin samples

Preparation of wax patterns:

Fifty-five wax patterns were fabricated by pouring molten modelling wax into the customized mold space of dimensions 4×4×2mm (Figure-2), coated with wax separator for easy retrieval of the wax patterns. The mold with the wax patterns was allowed to solidify in cold water and later retrieved for investment.

Preparation of PMMA resin samples:

Dental plaster was mixed according to the manufacturer instructions and poured into the

base of the dental flask. The wax patterns were invested into the dental plaster to half of their height (Figure-3). After the dental stone set, cold mold seal was applied and is allowed to dry. Dental stone is then mixed and poured into the flask until it is filled to form the second pour. After completing the stone's setting, de-waxing did by immersing the flask in the de-waxing unit at 100°C for 4 minutes. The flask removed from the de-waxing unit, and residual wax was flushed out using hot water (Figure-4).

After the mould is dried, a cold mold seal applied. The heat polymerized resin was mixed in a porcelain jar according to the manufacturer's instructions. The heat-cured acrylic resin packed into the stone mold in the dough stage in both the compartments, and the flask is placed under hydraulic pressure up to 1000 psi. Trail closure also was done, and the flask was removed.

The flasks are again placed back in the hydraulic press, and the resin was allowed to bench cure for 30 minutes. Flask was removed from the hydraulic press and then it was attached to the clamp and placed in a temperature-controlled acrylizer for polymerization. Short curing cycle was followed by putting flask at 74°C for 2 hours, and the temperature was raised to 100 °C and processed for 1 hour. Flask was allowed to cool down to room temperature and then removed from the water bath and deflashed. The heat-cured acrylic specimens were then retrieved carefully, trimmed by using the acrylic trimmer and sandpapered using sandpaper 80 µm, 120 µm, 220µm grit. Finishing and polishing are avoided to simulate the complete denture's intaglio surface.

Packing of the soft liner:

GC SOFT (auto-polymerized acrylic -based-long term) denture liner consists of the two-component system supplied as powder and liquid. The standard powder/liquid ratio is 2.2 g/1.8 g. It was measured into the glass jar and mixed for 30-60 seconds. The mixture was blended to avoid bubbles using a plastic spatula until all of the powder particles were moistened entirely.

The thickness of a soft liner to be packed over the surface of the heat-cured acrylic component was 2 mm (Figure-5). The heat-cured acrylic component was fabricated earlier with similar dimensions of 4mm × 4mm × 2mm (Figure-6) using a standardized aluminium mold. These heat-cured blocks were placed into the aluminium mold of size 4x 4 x 4mm. The bonding surface of the heat-cured acrylic component was dried thoroughly. Then the mixture is packed into mold space above the heat-cured acrylic resin block. The custom-made mould was then covered from the top by a cellophane sheet, and glass slab was pressed firmly against the mold to remove excess material and shape the specimens. Once the material was set, the sample was removed from the mold, placed in water and extra material was trimmed at the periphery using a heated BP blade. In total, 55 GC SOFT liner samples were prepared in the same manner as mentioned above. The samples were stored in a glass container with a lid that contained physiological saline.

Culture of Candida albicans:

Pure Lyophilized culture of *Candida albicans* MTCC 1637 was purchased and inoculated on Yeast Extract Peptone Dextrose growth medium (yeast extract, peptone, dextrose, agar) and incubated at 37°C for 24 hours. Subculture was done on Sabouraud's dextrose agar medium (dextrose, peptone, agar) (Figure-7) by taking 5µl of previous incubated culture growth on YEPD medium using a micropipette. It was then incubated for 48 hours at 37°C and checked for purity. Serial dilution is used in microbiology to estimate the concentration or number of cells/organisms in an incubated plate for a simple counting number of colonies. The culture was taken in a test tube and 9 test tubes, each with 9ml of

sterile diluents, and then at a rate of 0.9% saline.

Serial dilution of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , dilutions were done by taking 1ml of *Candida albicans* subculture and mixed with 9ml of physiological saline solution. 0.1ml of this solution from each serial dilution was spread on Sabouraud's dextrose agar medium with L shape glass rod to enable candidal growth. These Petri plates incubated at 37 °C for 48 hrs (Figure-8).

After this incubation period, colony counting was done to know the number of candidal colonies before disinfection (Figure-9).

Adherence testing:

A suspension containing 10^6 viable cells per millimetre was prepared in a saline solution using UV-Spectrophotometer at a wavelength of 600 nm, and the absorbance of -0.003 is obtained (Figure-10). Adherence testing was performed in an aseptic environment in a laminar airflow chamber using 24- wells plates for cell culture. For adherence testing, the Sabouraud's dextrose broth 350 µl, 50 µl of *Candida albicans* standardized suspension and one soft-lined acrylic block (sample) added to each well (Figure-11). And the plates were sealed with parafilm and incubated at 37° c for 24 hours.

After the incubation period:

The specimens were washed with 1ml of sterile distilled water and randomly divided equally into five groups according to the disinfecting solution tested.

Group A	Distilled water (control group)
Group B	100 % Tea Tree Oil
Group C	100 % Neem extract
Group D	2 % Chlorhexidine
Group E	0.5 % Sodium Hypochlorite

Disinfection of specimens:

According to the group, the specimens immersed for 10 minutes in 10 ml of the corresponding solution. The samples were then removed, washed with 2ml of sterile distilled water and transferred to test tubes containing 1ml of sterile physiological saline solution. The test tubes were agitated in a vortex stirrer for 60 seconds, and the adhered cells dispersed. (Figure-12)

Colony counting:

For proper colony count, the initial suspension diluted 10, 100, and 1000 times (Figure- 13) in physiological saline solution in the same manner as serial dilution which explained above and 0.1 ml of each suspension plated on growth medium. After 48 hours of incubation at 37°C, the number of colony-forming units per specimen determined using a colony counter (Figure-14). All the media and the armamentarium used for culture autoclaved at temperature 121 ° C for 30 minutes and 15 psi of pressure.

Results

It was observed that there exists a considerable difference between the disinfectants used in this study on adherence of *Candida albicans* to the soft denture liner. Tea tree oil demonstrated the highest inhibitory effect on the growth of *C. albicans* at each of the three

dilutions tested (Table 1). At dilutions 10 and 100, neem extract was observed to be having the next highest inhibitory effect on *C. albicans* after Tea tree oil; however, the efficacy of neem extract reduced with an increase in dilution with chlorhexidine demonstrating the second-highest inhibitory effect on *C. albicans* adherence at 10^3 dilutions. Post hoc tests were performed to analyze pairwise differences between the study groups at each of the dilutions used in this study. At a dilution of 10, tea tree oil demonstrated significant difference with all other disinfectants except neem extract. At a dilution of 100, similar observations were made with no significant difference in the candida adherence between Tea tree oil and neem extract. However, at a dilution of 1000, tea tree oil showed a significant difference only with sodium hypochlorite.

Table 1: - Comparison of efficacy of the disinfectants on adherence of *Candida albicans* to soft denture liner at various dilutions

Dilution	Disinfectant	Mean±SD	Mean rank	P-value
10	Distilled water	47.64 ±58.74	38.23	0.001*
	Tea tree oil	1.91 ± 3.08	13	
	Neem extract	19.73 ±38.82	22.23	
	Chlorhexidine	35.91 ±63.31	32.14	
	Sodium hypochlorite	12.73 ± 8.86	34.41	
100	Distilled water	7.18 ± 11.07	31.95	0.001*
	Tea tree oil	0.27 ± 0.64	13.32	
	Neem extract	2.36 ± 4.41	22.18	
	Chlorhexidine	5.91 ± 9.64	29.32	
	Sodium hypochlorite	12.27 ± 8.34	43.23	
1000	Distilled water	2.73 ± 3.63	27.86	0.016*
	Tea tree oil	0.91 ± 1.57	17.23	
	Neem extract	2.09 ± 1.92	29.23	
	Chlorhexidine	1.82 ± 1.83	25.5	
	Sodium hypochlorite	8.18 ± 10.4	40.18	

Except with Tea tree oil at a dilution of 100, no significant differences were found for distilled water with any other disinfectant at dilutions of 100 and 1000. At a dilution of 10, distilled water showed a significant difference in candidal adherence with Tea tree oil and neem extract.

In intra-group comparisons at various dilutions, only chlorhexidine showed a significant difference in an inhibitory effect on *C. albicans* with a change in dilution. All the other disinfectants did not show significant difference in their inhibition of *C. albicans* with increasing dilution.

Discussion

Pain and difficulty are experienced by many patients using dentures constructed with hard denture bases. On this basis, the resilient materials used to increase resiliency during function and under pressure. Soft lining materials can be defined as soft, resilient, elastic materials which form a cushioned layer between the hard denture base and the oral mucosa.⁵ Denture soft lining materials are broadly divided into two groups of materials. The first group includes the tissue conditioners and temporary soft lining materials typically based on polyethyl methacrylate, an aromatic ester, and ethyl alcohol. The second group comprises permanent soft lining materials based on silicone rubber or acrylic resin. Both of

the groups may be of self-cure (chemically activated) or heat-cure (heat activated).^{6,7,8}

Tissue conditioners or short-term soft liners are uncross-linked, which are formed by polymer chain entanglements. The polymer powder generally consists of poly ethyl methacrylate (PEMA) of molecular weights ranging between 1.79×10^5 and 3.25×10^5 with no initiator^{9,10,11}. The liquid comprises an ester-based plasticizer and 4–50 wt% ethyl alcohol (EtOH) and contains no monomer.^{9,12} Short-term soft liners are tissue conditioners which remain soft for a limited period. It should be replaced with a fresh mix every 2 to 3 days for adequate cushioning.¹³

This study aims to assess the efficacy of various disinfectant solutions on adherence of *Candida albicans* to soft denture liner. According to disinfecting solution tested, samples are divided into group A (distilled water) control group, group B (Tea Tree Oil), group C (Neem Extract), group D (2% Chlorhexidine), group E(0.5%hypochlorite). Each specimen was immersed in 10 ml the corresponding solution for 10 minutes. After immersion, the specimens were removed, washed with 2 ml of sterile distilled water. Then each sample was transferred to test tubes containing 1 ml of clean physiological solution. The tubes were agitated in a vortex shaker for 60 seconds to disperse adhered cells. To get proper colony count the initial suspension was diluted 10, 100 and 1,000 times in physiological solution and 0.1 ml of each suspension was taken and plated on Sabouraud dextrose agar. After 48 hrs. of incubation at 37° C, the numbers of colony-forming units per specimen (CFU/specimen) were determined using a colony counter.

From the results obtained, Table 1 depicts that there exists a significant difference between the disinfectants used in this study on adherence of *Candida albicans* to the soft denture liner. Multiple pairwise comparisons on the effectiveness of the disinfectants on adherence of *Candida albicans* to soft denture liner at multiple dilutions and Intra-group comparison of the efficacy of disinfectants on *Candida* adherence different dilutions. Tea tree oil demonstrated the highest inhibitory effect on the growth of *C. Albicans* at each of the three dilutions tested (Table1). At dilutions 10 and 100, neem extract was observed to be having the following highest inhibitory effect on *C. Albicans* after Tea tree oil; however, the efficacy of neem extract reduced with an increase in dilution with Chlorhexidine demonstrating the second-highest inhibitory effect on *C. Albicans* adherence at 1000 dilution. At a dilution of 10, tea tree oil showed significant difference with all other disinfectants except neem extract. At a dilution of 100, similar observations were made with no significant difference in the candida adherence between Tea tree oil and neem extract. However, at a dilution of 1000, tea tree oil showed a significant difference only with sodium hypochlorite.

The present study's result was congruent with the study conducted by **Pachava K R et al. (2015)**¹⁴. They showed that 15% of Tea Tree Oil had significant antifungal activity against *Candida albicans* on heat-cured acrylic denture base material. **Barua et al. (2017)**¹⁵ reported that the significant benefits of using neem are its easy availability, cost-effectiveness, good shelf life, low toxicity, and no micro-organisms resistance. **Dalwai S et al. (2014)**¹⁶ suggested that Tea tree oil exert a significant antifungal effect (similar to chlorhexidine gluconate 2%). Tea Tree Oil was effective in inhibiting *C. Albicans*.¹⁷ **Iqbal et al. (2016)**¹⁸ stated that Tea tree oil-modified tissue conditioners showed inhibitory and fungicidal activity on *C. Albicans* and effective in treating the denture stomatitis. Sodium hypochlorite was an effective agent in reducing adherent *Candida albicans* both in vitro and in vivo which justifies its application as a cleaning protocol.¹⁹ **R. Sushma et al. (2017)**²⁰ stated that Chlorhexidine has a broad spectrum of activity against various organisms, including *C. Albicans*, the susceptibility of *C. Albicans* biofilms to Chlorhexidine was significantly reduced compared to its action against suspended organisms.



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Conclusion

It was thus concluded that there exists a significant difference between the disinfectants used in this study on adherence of *Candida albicans* to the soft denture liner. Tea tree oil demonstrated the highest inhibitory effect on the growth of *C. Albicans*. At a dilution of 10, tea tree oil showed significant difference with all other disinfectants except neem extract. At a dilution of 100, similar observations were made with no significant difference in the candida adherence between Tea tree oil and neem extract. However, at a dilution of 1000, tea tree oil showed a significant difference only with sodium hypochlorite.

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Nil.

Conflicts of interest

There are no conflicts of interest

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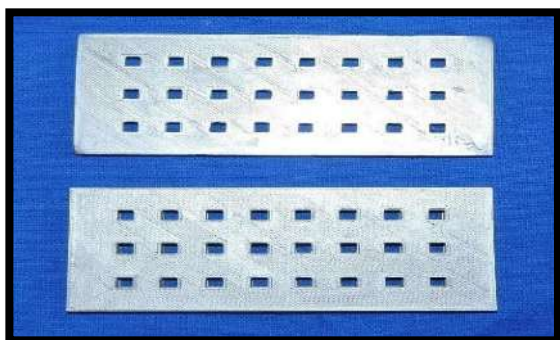


Figure- 1 Two Standard Aluminium blocks with mold size of 4mm x 4mm x 2mm & 4mm x 4mm x 4mm

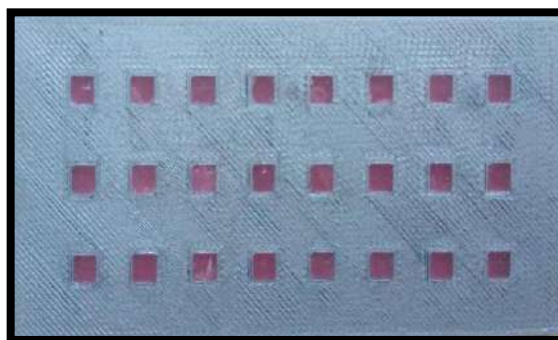



Figure- 2 Preparation of wax patterns



Figure- 3 Wax patterns invested into the dental plaster



Figure- 4 Dewaxing of wax patterns


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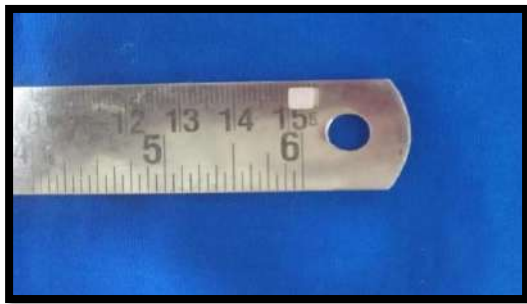


Figure- 5 Thickness of a soft liner – 2mm



Figure- 6 Heat-cured acrylic specimens-4 x 4 x2mm



Figure- 7 Subculture of Candida albicans



Figure- 8 Candida albicans growth after incubation

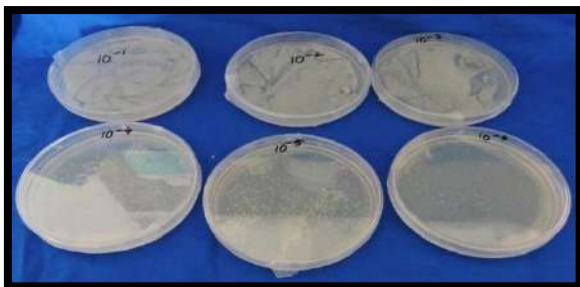


Figure- 9 Colony counting before disinfection

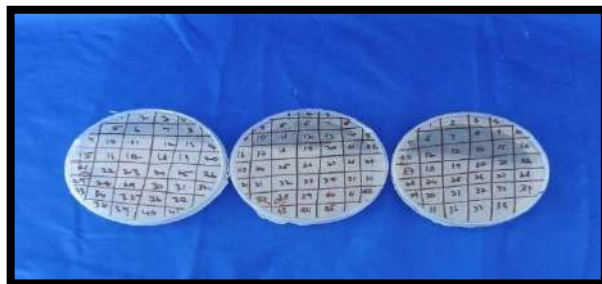


Figure- 10 UV-Spectrophotometer absorbance and wavelength reading

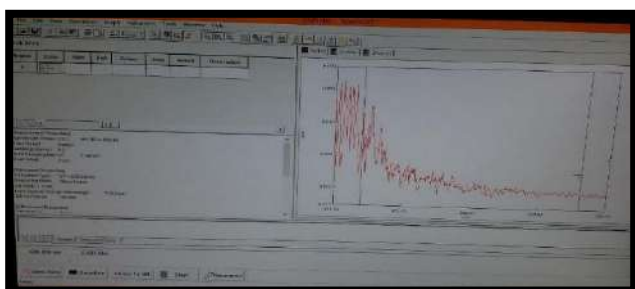


Figure- 11 Adherence testing by adding specimen with candida broth.

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Original Research

To determine the Correlation between the occlusal vertical dimension and length of thumb in coastal Andhra population

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ABSTRACT:

Aim of the study: To determine the Correlation between the occlusal vertical dimension and length of thumb in coastal Andhra population. **Materials and methods:** The present study was conducted on 220 subjects in coastal Andhra. Occlusal vertical dimension by Niswanger and Thomson method (two pinpoint markings were placed on the tattoo stickers of the tip of the nose and the most prominent point on the chin) was measured, and then the length of thumb finger (The proximal point on the radial side of the proximal crease over the first metacarpophalangeal joint and the distal point in the dactylion, the distal-most part of the thumb) was measured with a vernier calliper. The sample size was measured using G power 3.1.9.2 with α 0.05, Power 80% and the effect is 0.6, and the sample size obtained was 220. **Results:** Pearson correlation test was used to analyze the data. The thumb length was significantly correlated (0.001) with strong and positive values (Pearson's coefficient = 0.662 in the whole population). Regression analysis that thumb length was significantly related to the vertical dimension of occlusion. **Conclusion:** Considering the limitations of the study, the result implies that thumb length can be used as an alternative for establishing O.V.D. in the edentulous patients.

Keywords: Thumb, the vertical dimension of occlusion (O.V.D), Pearson coefficient, edentulous patients.

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INTRODUCTION:

The occlusal vertical dimension (O.V.D.) is defined as the distance between two selected anatomic points (usually one point on the tip of the nose and the other on the chin) when in the maximal intercuspal position. It is important for the functional, esthetic, physiological and psychological roles of the patient who loss their teeth and wants rehabilitation.¹

So, the establishment of proper occlusal vertical dimension (O.V.D.) is one of the important tasks for successful prosthodontic therapy.

There are several methods to determine the vertical dimension of occlusion, but each method has its own limitations because they were either tedious, time-consuming, require special instrument/equipment, or expose patients to radiation. So at least 2 or 3 methods should be done on the patient to accurately determine the V.D.O. of that particular patient.

Few studies shows that V.D.O. can be correlated clinically with anthropometric measurements, and

they can simple and precise method for estimating occlusal vertical dimension.² The aim of the study was to evaluate the Correlation between the O.V.D. and the length of the thumb.

MATERIALS & METHODS:

(a) SOURCE OF DATA:

The present study will be conducted on 220 subjects in coastal Andhra Pradesh.

(b) STUDY DESIGN:

Occlusal vertical dimension was measured by Niswanger and Thomson method (two pinpoint markings were placed on the tattoo piece on tip of the nose and the most prominent point on the chin) and then the length of thumb finger (The proximal point on the radial side of the proximal crease over the first metacarpophalangeal joint and the distal point in the



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dactylion, the distal-most part of the thumb) was measured.

- Attrition
- Trauma

(c) METHOD OF COLLECTION OF DATA:

The patients are selected according to the following criteria

Inclusion criteria: subjects with

- Full natural dentition (third molar not taken into account)
- Straight profile on visual examination

Exclusion criteria: Any history

- Orthodontic/surgical treatment,
- Presence of large carious lesions
- Abnormality or absence of thumb/s
- Hormonal abnormalities (e.g., gigantism, acromegaly)
- Open bite or deep bite cases
- Teeth anomalies

EVALUATION CRITERIA:

CLINICAL PARAMETERS:

- Occlusal vertical dimension was measured by Niswanger and Thomson method (two pinpoint markings were placed on the tattoo piece on the tip of the nose and the most prominent point on the chin)
- Length of thumb (The proximal point on the radial side of the proximal crease over the first metacarpophalangeal joint and the distal point in the dactylion, the distal-most part of the thumb)

Potential Risks and Benefits:

NIL

STUDY FLOW CHART

For occlusal vertical dimension-

Subjects should be seated comfortably in the dental chair in a fully upright position.

A head-rest was used to support the head with the camper's line of the subject in a horizontal position.

The subject was made to occlude the total teeth in maximum intercuspation.

Then two markings were placed on the tattoo marks on tip of the nose and the most prominent point on the chin, and the distance between them was measured with a vernier calliper.

For length of the thumb-

Each subject should be asked to place his or her hand on a graph paper keeping the fingers separated and the thumb placed comfortably

The proximal point of first meta carpophalangeal joint and the distal point in the dactylion, the distal-most part of the thumb, were to be marked.

The ends of the calliper were to be placed over these two landmarks, and the distance between them gives the maximum length of thumb

Data will be analysing using using G power 3.1.9.2 software.


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PROCEDURE:

Each volunteer was asked to place his or her hand on a graph paper with the palm facing downward and the fingers separated and the thumb placed comfortably. A sliding caliper was used to calculate the length of the thumb (Figure 1), as in the method used by Kumar et al. The proximal point on the radial side of the proximal crease over the first metacarpophalangeal joint and the distal point in the dactylion, the distal-most part of the thumb, were marked.



Figure 1: Measurement of thumb length.

The ends of the caliper were placed over those two landmarks of the subject, and the length between them measured the maximum length of thumb.

To measure the O.V.D., routinely used methods in the Department of Prosthodontics, Sibar Institute of Dental Sciences were employed (Figure 2). The subject was positioned comfortably in the dental chair in a fully upright position. A headrest was used to give support the head with the camper’s line of the

subject in a horizontal position. The subject was made to occlude the teeth in maximum intercuspation. Then two markings were placed on the tattoo points of tip of the nose and the most prominent point on the chin. The distance between them was calculated with a sliding caliper.



Figure 2: Measurement of O.V.D. with modified Vernier caliper

RESULTS:

The study was conducted among 220 students of the College of Sibar Dental Surgery. Pearson correlation test was used to analyze the data. The mean O.V.D. of the population was 5.58682 mm, and the mean thumb length (T.L.) of the studied population was 6.03000 mm(fig-3). The thumb length was significantly correlated (0.001) with strong and positive values (Pearson’s coefficient =0.662 in the whole population) (Fig-4). The graph shows the mean and range of various measurements (Fig-5)

Descriptive Statistics

	Mean	Std. Deviation	N
VDO	5.58682	.553329	220
LT	6.03000	.564886	220

Figure 3: Comparison of mean

Correlations

		V.D.O.	LT
V.D.O.	Pearson Correlation	1	.662**
	Sig. (2-tailed)		.000
	N	220	220
LT	Pearson Correlation	.662**	1
	Sig. (2-tailed)	.000	
	N	220	220

Figure 4.** The Correlation was significant at the 0.01 level (2-tailed).


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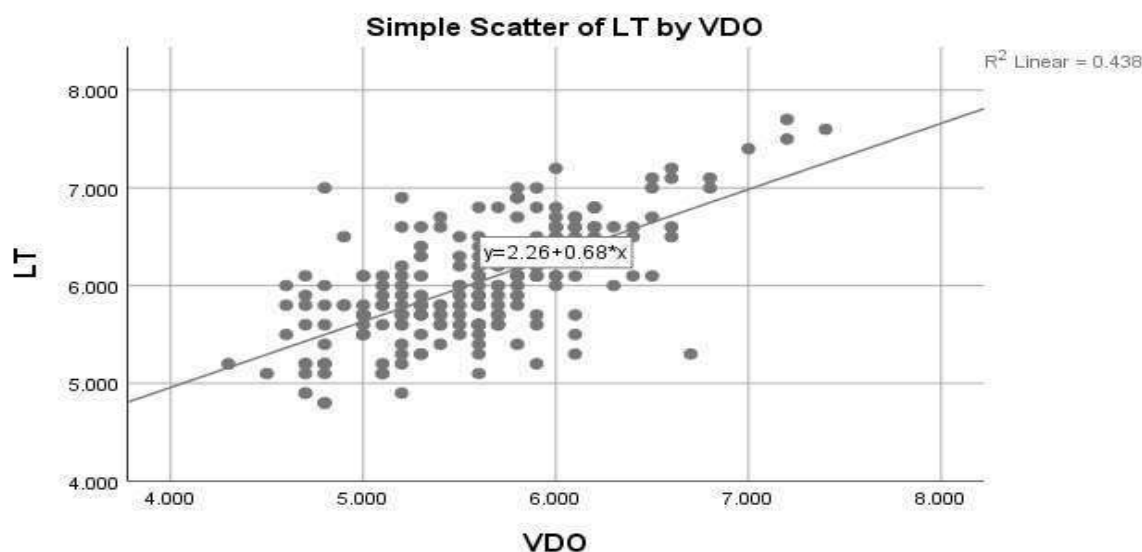


Figure 5: Range of variations in vertical dimension and thumb length

DISCUSSION:

The edentulism results in poor appearance of the lower third of the face. The rehabilitation of correct vertical dimension in those who have lost it should be in harmony with the upper part of the face. Prosthodontists have long been searching for universally accepted method of determining the O.V.D. There were methods like use of anterior teeth measurements⁷, closest speaking space⁸, swallowing method⁹, patient’s neuromuscular perception¹⁰, cephalometric radiographs^{11,12}, intra-oral and extra-oral anatomic landmarks¹³ and measurement of fingers⁶. All of these methods have disadvantages. Some methods have errors in measurement, while others are leads to difficult application, higher amount of cost, or longer time period for application. Any change in determining O.V.D. can be detrimental to the esthetics of facial soft tissues, causes difficulties in phonation, and leads to temporomandibular joint dysfunction.

Pre-extraction record is always superior to these methods. But recording the O.V.D. of all patients is not always possible¹⁵. A pre-extraction records, knowledge of aesthetics, relationship of teeth to the ridges, measurements relative to the lip length, use of phonetics and other methods are employed. This study was taken to investigate the relationship between the O.V.D. and the length of the thumb. The present study gave some idea about the relation between the facial measurement and length of the thumb their use in determining occlusal vertical dimension. The studies between the length of the thumb to the length of the lower facial height can help the clinician to establish the correct O.V.D. in the treatment of patients requiring restorations like the complete denture.

Geerts et al¹⁴ evaluate the correctness of the chin–nose distance measurement. The compressibility of skin cannot be avoided, so there may be some degrees of errors in measurement. Measurement by modified callipers invariably leads to some differences in the different studies. The present study did not compare these procedures of measuring the O.V.D.

LIMITATIONS:

The limitations of this study were errors occurred while measuring dimensions in big sample, and only one ethnic group are considered in the present study, so the findings cannot be extrapolated to other ethnic groups or races.

CONCLUSION:

Correlation of the length of the thumb to the O.V.D. was statistically significant. The Correlation between the length of the thumb and vertical dimension of occlusion was positive and strong in the total population. Within the limitations of this present study, the final result shows that thumb length can be used as an alternative for establishing O.V.D. in the edentulous patients.

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COMPARATIVE EVALUATION OF FLEXURAL STRENGTH AND ELASTIC MODULUS OF PROVISIONAL CROWN AND BRIDGE MATERIALS AT DIFFERENT STORAGE INTERVALS.

Prosthodontics

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ABSTRACT

Introduction: Provisional restorations are an integral part of fixed prosthesis and dental implantology. They serve as a key role in functional and esthetic tryin for design of final prosthesis. Fixed prosthesis prognosis depends on the quality of provisional restoration. In long term cases like full mouth rehabilitation and in implant supported prosthesis flexural strength (FS) and elastic modulus (EM) of the interim prosthesis play a vital role.

Aim: To evaluate and compare the flexural strength (FS) and elastic modulus (EM) of provisional crown and bridge materials stored at different time intervals in artificial saliva.

Materials And Methods: Ninety bar-shaped specimens (with dimensions of $25 \times 2 \times 2$ mm) as per American National Standards Institute (ANSI specification no 27) were fabricated with three provisional restorative materials Polymethyl Methacrylate (PMMA), Bis acrylic, Urethane dimethacrylate (UDMA) and are stored in artificial saliva at different time intervals. Further three point bending test were carried out in universal testing machine to calculate FS and EM. Changes were evaluated and data was analysed using one way ANOVA and paired t test.

Results: Results showed significant difference ($P < 0.05$) in FS values of provisional materials at different storage intervals except PMMA showed non-significant difference ($p > 0.05$) between 10days to 30days storage. There was non-significant difference ($p > 0.05$) of EM of bis-acryl samples between 24hrs to 10days and from 10days to 30days of storage.

Conclusion: Within the limitations of the study, it can be concluded that the FS and EM of Bis acrylic is comparatively better than PMMA and UDMA. Bis acrylic could be a better provisional restorative material for an extended period as less change was observed in elastic modulus of bis-acryl during long term storage compared to other materials.

KEYWORDS

Flexural strength, elastic modulus, Provisional restoration, Polymethyl methacrylate (PMMA), Bis-acrylic, Urethane dimethacrylate (UDMA).

INTRODUCTION

In fixed prosthodontic treatment it is important to protect the prepared teeth with provisional restoration so that the patient can be made comfortable during cast restoration fabrication¹. Materials providing more durability are required in situations like provisional FDPs that are expected to function for extended periods of time or when additional therapy is required before completion of definitive treatment as in case of prosthetic phase of dental implants where tissue contour issues should be solved and longer treatment duration as in full mouth rehabilitation.²

For longevity of a provisional restoration understanding its mechanical properties is necessary. Among mechanical properties, the flexural strength and elastic modulus of the interim prosthesis are important, particularly if the patient is expected to wear the restoration for a long period of time (e.g. full mouth rehabilitation)³. Flexural strength also known as transverse strength, is a measurement of the strength of a bar (supported at each end) under a static load. Elastic modulus describes the relative stiffness or rigidity of a material⁴.

The present study was carried out to evaluate and compare the flexural strength and elastic modulus of three provisional crown and bridge materials at different storage intervals in artificial saliva for 24hrs, 10 days and 30 days after setting.

MATERIALS AND METHODOLOGY

Following provisional crown Materials were used in this study

Table 1: Provisional Crown Materials

Product Name	Manufacturing Company	Lot no.
DPI (self-cure tooth moulding powder)	Dental Products of India, Mumbai, India	3452
REVOTEC LC (light cure resin for temporary crown, bridge, inlay & onlay)	GC Corporation., Tokyo, Japan.	1806061
TRANTEMP (bis-acrylic composite)	Nexobio co.ltd, Chungcheongbuk-do Korea.	80529

Metal mold consists of central metal plate and surrounding metal casing. Central metal plate has 5 slots of dimensions $25 \times 2 \times 2$ mm (**Figure 1**) in to which material was dispensed to get specimens of similar dimensions based on American National Standards Institute (ANSI) specification no 27 and surrounding metal casing which prevents movement of central metal plate that was split in between the slots for easy retrieval of samples.



Figure 1: Master Metal Mould

A total of 90 samples are included in this study and they are divided into 3 groups based on material used for fabrication. Each group is further divided in to 3 sub groups based on duration of immersion in artificial saliva i.e., 24hrs, 10days and 30days. (**Figure 2**)

All samples were fabricated using a split machined aluminum mold, sandwiched between two glass slabs. Bis-acrylic composite (Trantemp) was manipulated using automix cartridge loaded by an automix dispensing gun (1:10). The mixing tip of the cartridge was held at one end of the mould and material was expressed into the mould

moving the automix dispenser slowly to the other end to avoid incorporation of air bubbles. Light cure composite resin (Revotek LC putty stick) material was kneaded gently with fingers to soften it, dispensed and packed into the mould avoiding air bubbles or defects and glass slab is placed over mould. Care was taken to prevent over kneading of the material. Dispensed material was light cured according to the manufacturer's instructions i.e for 20secs. A required amount of the DPI Self Cure powder and liquid were measured in the ratio of 2:1 by volume, respectively and dispensed in a dappen dish. The polymer-monomer was mixed to a homogenous consistency using a stainless steel mixing spatula. The paste was then filled into a 2 cc disposable syringe and dispensed into the mould avoiding air bubbles. The specimens were subjected for testing under universal testing machine (DAK system Inc Series 9000) which uses 3 point bending test. The specimens were kept on the supports which are 20mm apart from each other. The cross head speed of this machine head is 0.5mm/min. Each specimen was gradually loaded and the point where it flexes and breaks were noted. (Figure 3)



Figure 2: Samples In Artificial Saliva



Figure 3: Testing Of Samples Under Universal Testing Machine

The flexural strength (S) and the elastic modulus (E) were calculated using the following formula for each sample:

Flexural strength (FS)
 $S = 3PL/2wt^2$

Elastic modulus (EM)

$E = PL^3 / 4wt^3\delta$ where

- P- Applied load (N)
- w- width of sample(m)
- t- Thickness of sample(m)
- L- span
- δ - Deflection

The values of each group were tabulated and statistically analyzed using ANOVA one way test and Paired t test.

RESULTS

The mean flexural strength and elastic modulus of all specimens are tabulated. Intra group comparison of Flexural strengths of PMMA, bis-acrylic and UDMA is done. In group-A there was significant difference (P<0.05S) in FS between 24 hr sample to 10days sample and non-significant difference(P>0.05) between 10days to 30days sample. In group-B there was non-significant difference (p>0.05) in FS between 24 hr sample to 10days sample and significant difference (P<0.05 S) between 10days to 30days sample. In group-C there was

significant difference (P<0.05 S) in FS between 24 hr sample to 10days sample and between 10days to 30days sample.

Intra group comparison of elastic modulus of PMMA, bis-acrylic and UDMA is done. In group-A there was significant difference (P<0.05 S) in EM between 24 hr sample to 10days sample and non-significant difference (p>0.05) between 10days to 30days sample. In group-B there was non-significant (p>0.05) difference in EM between 24 hr sample to 10days sample and between 10days to 30days sample. In group-C there was significant difference in FS between 24 hr sample to 10days sample (P<0.05 S) and 10days to 30days sample.

Table 2: Mean Comparison Of Flexural Strength In PMMA, BIS-ACRYLIC And UDMA

VARIABLE	TIME	MEAN	SD	F VALUE	P VALUE
PMMA (Group-A)	24HRS	57.19	2.22	19.557	<0.001 Significant
	10DAYS	51.02	2.01		
	30DAYS	50.07	3.74		
BIS-ACRYLIC (Group-B)	24HRS	79.92	2.96	11.612	<0.001 Significant
	10DAYS	82.25	1.78		
	30DAYS	77.48	1.67		
UDMA (Group-C)	2HRS	44.05	1.82	37.900	<0.001 Significant
	10DAYS	38.98	1.71		
	30DAYS	36.30	2.46		

Statistical Analysis: ANOVA one way test. Statistically significant if P<0.05

Table 3: Mean Comparison Of ELASTIC MODULUS In PMMA, BIS-ACRYLIC And UDMA

VARIABLE	TIME	MEAN	SD	F VALUE	P VALUE
PMMA (Group-A)	24HRS	951.32	8.43	470.552	<0.001 Significant
	10DAYS	760.10	7.96		
	30DAYS	760.14	25.35		
BIS-ACRYLIC (Group-B)	24HRS	2779.39	63.55	5.067	0.014 Significant
	10DAYS	2779.62	145.41		
	30DAYS	2615.03	167.93		
UDMA (Group-C)	2HRS	611.67	42.19	76.373	<0.001 Significant
	10DAYS	496.95	23.11		
	30DAYS	397.53	46.87		

Statistical Analysis: ANOVA one way test. Statistically significant if P<0.05

Table 4: Intra Group Comparisons Of Flexural Strengths PMMA, BIS-ACRYLIC And UDMA

TIME	PMMA		BIS-ACRYLIC		UDMA	
	MEAN±SD	P value	MEAN±SD	P value	MEAN±SD	P value
24hrs VS 10 Days	6.17±0.21	<0.001 S	2.33±1.18	0.103 NS	5.07±0.11	<0.001 S
10Days VS 30Days	0.95±1.73	0.408 NS	4.77±0.11	<0.001 S	2.68±0.75	0.011 S

Statistical Analysis: Paired t test. Statistically significant if P<0.05

Table 5: Intra Group Comparisons Of Of ELASTIC MODULUS Of PMMA, BIS-ACRYLIC and UDMA

TIME	PMMA		BIS-ACRYLIC		UDMA	
	MEAN±SD	P value	MEAN±SD	P value	MEAN±SD	P value
24hrs VS 10 Days	191.22±0.47	<0.001 S	0.23±81.86	0.997 NS	114.72±19.08	<0.001 S
10Days VS 30Days	0.04±17.39	0.996 NS	164.59±22.52	0.051 NS	99.42±23.76	<0.001 S

Statistical Analysis: Paired t test. Statistically significant if P<0.05

DISCUSSION

Autopolymerizing polymethyl methacrylate is the most commonly used material for fabrication of interim restorations but its use is on decline because of their disadvantages like polymerization shrinkage,

exothermic setting reaction, and the irritation associated with monomer and inaccurate marginal adaptation.⁵

Bis-acrylic composite based provisional restorative materials are gaining popularity because of their cartridge delivery system. This dispensary method is not only convenient but also may allow for a more accurate and consistent mix and thereby improving its physical and mechanical properties. Bis-acryl composite resins have low polymerization shrinkage, low exothermic response but this material has disadvantage of being costly and their repair is troublesome.⁶

The visible Light polymerized (VLC) material containing urethane dimethacrylate, a resin whose polymerization is catalyzed by visible light and initiated by camphoroquinone. UDMA usually incorporate filler such as microfine silica to improve physical properties like reduced polymerization shrinkage. Light-cured urethane dimethacrylates have an advantage of controllable working time, great wear resistance, low temperature changes. Their hindrances are poor peripheral fit, weak nature and high cost.⁷

Results in this study showed that flexural strength and elastic modulus of PMMA decreases from 24 hrs storage (subgroup IA) to 10 days storage (subgroup IB) in artificial saliva. There was no significant change in FS and EM from 10days storage to 30days storage which may be due to saturation.

Results in this study showed that flexural strength of Bis-acryl provisional material increases from 24 hr storage (subgroup IA) to 10 days of storage (subgroup IB) in artificial saliva. The reason may be the increasing conversion of reactive double bonds by radicals and, on the other hand, relaxation phenomena within the polymer network.⁸ There was slight decrease of FS from 10 days to 30days storage which can be due to leaching of filler content. There was no significant change in elastic modulus from 24hr storage to 10days storage and 10days storage to 30 days storage. Bis-acryl has a rigid central structure.⁹

Results in this study showed that flexural strength of UDMA decreases from 24 hr storage to 10 days storage and from 10days to 30days storage in artificial saliva. Glass fillers are slowly leached out in the presence of saliva, thus reducing the mechanical properties of the interim composites. Elastic modulus is less for UDMA as there are no phenol rings in its monomer when compared to Bis-acryl hence it has higher flexibility.¹⁰

Determination of flexural strength was unproblematic for composite based materials as all specimens broke after exceeding a certain stress maximum. In contrast, most specimens made of polymethylmethacrylate (group I) and UDMA (Group III) did not break but were distorted instead. Consequently, the results for group I and group II have to be interpreted with care. The lack of fracture can be related to the chemical nature of resins. The main monomer component of poly methylmethacrylate forming the matrix is methylmethacrylate which forms linear polymer chains without any cross-links. A similar phenomenon was observed by Lang et al who investigated the fracture resistance of provisional crown and bridge materials in artificial oral environment¹¹.

The results obtained in this study were in correlation with the study conducted by S Dagar et al on direct and indirect provisional materials and found that Protemp 2 has lesser flexural strength as compared to heat cure polymethyl methacrylate but more than self cure poly methyl methacrylate.¹² According to the results of the study among all groups flexural strength and elastic modulus of bis- acryl is highest when compared to other groups after 24hr storage in artificial saliva.

The mean values of flexural strength of Group I and Group II were similar with the results obtained in the study conducted by Haselton et al who compared the flexural strength of five auto-polymerising methacrylate-based resins and eight bis-acryl resins. The authors reported that most of the bis-acryl resins demonstrated significantly superior flexural strength over traditional auto-polymerizing methacrylate resins.¹³

The results obtained in this study were contradictory with the study conducted by Koumjian and Nimmo who tested 3 methyl methacrylate resins and an earlier generation bis-acryl resin. They evaluated the flexural strength immediately following polymerization, seven days of dry storage and 7 days of wet storage. They found that water storage

absorption resulted in a slight but insignificant decrease in the transverse strength of methyl methacrylate and bis acryl. Transverse strength varied widely in the repaired group, and all materials showed a statistically significant reduction compared with the seven day wet storage group. Although no data is available to compare the type of resin matrix or filler content of those bis-acryl materials, it is evident that the difference in flexural strength performance was material-specific.¹⁴

The results obtained in this study for Group I and Group II were in correlation with the study by Balkenhol et al who evaluated the flexural strength and elastic modulus of interim resin materials at different storage times and concluded that the mechanical properties of composite resin-based materials are superior to methacrylate resins and recommended a dual-curing interim resin material if a high mechanical strength is indispensable directly after fabrication. They explained that in dual-curing materials a large amount of polymerization takes place at the beginning because of the light curing initiation of the reaction.¹⁵

The results obtained in this study for group III were in correlation with the study done by Ireland & Dixon where the elastic moduli and moduli of rupture of Proviopont DC resin, Triad (autopolymerising poly methyl methacrylate) and a 50:50 mixture of jet acrylic resin and orthodontic resin was evaluated. Proviopont DC resin (dual-polymerizing resin) exhibited significantly higher elastic moduli and moduli of rupture values at the 24 hour test time. However, Proviopont DC resin exhibited the greatest decrease in these values over time. This result was explained by the fact that a large amount of polymerization takes place at the beginning for this provisional crown and bridge material caused by the light curing initiation of the polymerisation reaction.¹⁶

Limitations Of The Study

This in-vitro study evaluated flexural strength and elastic modulus of three provisional restorative crown and bridge materials.

1. In the oral cavity, the provisional restoration is exposed to forces of varying magnitudes acting in different directions, and there are also temperature variations. The same situation was not simulated in this in vitro study.
2. Provisional restorations are subjected to force immediately after fabrication but in this in vitro study samples are subjected to load after soaking in artificial saliva for 24hrs and 10 days. Therefore further investigations are required under more closely simulated clinical conditions.

CONCLUSION

Bis-acryl interim materials exhibited higher flexural strength than the polymethacrylate resins and urethane dimethacrylate provisional materials. Polymethylmethacrylate and urethane dimethacrylate had shown more deflection than bisacrylate. They have undergone more plastic deformation before breakage therefore Elastic modulus of polymethylmethacrylate and urethane dimethacrylate is less when compared to bis-acryl composite provisional material.

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Assessment of the Effect of Bone Density on Implant Stability: A Clinical Study

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Abstract

Background:

In this study, we determined the effect of bone density on implant stability.

Methodology:

Two hundred and sixty dental implant sites in 128 patients were assessed for resonance frequency analysis (RFA) following implant insertion, at 6 months and 12 months. The implant stability quotient (ISQ) was determined.

Results:

The mean \pm standard deviation bone density in anterior mandible was 862.8 ± 203.4 Hounsfield units (HU), in posterior mandible was 528.4 ± 115.6 HU, in anterior maxilla was 594.2 ± 95.2 HU, and in posterior maxilla was 438.1 ± 110.2 HU. The mean insertion torque in successful implants was 38.2 ± 7.1 Ncm and in failed implants was 22.4 ± 4.2 Ncm. The mean RFA value in successful implants was 65.4 ± 5.8 and in failed implants was 45.8 ± 4.1 ISQ. A statistically significant ($P < 0.05$) difference was obtained.

Conclusion:

Within the limitation of the study, we observed that successful implants exhibited higher RFA and insertion torque and were higher into failed implants suggesting a correlation of bone quality and implant stability parameters.

KEYWORDS: *Implant stability quotient, insertion torque, resonance frequency analysis*



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INTRODUCTION

Dental implants have been widely used for the correction of missing few or multiple teeth have become popular in the last couple of years owing to several benefits. The higher survival rate and limited surgical intervention have led treatment of choice among dentists and patients.[1] Although it has significant higher success rate, numerous factors affect its survival rate. These are procedure-related and patient-related factors. The quantity, i.e., amount of bone present and quality, i.e., type of bone present determines the process of osseointegration. Type of dental implant used as well as the surgical method adopted is procedure-related factors.[2]

According to Misch bone density classification, Type D1 has higher survival rate which is usually found in mandibular anterior region. Type D2 is seen in the mandibular posterior region, Type D3 in maxillary anterior region and Type D4 poorest of all is encountered in maxillary posterior teeth region. Type D1 is strongest of all with 80% stronger trabeculae, Type D2 has combination of dense and porous bone with 40%–60% stronger trabeculae than Type D3 with 50% weaker trabeculae than D2, and Type D4 has poor density with trabeculae strength 10 times weaker than Type D1.[3]

Dental implant stability is the deciding parameter which ensures success rate of dental implant. Insertion torque, periotest, and resonance frequency analysis (RFA) are way of checking dental implant stability. The insertion torque method measures the quantity of torque required to insert the implant in the bone.[4] Periotest calculates the degree of the periodontal integration of teeth and the stiffness of the bone/implant interface. RFA calculates the bone/implant interface stiffness using Osstell instrument.[5] We assessed the effect of bone density on implant stability.

METHODOLOGY

This study consisted of 260 dental implant sites in 128 patients of both genders conducted in the prosthodontics department. All enrolled patients were made aware of the study and their written consent was taken. Ethical clearance for the study was obtained before starting the study.

Data pertaining to all patients such as name, age, and gender were recorded. A thorough oral screening was done followed by computed tomography of the recipient implant site taken with Siemens Germany operating at standardized operating parameters (potential difference - 120 kVp, 90 mA tube current, 1 mm slice thickness, and slice intervals). The mean bone density of the potential

implant site was measured using software Siemens. The bone density was measured in Hounsfield units (HU).

Dental implants were inserted following all standardized surgical procedures. RFA was performed with an Osstell instrument following implant insertion, at 6 months and 12 months. RF values were recorded in terms of implant stability quotient (ISQ). High ISQ value (100) is showing high stability and low implant stability was given to lower number (1). The maximum insertion torque value was recorded with OsseoCare motor during implant placement [Figures 1–4]. A successful implant showed no pain, suppuration and any pathologic processes, no evidence of periapical implant radiolucency, sufficient implant stability, the implant provided attachment to prosthetic part based on criteria proposed by Lekholm and Zarb.[6] The results of the study were subjected to statistically analyzed with 0.05 level of set as significant.



Figure 1

Pre operative photograph showing partially edentulous area i.r.t 36



Figure 4

Postoperative photograph showing the primary stability of the implant using OSSTEL (resonance frequency analysis)

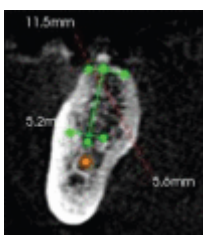


Figure 2

Preoperative cone-beam computed tomography radiograph showing 11.5 mm length and 5.6 mm width at crestal level i.r.t 36



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Figure 3

(a) Intraoperative photograph showing after incision flap was reflected using periosteal elevator. (b) Intraoperative photograph showing postoperative implant with abutment i. r.t 36

RESULTS

Out of 128 patients, 72 were male and 56 were female. Male had 140 and females had 120 dental implants. Male had eight and female had seven failed implants [Table 1].

Gender	Males	Females
Number of patients	72	56
Total implants	140	120
Successful implant	132	113
Failed implant	8	7

Table 1

Gender wise distribution of patients

Table 2 and Graph 1 show that mean \pm standard deviation (SD) bone density in anterior mandible was 862.8 ± 203.4 HU, in posterior mandible was 528.4 ± 115.6 HU, in anterior maxilla was 594.2 ± 95.2 HU, and in posterior maxilla was 438.1 ± 110.2 HU. A statistically significant ($P < 0.05$) difference was found between different locations.

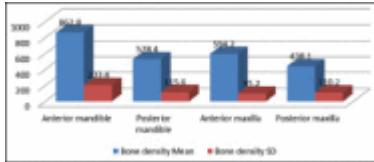
Location	Bone density, mean \pm SD	P
Anterior mandible	862.8 \pm 203.4	0.002
Posterior mandible	528.4 \pm 115.6	
Anterior maxilla	594.2 \pm 95.2	
Posterior maxilla	438.1 \pm 110.2	

SD: Standard deviation

Table 2

Assessment of bone density at different location

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Graph 1

Assessment of bone density

Table 3 shows that the mean insertion torque in successful implants was 38.2 ± 7.1 Ncm and in failed implants was 22.4 ± 4.2 Ncm. A statistically significant difference was found ($P < 0.05$).

Implants	Insertion torque, mean±SD	P
Successful implants	38.2±7.1	0.01
Failed implants	22.4±4.2	

SD: Standard deviation

Table 3

Assessment of insertion torque in successful and failed implants

Table 4 shows that mean RFA value in successful implants was 65.4 ± 5.8 and in failed implants was 45.8 ± 4.1 ISQ. A statistically significant difference was found ($P < 0.05$).

Implants	RFA, mean±SD	P
Successful implants	65.4±5.8	0.021
Failed implants	45.8±4.1	

RFA: Resonance frequency analysis, SD: Standard deviation

Table 4

Resonance frequency analysis value in successful and failed implants

Table 5 and Graph 2 show that mean ISQ value in the anterior mandible was 72.1, 71.4, and 73.6 at the time of surgery, at 6 months and 1 year, respectively. In the posterior mandible was 67.4, 66.7, and 69.7 at surgery, at 6 months and 1 year, respectively. In anterior maxilla at surgery, at 6 months and 1 year was 68.4, 69.2, and 70.1, respectively. In posterior maxilla was

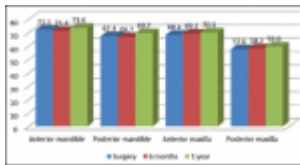
57.6, 58.2, and 59.6 at surgery, at 6 months and 1 year, respectively. A statically significant difference was found ($P < 0.05$).

Table 5
Change in implant stability quotient value at different interval

Location	Surgery	6 months	1 year	P
Anterior mandible	72.1	71.4	73.6	0.85
Posterior mandible	67.4	66.7	69.7	0.83
Anterior maxilla	68.4	69.2	70.1	0.82
Posterior maxilla	57.6	58.2	59.6	0.84

Table 5

Change in implant stability quotient value at different interval



Graph 2

Change in implant stability quotient value at different interval

DISCUSSION

Bone density is indispensable for the dental surgeon to arrive at the best treatment strategy and to finalize suitable implant design to achieve primary stability.[7] The advancement of designing in implants, surface treatments, and study of bone architecture proved beneficial in the field of implantology. The implant stability is the principal prerequisite to establish the prosthetic management that shall be initiated, which needs to be assessed before force insertion.[8] The present study was conducted to determine the effect of bone density on implant stability.

In the present study, males were 72 in number having 140 dental implants and females were 56 in number having 120 dental implants. Male had eight and female had seven failed implants. Turkyilmaz and McGlumphy[5] in their study on 300 implants in 111 patients determined effect of bone density on implant stability. Results showed mean bone density (620 ± 251 HU), insertion torque (36.1 ± 8 Ncm), and RFA value (65.7 ± 9 ISQ) at implant placement. There was significant correlation between insertion torque and bone density, ISQ values and bone density, and ISQ values and insertion torque, and bone density was observed. The mean bone density, insertion torque, and RFA at implant insertion area was 645 ± 240 HU, 37.2 ± 7 Ncm, and 67.1 ± 7 ISQ, respectively, and in 20 failed implants was 267 ± 47 HU, 21.8 ± 4 Ncm, and 46.5 ± 4 ISQ, respectively, with statistically significant differences ($P < 0.001$).

We found that mean \pm SD bone density in anterior mandible was 862.8 ± 203.4 HU, in posterior mandible was 528.4 ± 115.6 HU, in anterior maxilla was 594.2



± 95.2 HU, and in posterior maxilla was 438.1 ± 110.2 HU. Oliscovicz *et al.*[9] in their study used 32 Conexão® implants, eight conical, and 24 cylindrical. All were placed in Nacional® polyurethane using 15, 20, and 40 PCF densities. Results exhibited the best performance was the interaction implant CA x 40PCF substrate with difference from the other implants inserted in all substrates.

We found that the mean insertion torque in successful implants was 38.2 ± 7.1 Ncm and in failed implants was 22.4 ± 4.2 Ncm. The mean RFA value in successful implants was 65.4 ± 5.8 and in failed implants was 45.8 ± 4.1 ISQ. Cornellini *et al.*[10] inserted 40 implants in 20 patients, the mean ISQ values were 72 at implant surgery and 74.5 after 1 year, which was not statistically significant ($P > 0.05$).

We observed that mean ISQ value in the anterior mandible was 72.1, 71.4, and 73.6 at the time of surgery, at 6 months and 1 year, respectively. In posterior mandible was 67.4, 66.7, and 69.7 at surgery, at 6 months and 1 year, respectively. In the anterior maxilla at surgery, at 6 months and 1 year was 68.4, 69.2, and 70.1, respectively. In the posterior maxilla was 57.6, 58.2, and 59.6 at surgery, at 6 months and 1 year, respectively. Sjostrom *et al.*[11] studied 192 implants after bone-graft healing. Results showed that the ISQ value was 60.2 ± 7.3 at insertion time and 62.5 ± 5.5 after 6 months of prosthetic part insertion having significant difference between both periods. A ISQ of 61.9 ± 9.5 and 60.2 ± 7.3 was observed between implant insertion and abutment attachment with nonsignificant difference. A ISQ value of 62.5 ± 5.5 and 61.8 ± 5.5 was found at 6 months and 3 years of bridge loading, respectively.

The shortcoming of the study is small sample size and short follow-up.

CONCLUSION

Authors found that insertion torque and RFA was higher in successful implants as compared to failed implants suggesting correlation of bone quality and implant stability parameters.

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Conflicts of interest

here are no conflicts of interest.

Article information



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EVALUATION OF QUALITY OF LIFE WITH OBTURATORS IN PATIENTS POST MAXILLECTOMIES: AN ORIGINAL RESEARCH

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ABSTRACT

Introduction: In this study we evaluated the quality of life with obturators in patients post maxillectomies

Materials and Methods: Thirty-six subjects were enrolled with maxillary defects, irrespective of the cause, planned for definite obturator prosthesis, were recruited. Head and Neck version 1 of Quality of Life Questionnaire was used before surgical intervention and one month after definitive obturator. Questionnaire includes 35 questions related to the patient's physical health, well being, psychological status, social relation and environmental conditions. The data were processed with statistical package for social science (SPSS). Probability level of $P < .05$ was considered statistically significant.

Results: The quality of life after rehabilitation with obturator prosthesis was 81.48% (± 13.64) on average. On item-level, maximum mean scores were obtained for items problem with teeth (1.87 ± 0.94), pain in mouth (1.80 ± 0.92), trouble in eating (1.70 ± 0.88), trouble in talking to other people (1.60 ± 1.22), problems in swallowing solid food (1.57 ± 1.22) and bothering appearance (1.53 ± 1.04); while minimum scores were obtained for the items coughing (1.17 ± 0.38), hoarseness of voice (1.17 ± 0.53), painful throat (1.13 ± 0.43), trouble in having social contacts with friends (1.10 ± 0.40) and trouble having physical contacts with family or friends (1.10 ± 0.31).

Conclusion: Obturator prosthesis is a highly positive and non-invasive approach to improve the quality of life of patients with maxillectomy defects.

Key Words: Quality of life; Maxillectomy; Obturator prosthesis; Oral cancer

I. INTRODUCTION

Presently, the multitudinal impact of maxillofacial tumors on a patient's life has been recognized, which led various researchers to investigate the quality of life of those patients. However, studies evaluating the quality of life of patients with maxillectomy defects and the effect of prosthodontic therapy with obturator prostheses on their quality of life remain rare.

The significant areas of treatment concern after maxillary resection are reconstruction of the defect and restoration of oronasal functions while maintaining the facial contours. The obturator prosthesis fulfills most of these requirements and it also reduces the procedure time and offers the possibility of immediate rehabilitation. It is possible to examine the surgical site after removing the prosthesis, and recurrence may be detected at an early stage.⁵⁻⁸ So, the obturator can be considered as a highly positive approach for rehabilitation after maxillectomy. However, in some cases, impaired obturator functioning and handling may lead to deficits in speech, mastication, swallowing or facial disfigurement, thereby resulting in patient dissatisfaction.¹⁻¹⁰

Various investigators have found that orofacial deformities result in profound psychological and social consequences.¹¹⁻¹³ Such subjects are more likely to encounter social negligence and usually develop negative personality traits. Maxillo- facial injury rehabilitation represents one of the greatest challenges to public health service providers worldwide because of their high incidence and significant financial burden. They are often associated with morbidity and varying degree of physical, functional and aesthetic damages.^{12,13}

Hence, in this study we evaluated the quality of life with obturators in patients post maxillectomies.

II. MATERIALS AND METHODS

Thirty six subjects were enrolled in the study for the span of sixteen months. Ethical approval was obtained from the institution.

The instructions regarding filling of questionnaire were explained to the selected thirty six subjects, but for evaluation, only thirty subjects were available as six subjects had declined to be involved in the study. The Hindi version of European Organization for Research and Treatment of Cancer, Head and Neck version 1 of Quality of Life Questionnaire (EORTC QLQ-H &N 35) was used.

The questionnaire includes 35 questions related to patient's physical health, well being, psychological status, social relation and environmental conditions. The questionnaire was divided into two parts with initial 30 multiple choice questions, with scoring based on Likert scale of four-points, which were used to quantitatively measure the patient's perceived changes in the quality of life. Remaining 5 questions were dichotomous and were used for status evaluation of the patients. Patients were asked to complete questionnaire based on their experience during the past one week before surgical intervention, the same questionnaire was completed by the patient after definitive prosthetic rehabilitation. The quality of life of subjects was broadly divided into eight dimensions as follows:

Item Nos. 1-4 Pain

Item Nos. 5-8 Swallowing

Item Nos. 9-12 Teeth and Mouth Item Nos. 13-14, 22 Senses

Item Nos. 15-17 General Health Item Nos. 19-21 Eating

Item Nos. 18, 23-28 Social Item Nos. 29-30 Sex

The probability levels of $P < .05$ were considered statistically significant for all statistical analyses.

III. RESULTS

The patient's perceived quality of life after rehabilitation with obturator was calculated to be 81.48% (± 13.64). Majority of patients belonged to age group <30 years and age group 51-60 years respectively, showing a bimodal age distribution. There were only 2 patients in age group >70 years. Mean age of patients was 46.83 ± 16.98 years.

Squamous cell carcinoma was the most common clinical diagnosis (50%) responsible for maxillectomy. Surgery alone ($n = 19$; 63.3%) was the most common treatment modality used.

On item-level, maximum mean scores were obtained for items problem with teeth (1.87 ± 0.94), pain in mouth (1.80 ± 0.92), trouble in eating (1.70 ± 0.88), trouble in talking to other people (1.60 ± 1.22), problems in

swallowing solid food (1.57 ± 1.22) and bothering appearance (1.53 ± 1.04) while minimum scores were obtained for the items coughing (1.17 ± 0.38), hoarseness (1.17 ± 0.53), painful throat (1.13 ± 0.43), trouble in having social contacts with friends (1.10 ± 0.40) and trouble having physical contacts with family or friends (1.10 ± 0.31) (Table 1).

Minimum effect on quality of life was observed for the sex related QOL whereas maximum was observed for social life. At item-level, statistically significant reduction in mean scores was found for the items such as pain in mouth ($P=.032$), soreness in mouth ($P=.001$) and coughing ($P=.025$) (Table 1). A statistically significant increase in mean scores was observed for items such as problems in swallowing solid food, problem in opening mouth wide, trouble in eating, difficulty in eating food in front of family and other people, problem in enjoying food, difficulty in conversation to people and on the telephone, problem in making social contacts with friends, trouble in making public appearance and difficulty in making physical contacts with others. For all the other items the change was not significant statistically ($P>.05$). Overall, no significant change in mean scores was observed.

Table 1. Comparison of pre-treatment and post-treatment quality of life scores

	Mean	SD	Mean	SD	t	P
1. Pain in mouth	1.80	0.92	1.33	0.84	2.249	.032
2. Pain in jaw	1.50	0.97	1.13	0.35	2.009	.054
3. Soreness in mouth	1.40	0.62	1.00	0.00	3.525	.001
4. Painful throat	1.13	0.43	1.00	0.00	1.682	.103
5. Problem in swallowing liquids	1.27	0.74	1.53	0.97	-1.439	.161
6. Problem in swallowing pureed foods	1.23	0.63	1.27	0.52	-0.571	.573
7. Problems in swallowing solid food	1.57	0.90	2.03	0.89	-3.500	.002
8. Choking while swallowing	1.20	0.61	1.17	0.59	1.000	.326
9. Problem with teeth	1.87	0.94	1.90	0.76	-0.254	.801
10. Problem in opening mouth wide	1.47	0.78	2.10	0.76	-5.641	.000
11. Dry mouth	1.37	0.76	1.47	0.73	-0.902	.375
12. Sticky saliva	1.33	0.66	1.33	0.71	0.000	1.000
13. Problem with sense of smell	1.47	0.97	1.33	0.55	0.750	.459
14. Problem with sense of taste	1.20	0.48	1.23	0.43	-0.328	.745
15. Coughing	1.17	0.38	1.00	0.00	2.408	.023
16. Hoarseness	1.17	0.53	1.07	0.25	0.902	.375
17. Feeling of illness	1.37	0.67	1.23	0.43	1.072	.293
18. Bothering appearance	1.53	1.04	1.60	0.62	-0.441	.662
19. Trouble in eating	1.70	0.88	2.37	1.10	-3.247	.003
20. Trouble in eating in front of family	1.37	0.85	2.03	1.13	-3.162	.004
21. Trouble in eating in front of others	1.50	1.04	2.23	1.22	-3.717	.001
22. Trouble in enjoying meals	1.37	0.81	2.10	1.06	-5.117	.000
23. Trouble in talking to other people	1.60	1.22	2.37	1.25	-4.173	.000
24. Trouble in taking on the telephone	1.43	0.90	2.07	1.20	-3.072	.005
25. Trouble in having social contacts with family	1.23	0.57	1.37	0.85	-0.891	.380

26.	Trouble in having social contacts with friends	1.10	0.40	1.83	1.05	-4.253	.000
27.	Trouble going out in public	1.50	0.97	2.03	1.07	-3.565	.001
28.	Trouble having physical contacts with family or friends	1.10	0.31	1.53	0.90	-2.765	.010
29.	Less interested in sex	0.97	0.61	0.97	0.61	0.000	1.000
30.	Less joy in sex	0.97	0.61	1.03	0.67	-1.439	.161
	Total	45.80	13.61	46.67	12.27	0.437	.665

IV. DISCUSSION

The present study investigated the quality of life of patients with maxillectomy after rehabilitation with obturator prostheses. In spite of numerous researches regarding the quality of life after cancer therapy, only a few studies emphasize on the quality of life of maxillectomy patients rehabilitated with obturator.14-15

In the present study, thirty patients were investigated. Depprich *et al.*10 studied forty three patients, Rogers *et al.*11 interviewed ten patients, Hertrampf *et al.*6 evaluated seventeen patients.

In the present study, a 35-item head and neck module (EORTC QLQ-H&N35) was utilized. This standardized questionnaire allows a comparison between multiple study groups.

The quality of life after rehabilitation with obturator prostheses was calculated to be 81.48% (± 13.64). The direct comparison of these results with the previous studies are not possible as in different studies, different tests and scales were used to evaluate the quality of life.

In most of the previous studies, similar to the study conducted by Depprich *et al.*,10 only the quality of life after prosthetic rehabilitation was evaluated by a cross-sectional study whereas in the present study, the quality of life before prosthetic rehabilitation as well as the quality of life after prosthetic rehabilitation have been assessed by a longitudinal study, thereby enabling us to simultaneously evaluate the change in the quality of life scores, which was found to be in order of significance of change as 0.665.

In the present study, age of patients ranged from 20 to 76 years. Majority of patients belonged to age group <30 years and age group 51-60 years respectively, showing a bimodal age distribution. There were only 2 patients in age group >70 years. Mean age of patients was 46.83 ± 16.98 years. For younger patients, the quality of life score was 73.02% in comparison to the score of older age group which was 87.78% after prosthetic rehabilitation. Elderly patients, who anticipate to have age related physical illness, suffer less from distress related to cancer as compared with younger patients who feel that their life span has been shortened and their quality of life impaired due to the ailment.

Patients suffering from maxillofacial tumors develop coping strategies and so they gain an increase of quality of life after prosthetic rehabilitation. Most of the patients do not criticize their decision after knowing the treatment outcome and consider that being alive out-weighs the demerits of obturator therapy.

Good obturator function has been found to be responsible for improved quality of life.4,6,16,17 However, in the present investigation, only the quality of life of maxillectomy patients after obturator was assessed but other domains related to the obturator function and the effect of family behavior, which also contribute to the quality of life were not studied in detail.

The present study found that except for change in scores for senses, general health and sex, for all the other dimensions a significant change was observed. Except for pain, for all the other dimensions where significant changes were observed, mean scores were found to be significantly increased after treatment. For pain a significant reduction in mean scores was found. For both pre- and-post-treatment evaluations, minimum scores were observed for the dimension sex whereas maximum scores were obtained for the item eating. No significant change in physical status was observed following treatment ($P>.05$). The reduction in pain scores as found in this study is contradictory to the previous studies done by Hertrampf *et al.* and Rogers *et al.*

In the present study, at item-level, statistically significant decrease in mean scores was observed for the items pain in mouth, soreness in mouth and coughing. A statistically significant increase in mean scores was observed for items - problems in swallowing solid food, problem in opening mouth wide, trouble in eating, difficulty in eating food in front of family and other people, problem in enjoying food, difficulty in conversation to people and on the telephone, problem in making social contacts with friends, trouble in making public appearance and difficulty in making physical contacts with others. The above observations of the present study are supported by the results obtained from study conducted by Depprich *et al.*10. In the present study, surgery alone (n = 19; 63.3%) was the most common treatment modality availed followed by surgery + radiotherapy + chemotherapy (n = 5; 16.7%), surgery + radiotherapy (n = 4; 13.3%) and surgery + chemotherapy (n = 2; 6.7%). According to the study conducted by Depprich *et al.*10, the most common treatment modality was surgery only.

It was found that squamous cell carcinoma was the most common clinical diagnosis (50%) followed by adenoid cystic carcinoma of hard palate (n = 4; 13.3%). Malignant melanoma (n = 3; 10%) and nasopharyngeal angiofibroma (n = 2; 6.7%) were the next most common diagnosis. Ameloblastoma, cleft lip and palate, cystic lesion, giant cell tumor, osteoclastoma and sinonasal solitary fibrous tumor were present in 1 (3.3%) case each.

The hypothesis that the quality of life of maxillectomy patients after obturation is acceptable is justified by the results of the present study. Future research on defect related newer obturator designs may help to overcome the problems typically associated with obturator prostheses and will help to improve patient's quality of life after maxillectomy in the future.

V. CONCLUSION

Obturator prosthesis is a highly positive and non-invasive approach to improve the quality of life of patients with maxillectomy defects.

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Surgical approaches in successful maxillary sinus lift implant-a step by step detailed guided intervention

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Introduction

Implant dentistry has become an excellent treatment modality since its inception into the modern era of dentistry. It not only allows for a conservative and esthetic alternative to treating partial edentulism, but it also provides a stable foundation for treating complete edentulism. Dental implants can be a viable treatment option when there are sufficient quantity and quality of bone. However, when patients present with deficient alveolar ridges, it could jeopardize the application of implant dentistry. This problem is especially magnified in the posterior maxilla where ridge resorption and sinus pneumatization, compounded with a poor quality of bone, are often encountered. The procedure of choice to restore this anatomic deficiency is maxillary sinus floor elevation (sinus lift).⁽¹⁾

The maxilla itself is different in its function, physiology, and bone density than the mandible. These differences, in combination with the unique and varied anatomy of the

maxilla, pose a challenge to the surgeon in creating bone height and width sufficient for implant placement in harmony with planned prosthetic rehabilitation. However, a thorough knowledge of contemporary augmentation procedures mitigated by proper patient selection can lead to effective long-term solutions in the management of the deficient posterior maxilla⁽²⁾.

Implants can either be inserted simultaneously, when there is sufficient bone height for primary Stability (>4 mm), or can be inserted in a second procedure when bone-remodelling of the graft has taken place. This two-stage procedure is indicated when no good primary stability can be expected (bone height< 4 mm).⁽³⁾

Conditions such as sinus floor convolutions, sinus septum, and transient mucosa swelling and narrow sinus may form a (usually relative) contraindication for sinus floor elevation. Absolute contraindications are maxillary sinus diseases (tumours) and destructive former sinus surgery (like the Caldwell-Luc operation).⁽³⁾

Surgical techniques in sinus lift

Currently, there are mainly two approaches to the maxillary sinus floor elevation procedure, according to the literature. The first approach is lateral antrostomy, which is the classical technique and more commonly operated technique that was originally described by Tatum. More recently, Summers advocated a second approach known as the crestal approach, using an osteotome. This crestal approach is considered to be a more conservative method for sinus floor elevation.

Lateral Antrostomy: Lateral antrostomy is started with a crestal incision made on the alveolar ridge. Mostly, the incision is performed slightly palatal to the crest to preserve a wider band of keratinized attached gingiva for more solid wound closure and to avoid wound dehiscence. A Maxillary full-thickness flap is then raised to allow access to the lateral antral wall. Once the flap has been raised to the desired level, antrostomy is usually performed with a round bur to create a U-shaped trapdoor on the lateral buttress of the maxilla. Precaution must be taken so that the height of this trapdoor should not exceed the width of the sinus (it can be measured in a computerized tomogram) to allow for a final horizontal position of the new floor. The sinus membrane is then gently lifted from the bony floor by means of an antral curette. Marx and Garg suggested using a cottonoid soaked with a carpule of 2% lidocaine with 1:100,000 epinephrine and left in the space created for 5 minutes so as to limit bleeding and allow for better visualization for further dissection. It is important to free up the sinus membrane in all directions (Anteriorly, posteriorly, and medially) before attempting to intrude the trapdoor medially. Space is created after the sinus membrane has been elevated by the intruded trapdoor. This space is then grafted with different materials to provide the platform for implant placement; numerous research projects have been published to

evaluate the prognosis of implants under different grafting materials. Autogenous bone remains the gold standard in bone grafting. Iliac crest, chin, anterior ramus, and tuberosity have all been mentioned as common Autogenous donor sites in maxillary sinus lift. Hydroxyapatite mixed with Autogenous bone or used alone has also been shown to be viable alternatives. Care should be taken not to overfill the recipient site because it will cause membrane necrosis.

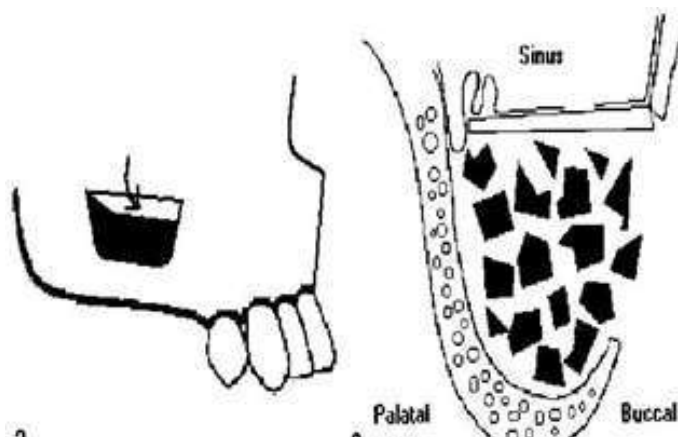


Fig. 1: Intruding the U-Shaped trapdoor. Corners of the trapdoor should be rounded.

Implants are placed either simultaneously with the graft (1-stage lateral antrostomy) or after a delayed period of up to 12 months to allow for graft maturation (2-stage lateral antrostomy). The initial bone thickness at the alveolar ridge seems to be a reliable indicator in deciding between these 2 methods. If the bone thickness is 4 mm or less, initial implant stability would be jeopardized. Therefore, a 2-stage lateral antrostomy should be carried out. The reverse holds true for a 1-stage procedure. A 1-stage procedure is less time-consuming for both the clinician and the patient. However, it is more technique-sensitive, and its success relies heavily on the amount of residual bone. **Crestal Approach:** One of the drawbacks of lateral antrostomy is that it requires the raising of a large flap for surgical access. Summers proposed a conservative crestal approach using osteotome for maxillary sinus floor

elevation in 1994. This technique begins with a crestal Incision. A full-thickness flap is raised to expose the alveolar ridge. An osteotome of the smallest size is then tapped into place by a mallet or drill into the bone. Preoperative bone height underneath the sinus is measured to determine the desired depth for osteotome extension. Osteotomes of increasing sizes are introduced sequentially to expand the alveolus. With each insertion of a larger osteotome, bone is compressed, pushed laterally and apically.

Techniques of maxillary sinus lift

The sinus lift surgical technique has developed over time, and several minor variations now exist like “Antral membrane balloon elevation” by Smiler, “Distraction osteogenesis” by Boyne etc.

Maxillary sinus augmentation techniques

Direct sinus lift or lateral window technique.	Indirect sinus lift
Piezoelectric bony window osteotomy: 2001.	Summers osteotomy technique: 1994.
Subantrosopic laterobasal sinus floor augmentation: 2002	Boyne’s distraction
Sinus/alveolar crest tenting (SACT) technique: 2003.	Elevation of maxillary sinus floor with hydraulic pressure: Sotirakis 2005.

Summer’s osteotome technique: 1994

As osteotomy preparation progresses toward the posterior, the surgeon usually notices a softer bone texture. The ability to drill accurately in the posterior maxilla diminishes with the loss of tactile sensitivity in the soft bone when using rotary instruments. Also, inadvertent sinus penetration and over the preparation of soft bone is

common with drills. Other factors, such as torquing of the handpiece and reproducing a consistent angle of penetration, become more demanding as bone density decreases in the posterior maxilla. Because of the problems of drilling in the maxilla summers developed a means of osteotomy preparation in which the bone is not removed. The objective of this technique is to maintain, if possible, all of the existing maxillary bone by pushing the bone aside with minimal trauma while developing an accurately shaped osteotomy.

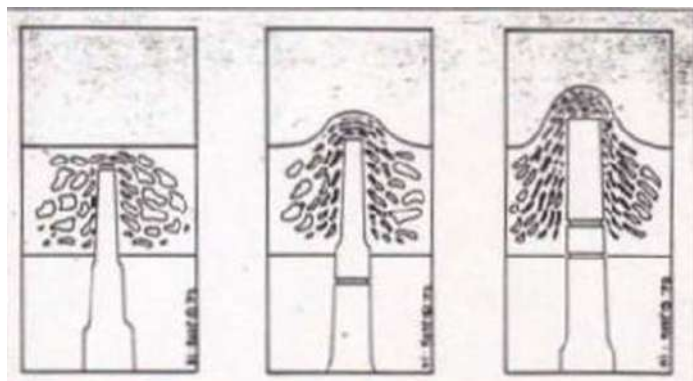


Figure 2: Lateral Antrostomy intruded with trap door grafting material.

At the osteotome sinus floor elevation, bone piles up in front of the penetrating osteotome, allowing the sinus floor to be displaced upwards.

The osteotome technique attempts to retain all of the bone that is present and to take advantage of the softer bone quality by relocating the bone to suit the needs of the surgery. In contrast to drilling, the osteotome technique improves maxillary anatomy by widening the ridge as the instruments are inserted. The osteotomes, developed by the author (Summers Osteotome Kit), are shaped so that the next larger osteotome tip fits into the opening created by the previous instrument. Bone buccal and palatal to the osteotomy is pushed laterally with successive penetrations of the larger osteotome. In a narrow ridge, expansion of the buccopalatal dimension of the site is an inherent beneficial characteristic of the osteotome technique. This

is called a ridge expansion osteotomy (REO), in contrast to a drilled site, in which the buccopalatal bone width is not changed.

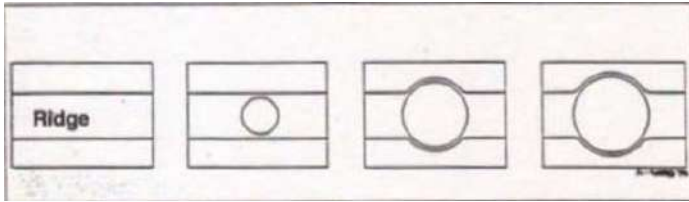


Figure 3

Ridge expansion osteotomy can be attempted at any location wider than 3mm.

Buccal and lingual bone moves laterally as the osteotome are inserted.

Other potential benefits of the osteotome technique include

1. Alteration of the anterior or posterior sinus boundary during a routine osteotomy.
2. More upright (less flared) positioning of implants. The osteotome technique provides greater flexibility for the surgeon to match opposing landmarks because of the REO feature.
3. Development of future implants sites.
4. Addition of bone into the osteotomy as the site is developed⁽¹⁷⁾.

Crestal core elevation (CCE) first described by Summers in 1995, is a modification of the osteotome sinus floor elevation (OSFE) and the bone-added OSFE (BAOSFE) approaches, which are suitable for immediate implant insertion but require ≥ 6 mm of bone between the sinus floor and the crest of bone. CCE may be a simple alternative to LWT (lateral window technique) when the quantity of bone does not permit immediate implant placement. Performing CCE involves vertical crestal drilling using a wide trephine bur up to the sinus cortex followed by displacement of the bony plug inward and raising the sinus floor with a wide concave osteotome. The technique may be implemented in patients with residual

bone height measuring 3 to 6 mm. A further modification of the summer's technique was suggested by Fugazzotto and De Paoli who used it concomitantly with extractions of the upper molars. The placement of deproteinized bovine bone mineral covered with an absorbable membrane or non-absorbable expanded polytetrafluoroethylene membrane minimized the loss of alveolar bone height and width after tooth removal and permitted implant placement after 4 to 8 months. (4). The osteotome sinus floor elevation procedure without grafting material, and immediate placement of tapered implants, might be applied in situations for which previously only the lateral approach was considered. (5). The osteotome technique can be recommended when more than 6 mm of residual bone height is present, and an increase of about 3 to 4 mm is expected. (6). The study consisted of 26 patients treated with 39 Brånemark implants (Nobel Biocare) placed Using the simplified osteotome technique between September 1997 and November 2004 (87 months). Implant length ranged from 10 to 15 mm, while the loading time . Ranged from 5 to 74 months (mean: 35.2 months). The success rate was 97.4%, according to Albrektsson's criteria. These preliminary data indicate that the simplified osteotome technique is an effective and safe technique. (7). Performing sinus elevations with osteotomes is a predictable technique that enables achieving an increase in bone height and successful results, similar to those of other techniques used in the placement of implants. (8).

The piezoelectric bony window osteotomy and sinus membrane elevation (Pbwo and Psme): 2001.

The most commonly used procedures, such as lateral bony window osteotomy technique and osteotome sinus elevation, have a problem of perforating the sinus membrane either with the burs during the osteotomy or with the manual elevators during the separation of the

membrane. If the bone graft is carried out in the case of a membrane lesion, it is very likely that parts of the bone graft will go to an ectopic site, usually on the surface of the respiratory mucous epithelium; this would entail the necrosis of the graft, followed by a suppurative process in the sinus cavity, generally visible in the Orosinus antral fistula. In 2001 Vercellotti et al. introduced a new technique for simplification of the sinus augmentation procedure, the piezoelectric bony window osteotomy and sinus membrane elevation (PBWO and PSME). This new technique uses a specifically engineered device, the Mectron Piezosurgery system, to perform the osteotomy.

Surgical procedure

With the blade of the 15 scalpels, a horizontal crestal incision is made at the top of the ridge from the distal aspect of the maxilla, continuing mesially until it reaches one or two of the anterior teeth, where a vertical releasing incision is made. Another releasing incision is made in the distal aspect under the Stenson's duct. A flap of the total vestibular thickness is raised. It is characterized by a broad vascular supply mesially and distally. The most apical parts of the two incisions are united by a horizontal periosteal incision to give greater elasticity to the mucous flap in the suturing phase.

Piezoelectric bony window osteotomy

To open the sinus window, the following surgical procedure is carried out. With the No. 1 scalpel from the Sinus Lift system by Mectron Piezosurgery, an outline is drawn. It begins with the most coronal horizontal incision, with a length of approximately 14 mm positioned approximately 3 mm apical to the residual crestal bone. Two vertical incisions of 6 to 7 mm are made and united at the top by another horizontal incision. The bony window is performed in the area of the second premolar–first molar. The outline is drawn in about 3 minutes, and the average thickness of the cut is approximately 1 mm. This

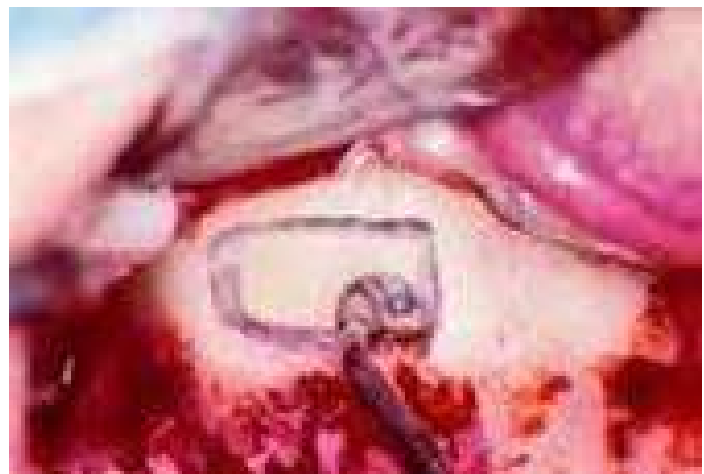
produces a bony window in which the frame is represented by the Schneiderian membrane (sometimes red in colour, sometimes blue). At this point, the osteotomy is completed by rounding the angles of the window.



PBWO made by No. 1 Piezoelectric scalpel. It is possible to observe the Schneiderian membrane, which appears as the frame of the bony window.



Different clinical case. The initial phase of PSME uses the overturned cone compressor.



Compressor is inserted into the frame of the window, separating the borders approximately 2 mm

Piezoelectric sinus membrane elevation

The No. 2 insert, a compressor in the shape of an overturned cone, is inserted into the edge of the frame of the membrane exposed by the osteotomy. It separates the borders approximately 2 mm with ease. At this point, the No. 3 insert (an angled periosteal elevator with rounded edges) or the No. 4 (a straight periosteal elevator), depending on the anatomic situation, is used in the following order. The first stage of the membrane elevation begins in the apical position.



PSME phase two: the elevator in position, ready to work.



First stage of the membrane elevation begins in the apical position, then in the mesial and distal aspects.



Once the membrane is elevated on three sides, it is possible to separate it from the floor of the sinus, where adhesions are very common, Therefore avoiding the risk of perforation.

The membrane separation in the apical direction depends on the length of the implants that will be placed in the second surgery. The insert is directed toward the mesial surface, separating the membrane until it meets the anterior walls of the sinus. The insert is then directed toward the distal walls, separating the membrane to obtain the volume required for the graft to build the future implant site. Finally, the insert is directed toward the crestal position, where it is possible to meet adhesions, particularly in the depths of the molar depressions. This maneuver is carried out last in a way that allows the separation of the membrane floor without tension, having already separated the membrane from the other sides of the window.

The sinus augmentation procedure is performed using an Autogenous bone graft mixed with Autogenous platelet-rich plasma gel. After the bone graft is performed, and the bioabsorbable membrane is positioned to cover the bony window and fixed to the bone with screws, horizontal mattress sutures are placed.⁽⁹⁾


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(Left) piezoelectric bone harvesting technique in the Mandible.



(Right) An autologous bone graft mixed with autologous platelet gel fills the sinus cavity.



(Right) Volume of bone chips is on average 2.5 to 3cm of cortex and Medullary bone.



(Left) Donor site after the completion of the bone harvesting.

A simple inexpensive method for precisely locating the floor of the maxillary sinus, as well as the presence of any septa, at the time of sinus augmentation surgery. Using an anaesthesia light wand placed transnasally to illuminate the sinus, the surgeon can reliably elevate the lateral maxillary wall overlying the sinus with relative ease without fear of placing the osteotomy cuts too far from the sinus floor. The same procedure can be used postoperatively to evaluate the density of the bone graft placed into the sinus prior to closure. (10)

Subantrosopic Laterobasal Sinus Floor Augmentation (Salsa) Technique: 2002

Engelke and Deckwer described a new endoscopically controlled technique for sinus floor augmentation. This technique involves trans alveolar mobilization of the sinus membrane controlled by sinuscopy, trans alveolar augmentation, and simultaneous implant placement and has been indicated for moderately reduced alveolar sites. Engelke and coworkers reported on a modified endoscopic technique, the laterodorsal tunnel technique, which allowed augmentation of multiple maxillary sites via 1 small laterobasal trepanation (unpublished data). Through this approach, a “tenting” of the complete sinus membrane from the premolar to the second molar site could be performed, thus allowing for large

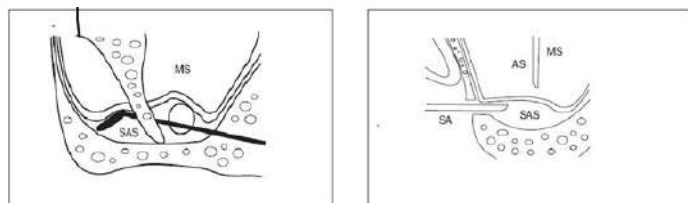

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augmentations in case of primary and secondary implantation. Both procedures are controlled with the endoscope placed in the lumen of the maxillary sinus via a puncture of the canine fossa.

Surgical procedure

The flap design depends on the number and location of implants planned. Typically, a crestal incision is made with a vestibular relief incision in the first premolar region. A full-thickness mucoperiosteal flap is then elevated, exposing the anterobasal aspect of the sinus wall, including the inferior third of the zygomatic buttress and the alveolar crest with the planned implant sites.

Microsurgical Access: A 5-mm-diameter laterobasal osteotomy is made directly anterior to the zygomatic buttress at the inferior aspect of the anterior sinus wall. The osteotomy is performed with a 4-mm diamond round bur under magnification with the support video endoscope technique. The osseous margin of the trepanation is then identified. The sinus membrane is displaced with the help of microsurgical elevators of 2 to 4 mm in diameter around the trepanation. The bony access is opened just enough to allow the introduction of 4-mm-diameter angulated mucosal elevators into the subantral space. The circular dissection of the sinus membrane is performed under continuous micro – endoscopic observation on a monitor.



Schematic representation of the SALSA technique. (Left) Preparation of the subantral space through the keyhole approach (Panoramic view). (Right) Endoscopic control during SALSA (Cross-sectional

view). MS=Maxillary sinus, SAS= Subantral space, AS= Antroscopy, SA=Subantroscope.



The 2.7-mm endoscope tip and SPS working end



Endoscope with SPS Mounted.



Microsurgical sinus Elevators : type O “dish-knives” for opening.

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Microsurgical elevators: type T for tunnel preparation

After circular detachment, the access hole is rounded and extended to a diameter of 5 mm. Its position is always located at the most inferior aspect of the alveolar recess to facilitate the laterobasal tunnelling.

Creation of the SAS: The SAS is created by tunnelling the sinus membrane with elevators of 0-, 45-, and 90-degree angulation under tactile control with the osseous basal floor. Primarily the membrane at the laterobasal angle of the sinus floor is detached in an anteroposterior direction. The detachment ended 5 mm dorsal to the projected most distal implant site. If necessary, the complete sinus floor is tunnelled this way. The tunnel then is extended at its medial and superior aspects.

The instruments have to be guided continuously in close contact with the bone to avoid tension or perforation of the sinus membrane, particularly if irregularities of the sinus floor or difficult anatomy are present. In case of septa or irregular shape of the sinus floor, endoscopic exploration helps to lead the elevators along the basal limits of the bony maxillary wall. The tunnel size depends on the height and volume planned for the augmentation and implants. Enough space has to be provided to place the graft material without tension on the sinus membrane.

Endoscopic Control of the SAS

After detachment of the sinus membrane, the subantral space is examined via the access trepanation using the

70-degree and 30-degree endoscopes. The examination includes circular identification of the boundaries of the SAS and inspection of the entire sinus membrane forming the roof of the SAS for perforations or tears.

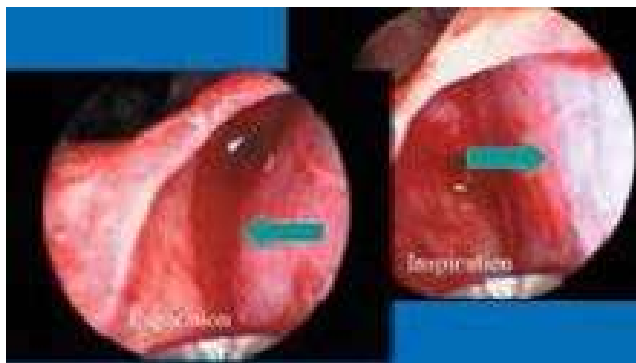


Microsurgical access to the subantral space.



Subantrosopic examination of the subantral space.

(Left) Normal appearance of the sinus membrane representing the roof of the artificial subantral space. (Right) Twist drill (Oraltronic, Bremen, Germany) entering the subantral space.



Sinus membrane examination. During respiration, alternating movement of the sinus membrane is

observed, giving evidence of the absence of perforations



Stepwise augmentation. Augmentation material is placed first at the distal and proximal ends of the subantral space and is controlled endoscopically before the periapical Spaces around the implants are filled.

If a perforation of the sinus membrane is detected, immediate repair is performed using polyglactine mesh (Vicryl, Ethicon). Finally, the length, height, and width of the subantral space are measured.

Preparation of Implant Cavities: Primary implant cavity preparation is carried out if primary Stability of the implants could be achieved. Within the subantral space, the sinus membrane is protected with elevators, while the basal bone is perforated with the implant burs. The implant cavity has to be surrounded by at least 5 mm of SAS to allow the membrane to tent up adequately during augmentation.

Endoscopically Controlled Stepwise Augmentation: The first portion of the augmentation is placed at the most distal part of the SAS. The desired “tenting up” of the membrane is checked endoscopically before covering the mesial aspect of the most distal implant with augmentation material. Proceeding from the distal extreme toward the entrance access hole, the inter implant spaces and periapical spaces around the implants are subsequently covered, with intermittent endoscopic control. Before the implant is placed adjacent to the access trepanation, the most mesial (anterior) aspect of the SAS is filled with

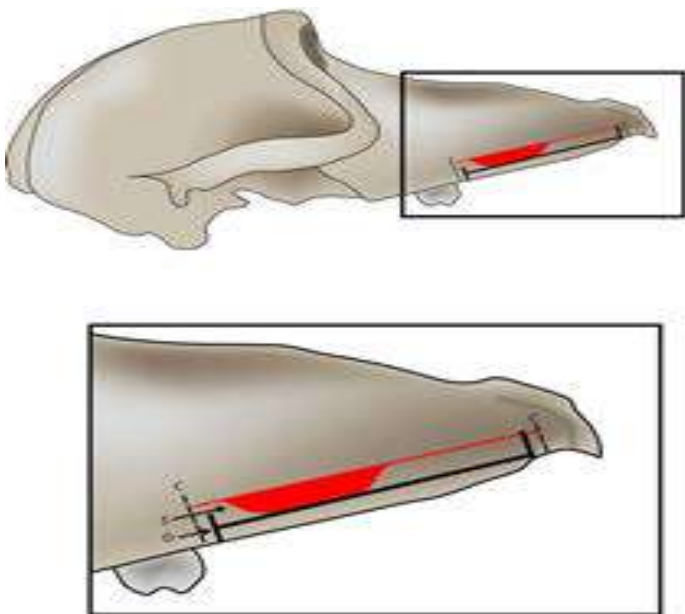
augmentation material. The entrance keyhole is then covered with a mucoperiosteal flap.

Primary or Secondary Implant Placement: Implants are placed primarily, if primary Stability could be achieved. In the absence of primary Stability or if the bone structure is obviously insufficient, secondary placement is carried out after at least 6 months of healing time. (11). From October 1999 to December 2000, of 92 sinus floor elevations, 18 were carried out endoscopically controlled with an osteotome technique. As augmentation material, -tricalcium phosphate (-TCP) or autogenous bone was used; 22 implants were placed. With the ECOSFE, (Endoscopically controlled osteotome sinus floor elevation) perforations of the sinus membrane can be visualized; however, they cannot be avoided. Although this technique is less invasive than the lateral window technique, it cannot be recommended as a standard procedure in the posterior maxilla because of the large amount of additional equipment needed and the technically demanding procedure. (12). Sinus floor augmentation has become a routine procedure with predictable results. Flapless implant placement is recommended for a series of indications with sufficient bone volume. Flapless surgery in the atrophic maxilla is presented as a refinement of the endoscopic Subantrosopic laterobasal sinus floor augmentation (SALSA) technique. Subantral space is augmented using the SALSA technique without raising a mucoperiosteal flap. Implants are placed transgingivally without raising a mucoperiosteal flap, with endoscopic control of the cover screw at the bone level. In a case series of 6 patients, 21 implants were placed and augmented simultaneously. The mean augmentation height was 10.7 mm (range, 5.7 to 16.6 mm); the mean residual bone height was 5.1 mm (range, 1.9 to 12.1 mm). Complications such as insufficient primary Stability and sinus membrane

perforation were treated without changing to an open surgical approach. (13).

Boyne's distraction osteogenesis technique

2004 Distraction osteogenesis is a biomechanical process of bone tissue formation, where the distraction forces which act between the bone segments affect the biological potential of the bone by forming a callus of determined length and height. Distraction osteogenesis is preceded by Corticotomy or sub-periosteal osteotomy and followed by fixation of the distractor on the segments and their gradual lengthening. Codvilla, in 1905 was the first to perform extremity lengthening by the application of external traction. The credit for popularizing the technique worldwide goes to Gavril Ilizarov, a Russian orthopaedic surgeon. In the late 1980s he published in America for the first time his research and clinical results on the bone distraction, causing a wave of developments in the bone distraction technique worldwide. Snyder et al., 1973 were the first to apply the Ilizarov principles of distraction osteogenesis is for the regeneration of the osteotomized mandible. (17).



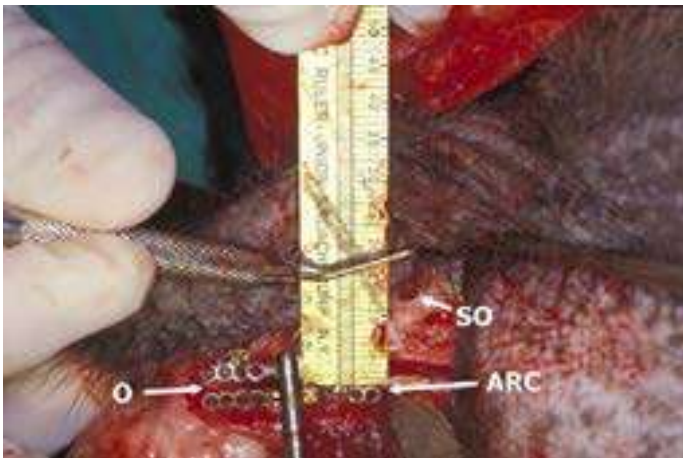
A diagrammatic representation of a Papio anubis dry specimen skull outlining the area of the osteotomy (O) for the DO in the maxillary canine, molar, and

premolar region. (The outline of the maxillary sinus is shown) (S). (C) indicates areas of vertical cuts made to obtain biopsies for control maxillary alveolar bone to compare with the DO regenerated area. (Bottom) A higher power view of the surgical site is shown.

In 2004 Boyne PJ et al have applied this distraction osteogenesis technique to increase height of the maxillary sinus floor. In this animal study the posterior maxilla's of 3 adult Papio Anubis baboons are rendered edentulous from the lateral incisors anteriorly, to the third molars posteriorly; and alveolectomies are performed to simulate post-extraction alveolar ridge atrophy. After 12 weeks of uneventful healing, osteotomies are performed bilaterally in the edentulous areas with the horizontal section extending from the lateral incisor area to within 5 mm of the mesial surface of the retained and fully erupted third molar tooth. The superior extent of the osteotomy section is located at the level of the sinus and nasal floors in the midposterior portion of the bony section. The location of the horizontal osteotomy for the DO is calibrated by making a small 3 mm osteotomy opening in the lateral wall of the nose and sinus. A periodontal probe is inserted between the nasal-antral membrane and the bony wall of the nasal sinus and extended down to the osseous floor. By this method of measurement, it is possible to determine how much bone remained between the sinus floor and the alveolar crest so that the osteotomy cut could be made according to the design of the study. The osteotomy cut is made to leave anteriorly and posteriorly approximately 1 to 1.5 mm of alveolar bone height to accept the screws for the distractor plate. The midposterior portion of the osteotomy is fashioned to actually interdict the floor of the antrum for a distance of approximately 8 to 10 mm in antral floor.



Intraoperative view of the Surgical site



A view of a small osteotomy (SO) made in the lateral wall of the maxilla and the insertion of a measuring probe by which it is possible to determine the height of the nasal and antral osseous floor remaining in the maxilla. By this method, it is possible to direct the periapical maxillary osteotomy section (arrow) to interdict the floor of the antrum in the mid posterior portion of the osteotomy cut. The nasal and antral mucosa was elevated from the inferior portion of the sinuses by a small curette placed through this osteotomy opening. Note the thin Alveolar ridge crest (ARC).

Fixation of the base plate portion of the DO device is obtained by placing the screws superior to the sinus floor into the lateral antral-nasal wall. The transport segment of the device containing the residual crest of the alveolar ridge is only 1 to 1.5 mm in height, which is minimally sufficient to accept the screws of the transport portion of

the DO device. After a latency period of 7 days, the distractor (12 mm; KLS Martin, Jacksonville, FL) is activated at the rate of 1mm per day for 10 days. The postoperative course is uneventful⁽¹⁸⁾.



A view of the positioning of the distractor which is being guided by the marking probe in the sinus wall. The screws securing the inferior transport segment are engaging the thin alveolar crest which measures only 1 to 2 mm in height below the osteotomy. The thinness of the alveolar crest (transport segment) (ARC) and the attachment of the base plate at the lateral maxillary wall are visualized.

Complications

Complications may be encountered during and after maxillary sinus elevation procedures. Surgical procedures involving bone grafting and implants in the maxillary sinus have potential complications that can be specific or non-specific for these procedures. For the sinus graft, perforations of the Schneiderian membrane are the main intraoperative complication, which occurs in 7% to 35% of the procedures. Postoperative complications are less common and consist mostly of acute or chronic sinus infection, bleeding, wound dehiscence, exposure of barrier membrane and graft loss. Intraoperative complications may lead to post-operative complications. Surgical complications did not significantly influence implant survival. When performing sinus augmentation, bone substitute materials are just as effective as autologous bone, whether used alone or in combination with autologous bone. Implant surface treatments can have an important

effect on implant survival, and it would appear that roughened surfaces are the best option. When implants are inserted simultaneously to grafting, a higher failure rate can be expected. In 100% of cases, the AAA (Alveolar antral artery) was found to be partially intra-osseous, that is, between the Schneiderian membrane and the lateral bony wall of the sinus, in the area selected for sinus antrotomy. Hence a sound knowledge of the maxillary sinus vascular anatomy and its careful analysis by CT scan is essential to prevent complications during surgical interventions involving this region.

Conclusion

An inadequate bone quantity and quality have been considered for many years as absolute contraindications for implant-supported rehabilitation. The risk of implant failure in the posterior maxilla is generally high because of the low bone density and the progressive ridge resorption caused by edentulism. Implant treatment in the atrophic posterior maxilla must be carefully planned and may require a pre-prosthetic surgical intervention of bone grafting. Maxillary sinus floor augmentation is often performed to create conditions adequate for implant placement. The implant success rate and the predictability of the maxillary sinus augmentation procedure depend on numerous factors. However, because of the improvement of surgical techniques and the progress of research in the field of biomaterials, excellent outcomes have been reported in the last years. Recent systematic reviews of the literature have demonstrated that sinus floor augmentation procedure is well documented with an overall implant survival rate well beyond 90%.

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Original Article

Effect of autoclaving on the cyclic fatigue resistance of nickel-titanium rotary instruments: An *in vitro* study

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Abstract

Aim: The aim of the study is to determine the effect of autoclaving on the cyclic fatigue resistance (CFR) in respect to three different rotary nickel-titanium (NiTi) systems.

Methods: Three rotary NiTi files with different manufacturing process were selected and arranged into three groups. Group 1: Neolix Neoniti files ($n = 15$), Group 2: Edge Files ($n = 15$), Group 3: NeoendoFlex files ($n = 15$). Each group was then subdivided into three subgroups containing 5 samples each based on the number of cycles of autoclaving (0, 1, 5 cycles). The instruments were subjected to autoclave without usage. The files of size 25, taper 6%, and 25 mm length were taken. After the autoclaving cycles, the files were rotated in cyclic fatigue testing device with simulated canal curvature of 60° until file separation. Time taken for the instrument separation (in minutes) was multiplied by the number of rotations per minute to attain the mean number of cycles to failure for every file. The obtained results were subjected to statistical analysis using two-way ANOVA and Newman-Keuls multiple *post hoc* procedures.

Results: Neolix Neoniti files exhibited the maximum CFR (98.80) than EdgeFiles (80.47) and Neoendo Flex files (41.80) ($P = 0.0001$). Reduction in CFR was observed with increase in the number of cycles of autoclaving (i.e., 0, 1, 5 cycles).

Conclusion: Autoclaving has a negative influence on the CFR of the rotary NiTi files used. Neolix Neoniti files exhibited greater CFR than EdgeFiles and Neoendo Flex files.

Keywords: Autoclaving; cyclic fatigue; EdgeFiles; Endodontic rotary file; Neoendo Flex files; Neolix Neoniti files; nickel-titanium

INTRODUCTION

In 1988, Walia suggested the use of nickel-titanium (NiTi) alloy named as nitinol in the manufacturing of rotary instruments owing to its superior mechanical properties such as shape memory and superelasticity.^[1,2] These properties of NiTi alloys are responsible for the austenitic to the martensitic phase transformation. Either the process

of cooling or the application of stress causes phase transformation in the NiTi alloy and is thermoelastic in nature. The mechanical properties and the transformation temperatures of Ni-Ti alloys are strongly dependent on their chemical composition and thermomechanical processing. A thorough knowledge of the physical properties and limitations of endodontic files can reduce undesirable procedural errors, like file separation in the canal, transportation, and strip perforation.^[3]

Cyclic fatigue is due to numerous cycles of tension and compression that eventuate when a file rotates in the

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curved canals. The rotary files tend to fracture at a certain point when it reaches its flexural limit.^[3] Most rotary NiTi endodontic instruments are produced by grinding. Some of the rotary NiTi files are produced by twisting the alloy after it had been subjected to heat treatment. Literature had revealed many studies regarding the effects of operating rate, instrument design, surface treatments, root canal irrigants upon the cyclic fatigue resistance (CFR) of rotary NiTi files. Possible strategies to improve the CFR comprises either improving the process of manufacturing or utilize novel alloys having excellent properties. Thermal processing methods optimize the microstructure of the NiTi alloy, thus affecting the CFR of the instruments.^[4-7]

Accurate determination of CFR requires utilization of natural root canals. However, after usage the root canal anatomy will change and the samples cannot be reused. Thus, it becomes difficult to standardize the experimental setting. Consequently, several equipments such as glass and metal tubes were used for determining CFR. Nonetheless, there is no international laboratory standard for determining the CFR of rotary NiTi files.^[8,9]

According to the manufacturers, rotary NiTi files are single use. However, in clinical settings, these are frequently reused after sterilization. After multiple uses, surface defects such as pitting, strip formation, microfractures/microcrack formation, and disruption of cutting edges are apparent on the instrument surface. Upon further use, the instrument may fracture.^[10]

Zinelis *et al.* stated that heat treatment of the apical 5-mm portion of a NiTi rotary instrument can extend its fatigue life by reducing its residual strain. Furthermore, NiTi files have more CFR when subjected to heat treatment at 430°C which results in superior elastic behavior and provides better shaping ability. When the instrument is heat treated at 600°C, the NiTi alloy undergoes martensitic and R-phase transformation. At this temperature, due to the recrystallization of NiTi alloy, decrease in the surface hardness will be evident. Hence, the heating temperature is an essential aspect closely related to the performance of NiTi alloy.^[11]

Literature search showed that autoclaving has effect on the behavior of rotary NiTi files with varying conclusions. Some researchers concluded that autoclaving negatively influences the physical properties of rotary NiTi files and causes files to fail prematurely. Other researchers have reported that autoclaving for 10 times did not impact the fracture predilection of rotary NiTi files.^[3,12] The unique properties of NiTi alloy such as superelasticity and shape memory are greatly influenced by the manufacturing procedure of the file.^[8,11] Yahata *et al.* had stated that the flexibility of the rotary NiTi instruments might improve when they are subjected to heat retreatment during

autoclaving.^[13] Sundaram *et al.* have suggested that sterilization temperatures above 125°C could reverse the NiTi deformation caused during clinical use.^[10]

Three different NiTi file systems were selected based on the criteria that they were produced by different manufacturing process and of different cross-sections. Neolix Neoniti files have nonhomogenous rectangular cross-section and are manufactured using wire-cut electric discharge machining (WEDM) process.^[14] The EdgeFile system has parabolic cross section and is manufactured using proprietary annealed heat treatment forming a branded fire wire.^[15] Neoendo Flex files have triangular cross section and are subjected to gold thermal treatment.^[16]

The main objective of the present research is to estimate whether autoclaving process has any influence on the CFR of newly introduced heat-treated rotary files, i.e., Neolix Neoniti, EdgeFiles, and Neoendo Flex file systems.

METHODS

Sample size calculation

The sample size was estimated using the G*power, version 3.1.9.4 (Franz Fauluniversitat, Kiel, Germany). Sample size was estimated with α -error is 5% and power of the test is 80% or 20% β -error with effect size of 0.6. Hence, it was calculated as 15 subjects in each group.

Three groups of rotary NiTi instruments of identical tip size of 0.25 mm and 6% taper were taken: Group 1-NeolixNeoniti files (Chatres-la-Forêt, France), Group 2-EdgeFile X3 (EdgeFile, Albuquerque, New Mexico, USA), Group 3-Neoendo Flex files (Orikam, India). Each file group consists of 15 instruments. It was further divided into three subgroups containing five instruments, each based on the number of autoclaving cycles, i.e., Subgroup A-no autoclaving, Subgroup B-one cycle of autoclaving, and Subgroup C-five autoclaving cycles. Autoclaving process was carried out at a temperature of 121°C for 15 min duration, 15lb pressure, which is followed by 15 min of drying. Finally, all the files were tested for CFR. A customized equipment was used for determining CFR. This equipment incorporates a stainless steel block with simulated canals [Figure 1]. The mounted handpiece enables correct positioning of every file into the simulated canal. Each file was tested in the customized artificial canal having 60° curvature angle and 5 mm of curvature radius. Each file was used at a rate of 300 rpm in a 16:1 contraangle reduction handpiece activated by a torque modulated endodontic motor (X Smart Endomotor, Dentsply Maillefer, Switzerland) till file separation had taken place. The time taken for the file separation was noted utilizing the stopwatch and video camera.



Number of cycles to failure (NCF) = Time taken for file separation (In minutes) × Number of rotations per minute.

Statistical analysis

Mean values of NCF and its standard deviations were evaluated for the samples. Two-way ANOVA test was used to calculate significant differences among the groups. Once the analysis revealed a significant difference, Neuman–Keuls *post hoc* test was done to calculate the mean differences (with $P < 0.05$). The data analysis was carried out utilizing SPSS 20.0 version software (IBM, Bangalore, Karnataka, India).

RESULTS

Higher NCF is observed in Group 1 (98.80) when compared to others. Least NCF is observed in Group 3 (41.80) (Graph 1). Mean NCF in Group 2 is 80.47. Two-way ANOVA comparison [Table 1] showed that the mean NCF is dissimilar in different groups which is statistically significant ($P = 0.0001$). The mean NCF of three subgroups (A, B, C) was also dissimilar which is statistically significant ($P = 0.0002$). However, there is no statistically significant difference in the interaction effect of three main groups (1, 2, 3) and three subgroups (A, B, C) with respect to NCF.

Pairwise comparisons of groups [Table 2] revealed a significant difference between Groups 1 and 2 ($P = 0.0211$),



Figure 1: Cyclic fatigue testing machine with mounted handpiece

Groups 1 and 3 ($P = 0.0001$), Groups 2 and 3 ($P = 0.0001$). The mean NCF is in the order of Group 1 >2>3. Pairwise comparison of subgroups revealed a significant difference only between Subgroup A and C ($P = 0.0262$). A nonsignificant difference was observed between Subgroups A and B ($P = 0.1105$) and Subgroups B and C ($P = 0.2895$).

DISCUSSION

The main purpose of autoclaving rotary files is to eliminate the microbial contamination, thereby increasing the chance for the success of root canal therapy.^[12] Several clinicians choose having fixed sets of particular files. However, all these files might not be used during the treatment. Therefore, the rotary files that have not been utilized are also exposed to multiple cycles of autoclaving. Majority of the studies in the literature had analyzed the influence of frequent autoclaving on the rotary NiTi file systems.^[2,17,18]

Autoclaving has a negligible influence on the physical properties of stainless steel instruments. Silvaggio and Hicks concluded that autoclaving of rotary NiTi files did not raise the probability of file fracture.^[10,19] Dissimilarities in the CFR among rotary NiTi files are owing to the differences in many factors such as the process of manufacturing, operational features, file design, characteristics, canal curvature, and the method of fatigue failure testing. Methods to improve the longevity of the NiTi instruments include the following: (i) thermal treatments before machining; (ii) appropriate machining conditions of the NiTi alloy; and (iii) electro-polishing.^[11]

According to Aminsobhani M *et al.*, the Austenite start temperatures of Neolix Neoniti file were in a manner that at the mouth temperature, the austenite phase existed. Neoniti reveals R-phase transformation.^[20] The austenite-finish temperatures of Neoniti were higher than human body temperature, therefore, material was in the phase transformation from martensite to austenite, gives the instruments more flexibility when used in the clinical situation.^[21] The EdgeFile files and Neoendo files are predominantly in austenite phase at room temperature.

In the present study, Neolix Neoniti files showed the highest CFR than EdgeFiles and Neoendo Flex files. It could be due

Table 1: Comparison of three main groups (1, 2, 3) and three subgroups (A, B, C) with mean number of cycles to failure by two-way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	P
Main effects					
Main groups	2	25,401.11	12,700.56	41.6124	0.0001 (S)
Sub groups	2	6574.71	3287.36	10.7708	0.0002 (S)
Two-way interaction effects					
Groups x subgroups	4	846.22	211.56	0.6931	0.6016 (NS)
Error	36	10,987.60	305.21		
Total	44	43,809.64			

S: Significant, NS: Nonsignificant

Table 2: Comparison between three main groups (1, 2, 3) and three subgroups (A, B, C) with mean number of cycles to failures by Newman-Keuls multiple *post hoc* procedures

Groups	Group 1	Group 2	Group 3	Sub Group A	Sub Group B	Sub Group C
Mean	98.80	80.47	41.80	89.40	71.67	60.00
SD	30.45	16.54	10.68	36.02	25.73	26.46
Group 1	-					
Group 2 (P)	0.0211*	-				
Group 3 (P)	0.0001*	0.0001*	-			
Sub Group A				-		
Sub Group B (P)				0.1105	-	
Sub Group C (P)				0.0262*	0.2895	-

*Significant. SD: Standard deviation

to differences in the manufacturing process of the three file groups. Neolix Neoniti files have a nonhomogenous rectangular cross-section, and it has multiple tapers in a single instrument. It is manufactured using the WEDM process. This manufacturing process improves the flexibility and shape memory of this system.

These observations are in compliance with previous studies.^[14,22] Neolix Neoniti files showed highest CFR than RaCe, Mtwo, Twisted file, and ProTaper Next X2 files.^[22] This may be due to the unique manufacturing process and heat treatment of this file. WEDM process creates an electrical discharge which produces sharp cutting ends, changing variable profiles, and flexibility in the rotary NiTi file systems.

Rubio *et al.* compared the cyclic fatigue resistance of 10 different types of endodontic files and concluded that the instruments manufactured with CM wire technology, i.e., Hyflex EDM and Neoniti were superior to other endodontic files, and there was no statistically significant difference between Hyflex EDM and Neolix files.^[23]

EdgeFiles have better CFR when compared to Neoendo Flex files. This can also be imparted to the manufacturing difference between the two file systems. The EdgeFile system has a parabolic cross-section. This system undergoes proprietary annealed heat treatment, forming a branded firewire that increases NiTi instruments' flexural strength and flexibility.

Adigüzel *et al.* evaluated the CFR of EdgeFile X3 files with One Curve, One Shape, and 2Shape files *in vitro*. They concluded that EdgeFile system showed highest CFR than the other groups tested. EdgeFile system was manufactured using Firewire technology that is responsible for its improved CFR over the other file systems.^[24]

According to Tanomaru-Filho *et al.*, the heat treatment CM that is applied to the ProDesign and HyFlex CM systems contributed positively to the best results presented in relation to instruments without heat treatment (MTwo) or with FireWire heat treatment (EdgeFile).^[15] Neoendo Flex files exhibited minimum CFR in the present study. The

current results are in accordance with the research done by Mahajan *et al.*^[16] They stated that Neoendo Flex files exhibited less CFR than One curve files. Other reason for better CFR of Neolix Neoniti files could be attributed to its rectangular cross section which contributes to bulk of the instrument when compared to parabolic cross section of edge file and triangular cross section of Neoendo files.^[14]

It is also observed that, after every autoclaving cycle, there was a marked reduction in CFR of rotary NiTi files. Hilfer *et al.* inferred that frequent autoclaving markedly reduced the NCF of size 25, taper 6% Twisted Files.^[12] Nair *et al.* conducted an atomic force microscopy and proposed that the roughness on the file surface became more pronounced with numerous autoclave cycles.^[25] Limitation of the present study is that the endodontic files were rotated in a stainless steel simulated canal. However, the files may behave differently when instrumented in a natural tooth. Hence, the results may slightly vary in the *in vivo* settings. Lubricating agent was not used in the present study. Hence, the effect of the presence of lubricating agents on the CFR of these files also needs to be evaluated.

CONCLUSION

This study concluded that Neolix Neoniti files, which were manufactured using EDM technology, exhibited highest CFR compared to EdgeFiles and Neoendo Flex files. Reduction of CFR in the rotary NiTi files was observed with an increase in number of cycles of autoclaving.

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Conflicts of interest

There are no conflicts of interest.


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Gayatri, *et al.*: Autoclaving and fracture resistance of rotary instruments

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Management of Bilateral Maxillary Second Molars with Extra Palatal Root: A Case Report

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ABSTRACT

A crucial understanding of teeth' internal and external anatomy is critical for the successful outcome of endodontic therapy. The presence of two palatal roots in maxillary molars, particularly in the second molar, is rare. The present case report addresses this anatomical variation in an 18-year-old female patient with a chief complaint of severe pain in the upper left back teeth region since one week. Preoperative radiographic evaluation of maxillary second molar did not reveal the aberrant anatomy due to its location and impaction of maxillary third molar onto the roots of the second molar. After access opening, inspection of the pulp chamber floor revealed a slightly deviated location of the palatal canal and a bleeding point mesial to the palatal canal. Intensive exploration for determining any additional canals was done using David Green (DG) 16 endodontic explorer under 3× magnification using dental loupes. A narrow field of view Cone Beam Computed Tomography (CBCT) was obtained to evaluate the unusual root canal morphology. Following a thorough analysis of CBCT, root canal treatment was performed employing a standard protocol. This case report highlights the successful diagnosis and management of the bilateral maxillary second molar with two separate palatal roots. With CBCT and magnification, determining the root morphology and canal configuration will be more accurate, further improving the quality of root canal treatment.

Keywords: Cone beam computed tomography, Maxillary molars, Magnification, Root morphology, Root canal therapy

CASE REPORT

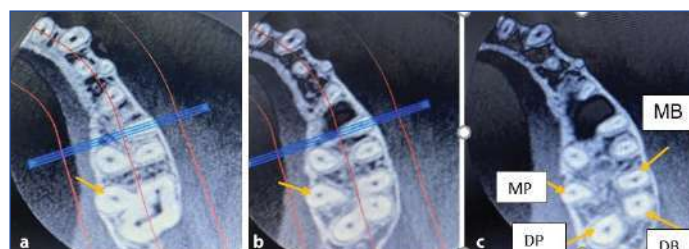
An 18-year-old female patient with a non contributory medical history presented to the Department of Conservative Dentistry and Endodontics with the chief complaint of pain in her maxillary left back tooth region for one week. The pain was intermittent throughout the day, non radiating, aggravated by taking cold, hot foods and on mastication. The patient gave a history of previously initiated root canal therapy from a private dental center one week back. Due to the Coronavirus Disease (COVID) pandemic, the previous clinician was unavailable and hence she visited our institute. Clinical examination revealed a tooth restored with temporary filling with respect to 27, tender on percussion with no associated swelling. Pulp vitality testing of the involved tooth showed no response to electronic pulp tester (SybronEndo, Orange, CA) and cold (Endofrost, coltene). Preoperative radiographic evaluation revealed an access cavity filled with radioopaque restorative material with a slight widening of periodontal ligament space with respect to 27.

Hence, a diagnosis of previously initiated root canal therapy with symptomatic apical periodontitis was established based on clinical and radiographic findings [Table/Fig-1]. Informed consent was taken and the tooth was anaesthetised with posterior superior alveolar nerve block using 2% lignocaine (Astra Zeneca Pharma India Limited, Bangalore, India) and isolated with a rubber dam (Coltène/ Whaledent, OH, USA). After temporary restoration removal, pulp chamber revealed three canals and a bleeding spot in the floor of the pulp chamber mesial to the palatal canal and was assumed as perforation. The Intraoral Periapical (IOPA) radiograph of the concerned tooth did not offer any conclusive information. Inspection of the chamber floor for determining any additional canals was done using DG 16 endodontic explorer (Hu-Friedy, USA) under 3X magnification using dental loupes (Daray, Derbyshire). Intensive exploration revealed a catch at the bleeding point. A sterile cotton pellet was placed inside the pulp chamber, and the access cavity was sealed with Intermediate Restorative Material (IRM) (Caulk, Dentsply, USA).

A CBCT (Carestream/Trophy, Marne-la-Vallée, France) of the partial arch was taken with exposure parameters of 120 kV and 5.0 Ma to evaluate whether it is an extra canal or an extra root and also to study its anatomy. An ultra-low radiation dose scan was done using a focused field of view. The images were reconstructed at 0.2 mm thickness increments. Axial view of CBCT with respect to 27 showed the presence of an additional palatal root [Table/Fig-2a-c].

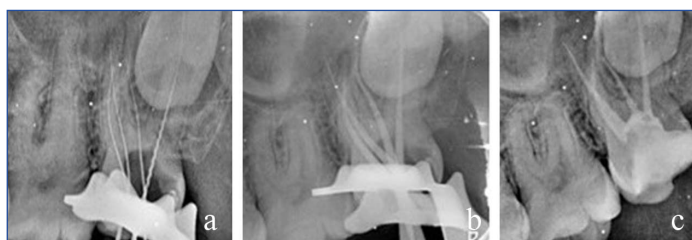


[Table/Fig-1]: Preoperative radiograph in which access opening has been done #27 with arrow mark depicting widening of periodontal ligament space at the periapex.



[Table/Fig-2]: a,b&c) Transverse section of CBCT showing four roots with four canals at a) cervical, b) middle, c) apical third respectively # 27 (MB mesiobuccal DB distobuccal MP mesio-palatal DP disto-palatal respectively).

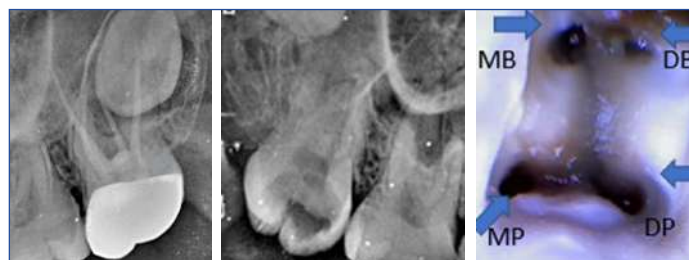
Tooth 27 had four canals with four roots mesiobuccal, distobuccal, mesio-palatal, distopalatal. Initial instrumentation was performed using a 10 k-file (Mani, Inc, Tochigi, Japan). Coronal enlargement was done with Hyflex Electrical Discharge Machining (EDM) orifice opener (Coltene- Whaledent, Allstetten, Switzerland) to obtain straight-line access. Each canal's working length was determined using an electronic apex locator (Root ZX, J Morita Mfg. Corp., Japan) and confirmed radiographically with an IOPA [Table/Fig-3a]. Glide path was established with 10/ 05 Hyflex EDM file ((Coltene-Whaledent, Allstetten, Switzerland) followed by which canals instrumented with Hyflex EDM rotary files till 25/~ (Coltene-Whaledent, Allstetten, Switzerland). Copious irrigation of the canals was done with 5ml of 3% NaOCl (Sodium Hypochlorite) (Vensons India, Bengaluru, India) and 5ml of 17% Ethylene Diamine Tetra-acetic Acid (EDTA) (Prime dental Pvt., Ltd., Thane) liquid between each file. With each irrigation flush of NaOCl and EDTA, sonic agitation of irrigant was done using an endoactivator (Dentsply Sirona) for one minute. Distilled water (Milli-Q water system, Millipore Corporation, Bedford, MA, USA) was used as the final irrigant. Root canals were dried with paper points and obturated with corresponding 25 size gutta-percha (Coltene-Whaledent, Allstetten, Switzerland) and AH plus sealer in the same visit (Dentsply, Konstanz, Germany) [Table/Fig-3b]. Finally, the tooth was restored with a bulk-fill composite restoration (EverX Posterior (EP)/GC Europe [Table/Fig-3c]. At one month follow-up, due to financial constraints and less interocclusal clearance, cast metal crowns were delivered for the patient. After one year, the clinical and radiographic follow-up revealed that the patient was asymptomatic and periapical tissues were healthy [Table/Fig-4].



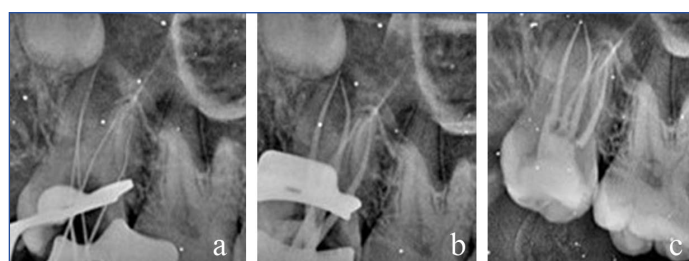
[Table/Fig-3]: a-c) Radiograph showing working length of all four roots #27, Master cone placement #27, Postoperative radiograph of maxillary second molar restored with bulk fill composite #27 respectively.

As the same patient was asymptomatic with relation to 17 during the initial visit and due to financial constraints, she deferred the endodontic treatment. However, she developed severe pain in the upper right back tooth region of the jaw after ten days. The patient complained of lancinating pain, which was intermittent, non radiating, aggravated on mastication, cold foods, and relieved on taking medication, and there was no associated swelling #17. The clinical examinations revealed deep occlusal caries wrt. maxillary right second molar which was tender on percussion [Table/Fig-5]. Vitality testing of the involved tooth showed lingering pain to cold test and premature response to electric pulp tester (SybronEndo, Orange, CA). Based on the findings, a diagnosis of symptomatic irreversible pulpitis with symptomatic apical periodontitis was made. The root canal treatment procedure was explained, and informed consent was taken. Preoperative radiographic evaluation revealed atypical root canal anatomy. Careful periodontal probing from mesial to distal side on the palatal aspect revealed a concavity on palatal surface and the probe dipped in the centre of palatal surface indicating a furcation. On either side of this concavity, two contours were appreciated hence the clinical examination confirmed two palatal roots. The tooth was anaesthetised with a posterior superior alveolar nerve block with 2% lignocaine (Astra Zeneca Pharma India Limited, Bangalore, India) and isolated with a rubber dam. Following access opening, based on the clinical experience gained from the previous tooth #27 and slightly deviated location of the palatal canal, the access cavity was modified from conventional triangular

to trapezoidal shape to achieve straight-line access for all canals [Table/Fig-6]. The second palatal canal orifice was explored with the aid of a DG-16 explorer (Hu- Friedy, USA), and its presence was confirmed with a dental operating microscope at 5X magnification (Labomed Dental Microscope Prima DNT, USA). It was found mesial to the usual location of the palatal canal [Table/Fig-8]. The mesio-palatal root showed moderate apical curvature towards the distal root, while the distopalatal root was relatively straight. The distance between the two palatal canal orifices was more trapezoid formed upon joining the imaginary lines connecting the four orifices was wider on the palatal side and narrower on the buccal side. The same treatment protocol was followed as in case report one [Table/Fig-7a-c]. After one year, the clinical and radiographic follow-up revealed that the patient was asymptomatic and periapical tissues were healthy [Table/Fig-8].



[Table/Fig-4]: A one year follow-up radiograph. **[Table/Fig-5]:** Preoperative radiograph depicting wide occlusal caries with pulpal involvement #17. **[Table/Fig-6]:** Clinical image under dental operating microscope. (Image from left to right)
MB: Mesio-buccal; DB: Distobuccal; MP: Mesio-palatal; DP: Disto-palatal



[Table/Fig-7]: a-c) Working length determined Radiographically #17, Master cone placement #17, Obturation followed by postendodontic restoration #17.



[Table/Fig-8]: A one year follow-up radiograph.

DISCUSSION

Successful root canal treatment depends on thorough biomechanical instrumentation and three-dimensional obturation of the entire pulp space. Undetected extra roots or canals are recognised as a significant reason for the failure of root canal treatment [1,2]. Maxillary second molars have the most complicated root canal systems [3]. Kim Y et al., reported that in maxillary second molars

4.63% had one root, 10.72% had two roots, 82.7% had three roots, and 5.6% had four roots [4]. Four rooted molars with double palatal roots were reported to occur in 1.1 to 1.5% of maxillary second molars [5,6]. The bilateral occurrence of this phenomenon has also been documented [7].

In the present case, owing to the anatomical location of maxillary second molars and impaction of the third molar onto the roots of second molars, the unusual anatomy was not appreciable on a radiograph. Therefore, the concept of the narrow field of view CBCT provided a three dimensional (3D), low-radiation/high-resolution solution to assist successful endodontic diagnostic and treatment problems in case of variations in tooth anatomy [8,9].

With regard to tooth no 27, the extra root was successfully diagnosed and treated with the assistance of CBCT. Matherne RP et al., researched the benefit of CBCT imaging in endodontic treatment; they concluded that CBCT images always identified a greater number of root canal systems than digital images [10].

Purushotham M and Sahoo H treated a maxillary molar with the aid of a dental operating microscope and careful exploration of the pulp chamber with ultrasonic tips. Upon locating a bleeding point CBCT was taken to rule out any further complex anatomy similar to the present case report [11].

CBCT slices revealed four distinct roots with four canals. Moreover, mesiobuccal and distobuccal canals were relatively closer than the mesiopalatal and distopalatal canals. Thus, the box formed by joining the imaginary lines connecting the four orifices was wider on the palatal side, giving a trapezoidal shape [12]. A similar wider location of palatal orifices than palatal orifices was observed by Alani AH, Patel S and Patel P [7,13] Carlsen O and Alexandersen V classified these teeth according to the side of the crown with which the accessory palatal root is associated [14]. Hence according to this classification, this case belongs to radix mesiolingualis- the accessory root has an affinity to the pronounced mesiopalatal part of the crown. According to Ahmed HMA et al., the root canal configurations are depicted as 427 Mesio-buccal (MB)1 Disto-buccal (DB)1 Mesio-palatal (MP)1 Disto-palatal (DP)1, 417 MB1 DB1 MP1 DP1, respectively [15].

Not all scenarios need CBCT imaging. Al Qahtani A et al., published a case report describing the retreatment of maxillary second molars with two palatal roots. As the preoperative radiograph revealed four roots, they performed retreatment based on anatomic guide lines [16]. By relying on the experience and use of magnification, such variations could be treated successfully. The same was followed in case of tooth 17. Magnification aids like dental operating

microscopes and loupes help to locate anatomic aberrations better by improving visualisation [13]. Thus precise access cavity and diagnostic aids such as dental operating microscope eliminate many potential problems such as missing additional canals and provide a better insight into root canal anatomy.

CONCLUSION(S)

Any variations in root canal morphology can be identified with the recent innovations in diagnostic and operating aids. Anatomical deviations can occur in any tooth; therefore, clinicians should anticipate these variations and utilise all the available diagnostic tools to manage them.

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Effect of nonthermal atmospheric plasma on the shear bond strength of composite resin after using different tooth-whitening systems: An *in vitro* study

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Abstract

Aim: We aimed to evaluate the effect of nonthermal atmospheric plasma (NTAP) on the bond strength of composite resin after using different tooth-whitening systems.

Methods: Eighty maxillary central incisors ($n = 80$) were divided into two groups based on the tooth-whitening procedure used – Group 1: bleaching (Pola Office, USA), Group 2: microabrasion (Opalustre, Australia). The samples were subdivided into four groups depending on the surface treatments – Group A: control (no surface treatment), Group B: plasma, Group C: antioxidant (sodium ascorbate), and Group D: buffering agent (sodium bicarbonate). After the surface treatments, the specimens were stored in artificial saliva for 24 h and composite resin was bonded to the labial surface of the teeth perpendicular to the long axis. The samples were then subjected to shear bond strength test under the universal testing machine.

Statistical Analysis: The results received from shear bond strength analysis were subjected to statistical analysis using a two-way ANOVA test, independent *t*-test, and Tukey's multiple *post hoc* tests. The *P* value set was <0.05 .

Results: The highest mean bond strength value was seen in Group 2B (NTAP treatment after microabrasion) followed by Group 1B (NTAP treatment after bleaching). Mean shear bond strength values have suggested a statistically significant difference between all the other groups ($P < 0.05$) except between Groups 1A, 2A and 1D, 2D.

Conclusions: Within the limitations of the current *in vitro* study, bleaching and microabrasion followed by surface treatment using NTAP showed the highest bond strength than other groups.

Keywords: Bleaching, bond strength, microabrasion, nonthermal atmospheric plasma

INTRODUCTION

Esthetics play a vital role both socially and professionally among individuals of all age groups, thus increasing the demand for cosmetic dentistry.^[1] Some intrinsic and extrinsic enamel defects may reduce the quality of smile.^[2]

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Various treatment methodologies have been employed to overcome those problems and achieve a pleasing smile, which include bleaching, microabrasion, ceramic or composite veneers, as well as crowns.^[3] Bleaching and microabrasion have gained popularity, being conservative technique to improve the appearance of discolored teeth.^[4] Bleaching of tooth surface involves the release of oxygen, resulting in the oxidation of the pigments causing stains.^[5] On the other hand, microabrasion removes the surface stains with an abrasive/acid compound-containing

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paste of 18% hydrochloric acid and flour of pumice, which involves an insignificant and unrecognizable loss of enamel.^[6,7] Immediate or delayed composite veneering or restoration is considered as the next minimally invasive treatment in certain clinical scenarios when the above tooth-whitening procedures do not give a satisfactory result. Despite being conservative approaches, bleaching and microabrasion resulted in the alteration of surface topography of enamel that might reduce the bond strength of direct or indirect veneers.^[11] Several researchers have postulated the presence of active chemicals or free radicals as one of the reasons for reduced bond strength followed by bleaching.^[8] Surface treatment either with a biocompatible and neutral antioxidant such as sodium ascorbate or a buffering agent before bonding may reverse this effect and neutralize the acidic pH.^[9,10] In the past few years, nonthermal atmospheric plasma (NTAP) has been widely experimented in biomedical and dental applications, attributed to its ability in altering the surface characterization and enhancing the adhesion.^[11]

The current study evaluated the effect of NTAP on the bond strength of composite resin after using different tooth-whitening systems.

METHODS

Specimen preparation

Eighty intact permanent maxillary central incisors, freshly extracted due to periodontal diseases, were selected for the present study. Preference was given to teeth with intact crowns regardless of the root structure. The sample size was calculated using Raosoft online^[12] (<http://www.raosoft.com/samplesize.html> accessed on December 2019) sample size calculator, and the power of the study set was 80%. The teeth with visible fractures, cracks, teeth not stored in any media, and teeth with developmental defects were excluded from the study. The collected teeth were stored in distilled water containing 0.2% thymol solution (Chemigens Research and Fine Chemicals, Mumbai, India). The teeth were then embedded in self-cure acrylic (DPI products, India) resin blocks till cemento-enamel junction. The labial enamel surfaces of all samples were cleaned using a prophylactic paste (DPI Propol, India) with a rubber cup attached to slow-speed handpiece (Yoda Greentech, Gujarat, India) to remove any surface debris.

Nonthermal atmospheric plasma jet apparatus

Compressed helium gas with a pulse head of 2 GHz at 3.25 standard liter was used to generate the plasma jet. The delivery pressure was maintained <2 pascals with a mean power of 2 Watt. Each application was carried for a total period of 15 s in the installments of 5 s each with a rest period of 5 s in between. The distance between the

applicator tip and the tooth surface was maintained at 5 mm. All the parameters were controlled by a microwave generator-connected module (Acxys Smart Systems, Germany). The temperature changes at the enamel surface were monitored using a thermocouple and found to be in the range of 27°C–29.4°C which is well tolerated by dental pulp.^[13] All the plasma exposures were done in an isolated chamber to prevent any possible environmental contamination.

Experimental procedure

The teeth were randomly divided into two main groups depending on the tooth-whitening system: Group 1 – bleaching and Group 2 – microabrasion. In Group 1, the labial surface of the teeth was blot dried using blotting paper. Contents from the Pola Office syringe (SDI limited, Australia) containing 35% hydrogen peroxide were released into the pot containing bleach powder (73.26% thickeners, 26.2% catalysts, 0.04% dye, and 0.5% desensitizing agents) and mixed immediately using a brush applicator until the gel is homogeneous. A uniform layer of gel was applied to the labial surface of the teeth and left for 6–8 min [Figure 1a] and was later rinsed off using distilled water.

In Group 2, a fine grit (TC-11EF/20–30 μm) water-cooled diamond bur (Mani, Inc., Japan) was used to superficially abrade the labial surface in two to three gentle strokes to initiate microreduction. A plastic White Mac Tip (Ultradent, USA) was attached to the Opalustre syringe and a uniform layer was applied to the labial surfaces. A slow-speed handpiece (Yoda Greentech, Gujarat, India) was used at 250 RPM, with the Opal prophycups (Ultradent, USA) [Figure 1b] directly on the enamel surfaces for 60 s and later rinsed off with distilled water.

Both the groups were further divided into four subgroups depending on the surface treatment performed:

- Group A: Control group – No surface treatment was done (1A and 2A)
- Group B: Plasma group – The labial surface of the samples was subjected to NTAP for 15 s [Figure 1c] (1B and 2B)
- Group C: Antioxidant group – The labial surface of the teeth was treated with 10% sodium ascorbate liquid (Avi Chem Industries, India) for 30 s by applying gentle pressure using a brush and then rinsed off [Figure 1d] (1C and 2C)
- Group D: Buffering group – The labial surface of the teeth was treated with 10% sodium bicarbonate liquid (Avi Chem industries, India) for 30 s by applying gentle pressure using a brush and then rinsed off [Figure 1d] (1D and 2D).

All the specimens were stored in artificial saliva for 24 h at 37°C in an incubator (Nabertherm lab incubator, India).

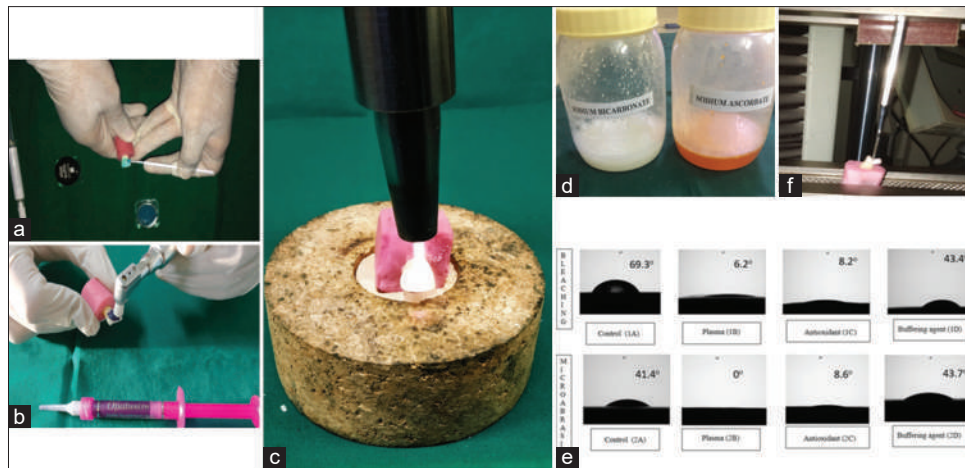


Figure 1: (a) Bleaching on the labial surface of tooth (Pola Office). (b) Microabrasion on the labial surface of tooth (Opalustre). (c) Plasma treatment on the labial surface of tooth. (d) Antioxidant solution and buffering solution. (e) Contact angle measurement. (f) Instron machine testing for shear bond strength

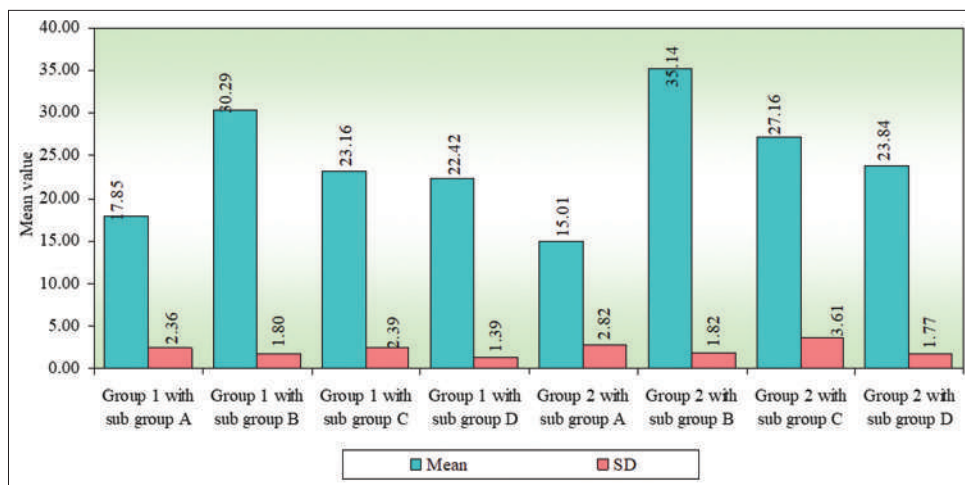


Figure 2: Comparison of interactions of two main groups (1 and 2) and four subgroups (A, B, C, and D) with shear bond strength

Contact angle measurement

Six of the samples with near-flat enamel surface from each group were selected and contact angle has been evaluated with the help of a goniometer (Rame-Hart Contact angle goniometer). Bidistilled water was used as the contact medium in the process [Figure 1e].

Composite restoration

Self-etch adhesive (Single Bond Universal adhesive, 3M) was applied on the labial enamel surfaces of all the above specimens as suggested by the manufacturer using an applicator tip. The first coating of the bonding agent was applied, left for 10 s, and was air-dried gently to allow the evaporation of any excess material. The second coat of bonding agent was applied in the same manner and photo-polymerized (LED Curing Light, Woodpecker – 5W high power blue light LED, 420–480 nm, 650 mW/cm²–800 mW/cm²). A custom-made cylindrical plastic matrix of 2 mm diameter and 2 mm height was used

as a mold to pack the composite material. The plastic mold was placed on the labial surface of the tooth perpendicularly followed by incremental layering (1 mm each) of composite material using a plastic filling instrument (Ivoclar, USA). Each increment was photo-polymerized for 20 s. After polymerizing, the plastic matrix was separated carefully. All the specimens were stored in artificial saliva for 24 h at 37°C in an incubator (Nabertherm lab incubator, India) to simulate the oral environment after the composite restoration.

Shear bond strength test

After completion of incubation, the shear bond strength of composite to the tooth surface was determined using a Universal Testing Machine (Instron Universal Testing Machine, Model 8801). A chisel-edge plunger was mounted onto the movable crosshead of the testing machine with the leading edge aimed at the composite and labial surface interface at a crosshead speed of 1 mm/min. The force required to de-bond the restorative material was

Table 1: Comparison of interactions of two main groups (1 and 2) and four sub groups (A, B, C, D) with shear bond strength by Tukeys multiple posthoc procedures

Groups	Group 1 with sub group A	Group 1 with sub group B	Group 1 with sub group C	Group 1 with sub group D	Group 2 with sub group A	Group 2 with sub group B	Group 2 with sub group C	Group 2 with sub group D
Mean	17.8460	30.2866	23.1562	22.4222	15.0090	35.1395	27.1649	23.8410
SD	2.3598	1.8023	2.3935	1.3914	2.8181	1.8227	3.6096	1.7697
Group 1 with sub group A	-							
Group 1 with sub group B	$P=0.0001^*$	-						
Group 1 with sub group C	$P=0.0002^*$	$P=0.0001^*$	-					
Group 1 with sub group D	$P=0.0011^*$	$P=0.0001^*$	$P=0.9968$	-				
Group 2 with sub group A	$P=0.1371$	$P=0.0001^*$	$P=0.0001^*$	$P=0.0001^*$	-			
Group 2 with sub group B	$P=0.0001^*$	$P=0.0005^*$	$P=0.0001^*$	$P=0.0001^*$	$P=0.0001^*$	-		
Group 2 with sub group C	$P=0.0001^*$	$P=0.0722$	$P=0.0064^*$	$P=0.0007^*$	$P=0.0001^*$	$P=0.0001^*$	-	
Group 2 with sub group D	$P=0.0001^*$	$P=0.0001^*$	$P=0.9979$	$P=0.8745$	$P=0.0001^*$	$P=0.0001^*$	$P=0.0437^*$	-

measured, and the shear bond strength was calculated by dividing the peak load values and the restorative material base area (N/mm^2) [Figure 1f].

RESULTS

The results obtained were then analyzed statistically using the Statistical Package for the Social Sciences (SPSS) (SPSS software version 20.0; SPSS Inc., Chicago, IL, USA). Data were analyzed using a two-way ANOVA test for overall significance, independent *t*-test, and Tukey's multiple *post hoc* tests for inter- and intragroup comparison, respectively. The highest mean bond strength values were seen in Group 2B (microabrasion followed by plasma), followed by Group 1B (bleaching followed by plasma). The lowest mean bond strength values were seen in Group 2A and Group 1A [Table 1 and Figure 2]. Mean shear bond strength values have suggested a statistically significant difference between other groups ($P < 0.05$) except between Groups 1A, 2A and 1D, 2D.

DISCUSSION

Plasma is portrayed as the fourth state of matter and is extremely a reactive material comprising strong electric field, radicals, and charged particles.^[14] NTAP generated at atmospheric pressure, when used as an adjuvant increased the outcome of the bonding technique. For the above reasons, it has recently gathered attention not only in industrial sector but also in the medical and dental fields.^[15]

Esthetic reconstructions, laminate and composite veneers, are often necessary after the tooth-whitening procedures.^[16] Tittley *et al.* explained the primary mechanism of bleaching as ionic dissociation of hydrogen peroxide and increased formation of free radicals such as nascent oxygen and the hydroxyl radical on the enamel surface.^[17] Although bleaching has proven to be a safe and effective treatment, it may present specific antagonistic effects on the enamel, such as surface morphological changes, compositional changes, alterations in the surface microhardness, and surface roughness.^[18] Furthermore, there is evidence

that tooth bleaching led to a reduction in bond strength of composite resin applied to previously bleached enamel.^[17] Glasspole *et al.* stated that the reduced bond strength was due to the prevention of resin infiltration and polymerization because of oxygen-free radicals.^[19] Various additional surface treatments after subjecting the tooth to tooth-whitening procedures were indicated lately to reverse the effect of such methods on the bonding properties of the tooth.^[20]

It was found that NTAP increased the bond strength at the dentin-composite interface by 60%.^[21,22] This interface-bonding enhancement was reported to significantly improve composite performance, durability, and longevity.^[22] In the current study, a significant increase in the bond strength was seen in the groups that were subjected to plasma irrespective of the tooth-whitening procedure used. Plasma enhances the hydrophilicity of the tooth surface, surface energy, and wettability and changes the chemical structure of exposed collagen fibrils by temporarily uncovering the functional groups and partially dispersing them.^[23] In case of dentin, it has been proven that the surface modification plays a lesser role compared to alteration of the surface chemistry due to the presence of higher organic content and more collagen fibrils. In the present study, since the evaluated area is mostly on the enamel, alteration of surface properties seems to play a major role than surface chemistry which is evident by reduced contact angle. Plasma-treated samples have shown the lowest contact angle compared to all the study groups^[23] [Figure 1e].

When NTAP was used along with hydrogen peroxide, increased molecular breakdown and superior bleaching ability were observed.^[24,25] However, there is minimal or no literature evaluating the effect of plasma on the bond strength after treating the tooth surface with bleaching or microabrasion. The present study evaluated the same.

It was found that application of plasma after microabrasion significantly improved the bond strength compared to plasma treatment after bleaching. The superior bond

strength might be due to the nonselective etching, enamel demineralization, and increased surface roughness by the aggressive hydrochloric acid. The further application of plasma enhanced the adhesive-tooth bond strength.^[26] However, the efficacy of plasma is reduced due to the residual free radicals after bleaching resulting in inferior bond strength compared to microabrasion.^[19]

Several authors postulated that the application of antioxidants on the bleached tooth surface caused the reversal of detrimental effects.^[27-29] In the current study, the use of sodium ascorbate showed increased bond strength values compared to the control group similar to a previous study conducted by Kaya *et al.*^[30] This might be due to the potential of antioxidant agents to prevent the interference of residual radicals with the polymerization of adhesives, which in turn showed the improvement of the bond strength of resin composites to bleached enamel.^[31,32] After the bleaching procedure, increased free radicals are effectively reduced by antioxidant treatment resulting in better bond strength compared to the control group. However, after the microabrasion procedure, the released free radicals are minimal and also the available collagen content is more compared to bleached tooth surface due to the greater surface depth. Further attributed to the collagen-cross-linking ability of the antioxidants, in the current study, the bond strength of the composite in Group 2C (microabrasion and antioxidant) is greater than Group 1C (bleaching and antioxidant).

On the other hand, various studies evaluated the effect of surface treatment using a buffering agent on the bleached enamel and its influence on composite resin bonding.^[10] It was reported that there was an improvement in the bond strength values after using a buffering agent. The outcomes of the present study are in conjunction with the previous literature stating that the application of a 10% sodium bicarbonate solution after bleaching procedure could be an alternative prerestorative treatment.^[33] The mechanism of action of sodium bicarbonate built on its high pH (approximately 8.67) can disrupt the peroxide molecule and result in its decomposition and inactivation.^[10] Buffering agent in combination with microabrasion performed better than bleaching. This is evident as there is no much reduction in the pH after bleaching procedures as compared to microabrasion revealing the effectiveness of buffering agent. However, there is no statistically significant difference in the bond strength between both the groups (Group 1D and 2D).

The plasma-treated group showed the best results which could be due to the improvement in the interface properties and modification of the tooth surface, thus increasing the tooth/adhesive interfacial bonding. Still, further studies are required to clinically evaluate the effect of plasma on

bond strength, microabrasion, and bleaching in the near future. It is of valuable interest to know if the application of plasma after using antioxidant or buffering agent enhances the bonding further in the upcoming studies.

CONCLUSIONS

Within the limits of the study, it can be concluded that

1. Irrespective of the tooth-whitening procedure performed, surface treatment using plasma showed a significant increase in the mean bond strength values
2. No significant difference in the mean bond strength values was seen between bleaching and microabrasion without any surface treatment
3. Application of sodium ascorbate and sodium bicarbonate showed an increase in mean bond strength values compared to control groups.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Effect of nonthermal atmospheric plasma on the push-out bond strength of epoxy resin-based and bioceramic root canal sealers: An *in vitro* study

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Abstract

Aim: The aim of this study was to evaluate the effect of nonthermal atmospheric plasma (NTAP) on the bond strength of epoxy resin-based and bioceramic root canal sealers.

Materials and Methods: Freshly extracted forty ($n = 40$) single-rooted mandibular premolar teeth were divided into four groups ($n = 10$) based on the sealer used and NTAP application – Group 1: Epoxy resin-based sealer (AH Plus) without NTAP application, Group 2: Epoxy resin-based sealer (AH Plus) with NTAP application for 30 s, Group 3: Bioceramic sealer (BioRoot RCS) without NTAP application, and Group 4: Bioceramic sealer (BioRoot RCS) with NTAP application for 30 s. After NTAP application in Groups 2 and 4, all the samples were obturated using sealers according to their grouping protocols. Two-millimeter slices were obtained from each sample using hard tissue microtome, which were subjected to push-out bond strength (PBS) under the universal testing machine. Data were subjected to statistical analysis using one-way analysis of variance followed by the Post hoc tukey test. The level of statistical significance was set at $P < 0.05$.

Results: The PBS values were observed to be significantly higher in bioceramic sealer with NTAP application (Group 4) followed by epoxy resin-based sealer with NTAP application (Group 2).

Conclusion: NTAP application enhanced the PBS of bioceramic (BioRoot RCS) and epoxy resin-based (AH Plus) sealers compared to their control groups.

Keywords: AH Plus sealer; bioceramic sealer; nonthermal atmospheric plasma; push-out bond strength

INTRODUCTION

Successful endodontic treatment is dependent on the meticulous cleaning of the root canal system, the eradication of pathogenic microorganisms, and a three-dimensional filling of root canal space with an inert root canal filling material and attaining a fluid tight seal to prevent the ingress of microorganisms

from the oral cavity and its spread to the periapical tissue.^[1]

Root canal sealers are used as a thin tacky paste which functions as a lubricant and luting agent during obturation allowing the core obturation material such as gutta-percha or other rigid materials to slide in and forms a fluid tight seal within the root canal. Sealers can fill voids, lateral canals, and accessory canals where core obturation materials cannot infiltrate. If the sealer does not perform its function, microleakage may cause endodontic treatment failure through the clinically undetectable passage of bacteria, fluids, molecules, or ions between the tooth and

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Garlapati, *et al.*: Effect of NTAP on bond strength of root canal sealers

the restorative material. A great variety of endodontic sealers are available commercially, and they are divided into different groups according to their chemical composition.^[2]

Traditionally used root canal sealers are zinc oxide eugenol, calcium hydroxide, and glass ionomer-based sealers. Newer root canal sealers such as epoxy resin and bioceramic based are being developed to provide the improved properties. AH Plus sealer (Dentsply Maillefer, Ballaigues, Switzerland) is an epoxy resin-based sealer which contains diepoxide, calcium tungstate, N, N-dibenzyl-5-Oxanonane-diamine-1,9 TCD-diamine-1-adamantane amine, and zirconium oxide. AH Plus is a widely used sealer for root canal filling due to its acceptable physical properties, low solubility, disintegration, apical sealing ability, good adhesion, antimicrobial action, and good biological properties.^[3] Baechtold *et al.* evaluated the adhesion capability of two sealing cement, namely MTA Fillapex and AH Plus, and reported that AH Plus sealer has greater adhesion than the MTA Fillapex.^[4]

Bioactive endodontic sealers have been developed to improve the quality of root canal obturation. BioRoot RCS (BC) (Septodont, St. Maur-des-Fosses, France) is the newest development of a bioceramic sealer. The powder mainly consists of tricalcium silicate, povidone, and zirconium dioxide. The liquid is an aqueous solution of calcium chloride with polycarboxylate. Contemporary studies on BC sealer have documented its several adequate characteristics, including its adhesive property, low dimensional change, proper radiopacity, and low solubility.^[3]

Adhesion and penetration are two important aspects to be considered in sealer selection. Studies have shown that bond strength and sealer penetration may be affected by pretreatment of root canal walls and by the type of sealer used. These procedures change the physical conditions of dentin by removing the smear layer, thereby opening the dentinal tubules and increasing the wettability of root canal dentin.^[5]

Recently, the possibility to generate nonthermal atmospheric plasma (NTAP) has enabled extending plasma applications to the treatment of root canal surfaces. The interest in plasma applications in dentistry is due to its several unique advantages over conventional approaches, such as the ability to penetrate into small and irregular root canal recesses and achieve bacterial decontamination without using potentially dangerous chemicals.^[6] NTAP has improved the bond strength between dentin and adhesive materials.^[7]

Previous studies have demonstrated that NTAP was effective and efficient in sterilization of endodontic instruments, other surgical instruments, elimination of persistent,

nonaccessible biofilms, sterilization, and thorough disinfection of root canals. Raymond E. J. *et al.* used a plasma needle to observe the interactions between NTAP and dental tissues and concluded that plasma treatment allows cleaning of irregular and narrow channels and it is a novel tissue-saving technique. NTAP was an effective source of free radicals and have the property of eradicating microbial disinfection without causing destruction of the tissue. Whittaker *et al.* suggested that sterilization of surgical instruments with NTAP decreases the cross contamination during endodontic treatment.^[8]

Lu *et al.* used a reliable and user-friendly plasma jet device, which could generate NTAP, and it is directed into the root canal for disinfection without causing any painful sensation.^[9] Pan *et al.* investigated the feasibility of using NTAP in root canals infected with *Enterococcus faecalis* biofilms and concluded that NTAP was highly efficient in disinfecting the *E. faecalis* biofilms during root canal treatment.^[10]

To the best of our knowledge, there are no studies in the literature reporting the effect of NTAP on the bond strength of epoxy resin-based (AH Plus) and bioceramic (BioRoot RCS) root canal sealers. Hence, the main purpose of the present *in vitro* study was to evaluate and compare the effect of NTAP application on the push-out bond strength (PBS) of epoxy resin-based and bioceramic root canal sealers.

MATERIALS AND METHODS

Specimen preparation

Freshly extracted forty ($n = 40$) single-rooted mandibular premolar teeth were selected. Crowns were decoronated below the cemento-enamel junction using a low-speed hand piece, so that the lengths of all roots were adjusted to 15 mm. Patency of each root canal was checked using a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) and working length was established 1 mm short of the apex.

Cleaning and shaping procedures were performed using ProTaper Gold (Dentsply Sirona, USA) rotary nickel-titanium instruments. Canals were irrigated with 2 mL of 5.25% sodium hypochlorite (NaOCl) (Prime Dental Products, Maharashtra, India) between each file change followed by irrigating with 2 mL of 17% ethylenediaminetetraacetic acid (MD-Cleanser, META Biomed, Korea) to remove the smear layer formed after instrumentation. Root canals were finally flushed with 5 mL of distilled water and dried with paper points. All the samples ($n = 40$) were divided into four groups ($n = 10$):

- Group 1 ($n = 10$): Epoxy resin-based sealer (AH Plus) without NTAP application
- Group 2 ($n = 10$): Epoxy resin-based sealer (AH Plus) with NTAP application

- Group 3 ($n = 10$): Bioceramic sealer (BioRoot RCS) without NTAP application
- Group 4 ($n = 10$): Bioceramic sealer (BioRoot RCS) with NTAP application.

Nonthermal atmospheric plasma application

After cleaning and shaping of samples in Group 2 and Group 4, they were subjected to NTAP application. This was conducted at the Department of Chemistry, Indian Institute of Space Science and Technology, Thiruvananthapuram, Kerala. A glass reactor was used for NTAP application. This glass reactor consists of a 5-cm diameter and 30-cm long tube, evacuated by a mechanical pump, down to pressures lower than 2 Pa. Gas was allowed to fill the glass reactor up to a pressure of 10 Pa. NTAP was generated within the glass cylinder under vacuum by the action of an induced magnetic field from the current passing through an electrical coil surrounding the cylinder. Surfaces of the samples were treated using helium–argon gas at 60 W for 30 s. At the end of the process, radiofrequency was turned off before the samples were exposed to air.

In the control and experimental groups (after NTAP application), all the samples were obturated using the single-cone technique with size 25/.08 gutta-percha points (Dentsply Maillefer, Ballaigues, Switzerland) using AH Plus in Group 1 and Group 2 and BC sealer in Group 3 and Group 4, respectively. The roots were mounted on the self-cure acrylic resin cylinders and were subjected to hard tissue microtome (SRM Institute of Science and Technology, Kattankulathur, Chennai) to obtain 2-mm slices from the middle third of the root from each sample.

Push-out bond strength test

The obtained 2-mm slices were subjected to PBS test, and loading was performed using a universal testing machine (Instron Servohydraulic Testing System, Mechanical Department, GITAM, Visakhapatnam). The samples were placed on a base of a metal slab of universal testing machine to allow the free movement of the plunger. The compressive load was applied by exerting a downward pressure on the surface of the test material in each sample, with the Instron probe moving at a constant speed of 1 mm/min. The plunger size of 1 mm had a clearance of approximately 0.2 mm from the margin of the dentinal wall to ensure contact only with the test materials. The maximum force which was applied to materials at the time of dislodgement was recorded in newtons. The PBS in megapascal (MPa) was calculated by dividing this force (N) by the surface area of the test material where $N/2p \times r \times h$, p is the constant 3.14, r is the root canal radius, and h is the thickness of the dentin slice in millimeters.

Statistical analysis

Data were entered in MS-Excel and analyzed in Version 21.0 (IBM Corp. Chicago, USA). Shapiro–Wilk test was applied to find the normality. Descriptive statistics were represented with mean and standard deviation (SD). 95% confidence intervals were calculated. One-way analysis of variance, followed by Post hoc tukey test, was applied to find significance. $P < 0.05$ was considered as statistically significant.

RESULTS

Table 1 shows the mean values and SD of the PBS (MPa) of all the groups. Results shown that NTAP-applied samples, i.e., Group 2 and Group 4, had a statistically significant increase in bond strength, more than twice that of their respective control groups, i.e., Group 1 and Group 3 ($P < 0.05$). Bioceramic sealer (BioRoot RCS) showed significant bond strength values compared to AH plus sealer without NTAP application ($P < 0.05$).

DISCUSSION

The aim of endodontic therapy is not only elimination of microorganisms by cleaning and shaping of the root canal but also to ensure that the root canal system to be fluid free and that a single-block configuration is created that seals hermetically the root canal space. The major function of a root canal sealer is to fill imperfections and increase adaptation of the root canal filling material to the canal walls, failing which the chances of leakage and failure increase.

The ability of the PBS test is to evaluate the bonding strength surpasses that of other tests because it generates parallel fractures in the interfacial area of dentin bonding. However, a limitation of the PBS test is that it creates nonuniform stress distribution. In the present study, this limitation was prevented using 2-mm thick slices.^[3]

Root canal disinfection using NTAP can be considered as a special type of decontamination because of its importance in the endodontic procedures for a successful outcome. It has been reported that bacteria can enter dentinal tubules as deep as 500–1000 μm . NTAP has a capability of reaching deep into the complex root canal, i.e., up to 800 μm . NTAP creates reactive oxygen species, causing serious damage to microorganisms through irreversible oxidation of cell components. There is an unique advantage of direct contact with the bacteria using NTAP, which is not possible with conventional methods.^[11]

With respect to NTAP application, bioceramic sealer (Group 4) showed higher bond strength than epoxy

resin-based sealer (Group 2). Lehman *et al.*^[6] reported that without NTAP application, the intertubular areas appear slightly roughened on etched dentin, smear plugs were removed from the tubules, and the collagen network is visible within the tubules and in the intertubular areas. With NTAP application, no collagen fibers are visible on this surface and the orifices of dentinal tubules appear irregularly enlarged, which results in the better penetration of root canal sealer into the dentinal tubules and could ensure higher mechanical retention of the root canal filling materials. This might be the possible reason for the increased PBS values of Groups 2 and 4 twice that of control groups, i.e., Group 1 and Group 3, in the present study.

The application of NTAP into the root canal walls provides a better bond strength while using bioceramic-based root canal sealers for obturation procedure.^[12] Hence, NTAP can be used as an adjuvant during endodontic treatment to increase the adhesion property of root canal sealers which might result in a successful outcome.

In the present study, compared to epoxy resin-based sealer (Group 1), bioceramic sealer (Group 3) showed higher bond strength. This may be attributed to the true self-adhesive nature of bioceramic sealer, which forms a chemical bond (through the production of hydroxyapatite during setting) with dentin. Moreover, it is hydrophilic and possesses low contact angle allowing it to spread easily over the root canal walls providing adaptation and good fluid tight seal.^[13] The results are in accordance with a previous study done by Ana Carrillo-Varguez AG *et al.* (2017)^[14] who reported that BC sealer with single-cone technique performed better adhesion than AH Plus to root canal dentin.

Srivastava *et al.* from their study observed that BioRoot RCS showed better bond strength to root dentin compared to AH Plus after using different irrigating solutions. BioRoot RCS showed better bond strength to root dentin compared to AH Plus probably due to setting reaction of the bioceramic-based sealer. It absorbs water from the dentinal tubules and forms calcium silicate hydrogel and hydroxyapatite compound. The hydroxyapatite which is present in the sealer undergoes a continuous process of crystal growth and binds chemically with the dentin. Being resin free, it is capable of flowing into the dentinal tubules with no shrinkage and provides excellent adhesion

to dentin and gutta-percha and its ability to seal auxiliary canals.^[15] A study done by Dayanand Chole *et al.*^[3] reported that BioRoot RCS showed the highest bond strength than AH Plus and MTA Fillapex due to its true self-adhesive nature. According to Han and Okiji,^[16] the formation of a tag-like structure was suggested to be responsible for the BC sealer sealing ability and their bond strength to dentin.

Sagsen *et al.* (2011)^[17] evaluated the bond strength of two new calcium silicate-based and AH Plus endodontic sealers and found that AH Plus sealer had higher PBS values than MTA Fillapex because of the better flow and sealing ability of AH Plus sealer. In a study by Kumar *et al.*, results revealed that the dislocation resistance of AH Plus to root canal dentin was significantly decreased by the use of NaOCl as a final irrigant. Reversal of the compromised PBS and also improved adhesion of epoxy resin-based sealer (AH Plus) to NaOCl-treated dentin was significantly increased when proanthocyanidin and bamboo salt were used as final irrigating solutions. Epoxy resin-based sealers (AH Plus) produces a better seal in the root canal, as they do not shrink, rather they expand and seal the root canal.^[18]

In a study done by Huang *et al.*, bioceramic root canal sealers have shown excellent flow and appropriate film thickness, as well as favorable properties, including high calcium ion release, proper radiopacity, low dimensional change, and low solubility. From their observations, they concluded that BC sealers showed superior wetting and adhesion properties to the root canal dentin. BC sealers having excellent flow would obturate irregular spaces and penetrate into dentinal tubules, thus enhancing the seal between the root canal surface and sealers, this might contribute to the increased bond strength value of BC sealer in the present study.^[19]

As per the literature, the current *in vitro* study is the first one to evaluate and compare the effect of NTAP on the PBS of epoxy resin-based (AH plus) and bioceramic (BioRoot RCS) root canal sealers. However, this being an *in vitro* study, it cannot mimic the *in vivo* situation. The application of the NTAP in an *in vivo* condition is yet to be evaluated. For NTAP to reach the clinical use, future research activities should involve increasing numbers of dentists and move towards animal testing; on this path, assessing the safety of NTAP treatments and employing plasma sources that are realistically applicable in a clinical environment will be inevitable steps.

Table 1: Mean push-out bond strength value of four different experimental groups

Group	n	Minimum	Maximum	Mean	SD	P
GROUP 1	10	1.90	4.31	3.40	0.72	<0.001
GROUP 2	10	4.94	7.24	6.13	0.84	
GROUP 3	10	3.25	4.62	3.90	0.45	<0.001
GROUP 4	10	8.43	9.67	9.04	0.41	

CONCLUSION

Within the limitations of this *in vitro* study, NTAP application significantly increased the PBS values of epoxy resin-based (AH Plus) and bioceramic (BioRoot RCS) root canal sealers. Bioceramic sealer with and without NTAP

application has shown higher PBS values than that of epoxy resin-based sealer.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

Effect of indirect ultrasonic activation of modified bioceramic materials on the bond strength and tubular penetration in root canals

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ABSTRACT

Background: To maintain the integrity of the interface between root canal filling and radicular dentin an ideal endodontic biomaterial should have good adhesion. This study was aimed to evaluate the bond strength and tubular penetration of modified bioceramic materials by indirect ultrasonic activation.

Materials and Methods: In the present *in vitro* experimental study, 120 coronal root slices of 2 mm were prepared from mandibular premolars and randomly divided into six groups ($n = 20$) in accordance to placement techniques: Group I: Nano Biodentine-manual, Group II: CaCl_2 modified ProRootMTA-manual (MM), Group III: Biodentine-manual, Group IV: Nano Biodentine: Ultrasonic, Group V: CaCl_2 modified ProRootMTA-ultrasonic, and Group VI: Biodentine-ultrasonic (BDU). The samples were kept in humidifier for 4 days at 37°C and push out bond strength, sealer penetration were evaluated using an universal testing machine and confocal laser scanning microscope respectively. Data were subjected to statistical analysis using SPSS software by using One-way ANOVA for overall significance and Tukey's multiple *post hoc* test for intergroup comparison ($P < 0.05$).

Results: Highest push out bond strength and greater tubular penetration were observed with Group VI (BDU), while the lowest bond strength and tubular penetration were associated with Group II (MM).

Conclusion: Within the limitations of current study it was observed that Biodentine with indirect ultrasonic activation has resulted in highest pushout bond strength among all the study groups.

Key Words: Confocal laser scanning microscopy, mineral trioxide aggregate, nanomaterials, tricalcium silicates

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INTRODUCTION

Endodontic materials should be resistant to dislocating forces such as functional pressure or condensation for good long term prognosis. Since past decade there has been a greater utilization of bioceramic materials in endodontics. Mineral trioxide aggregate (MTA), a calcium silicate based hydraulic cement (CSC) is widely used for perforation repair,

root-end filling, pulpotomy, apexification and regenerative procedures. MTA has several desirable properties such as biocompatibility, superior sealing ability, and the ability to set in the presence of moisture and wet environment. However, MTA possesses some notable shortcomings such as long

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setting time, poor immediate washout resistance and difficult handling property. In few recent studies, to improve the physicochemical properties of MTA, the addition of calcium chloride (CaCl_2) to MTA^[1] with the concentration of 2%–15% has been proposed^[1] to reduce the setting time of the material^[2,3] with similar biocompatibility.^[4]

Biodentine (Septodont, Saint Maur des Fosses, France) is a more recent CSC with improved physical properties and reduced setting time as compared to MTA.^[1] Biodentine powder is mainly composed of tricalcium silicate, calcium carbonate (filler material) and zirconium oxide (radiopacifier), whilst the liquid consists of CaCl_2 (used as a setting accelerator) and a hydro soluble polymer (water-reducing/super plasticizing agent). This biomaterial is considered as a biocompatible and bioactive dentine substitute and has been indicated for coronal and radicular restorations.^[1]

Nanotechnology can be beneficial in producing and constructing advanced biomaterials with exclusive biological, chemical and physical properties.^[5] In present study Nano Biodentine (NBD) has been included to evaluate its adhesive properties compared to Biodentine.

Indirect Ultrasonic agitation has been proposed as an effective mode of increasing the packability and density of endodontic materials as well as the compressive strength of hydraulic cements.^[6]

Till date, a little information is available on the bond strength and adhesive properties of CaCl_2 modified MTA and NBD to radicular canal dentin with the indirect ultrasonic activation. This current study evaluates the push out bond strength of CaCl_2 modified MTA and NBD with manual placement and indirect ultrasonic activation techniques.

MATERIALS AND METHODS

An *in vitro* experimental study was conducted on recently extracted human mandibular premolars with single root, extracted due to periodontal and orthodontic considerations between July and September 2018 at department of conservative dentistry and endodontics, SIBAR institute of dental sciences, Takkellapadu, Guntur, and Andhra Pradesh, India. Institutional ethical clearance was obtained. The sample size was calculated using Raosoft online sample size calculator and the power of study set was 80%. The criteria for teeth selection included the

presence of single rooted teeth with canal curvature between 5° and 10° , the absence of any micro cracks on dentinal walls under stereomicroscope at 4X magnification, absence of internal and external resorption or calcification and with complete formation of root apex. Each tooth was radiographed both buccolingually and mesiodistally to detect any possible calcifications. After a thorough screening, a total of 120 samples were considered for the inclusion in this *in vitro* study.

Specimen preparation

The collected single rooted human mandibular premolars were decoronated at the cemento-enamel junction with a diamond disc (Wuxi xiangsheng industrial and trading co.) mounted on a micro motor (NSK Ti-max Nao 95LS) under water coolant. A total of 120 samples were selected, which were sectioned perpendicular to the long axis at mid coronal third of root to attain dentin discs of 2mm thickness using Isomet precision cutter (Buehler Inc). While selecting, these discs were gauged with a customized acrylic gauge for a standardized internal diameter. The root sections were immersed in 17% EDTA (Vista Dental Products, U.S.A) followed by water rinse and then in 2.5% sodium hypochlorite (PrevestDenpro Dental Products, India) each for 1 min to remove the smear layer. They were then cleansed with distilled water in an ultrasonic bath. Later, the root canal surfaces of the samples were dried with a dehydrating solution using absorbent points (Hydrol, septodont).

Experimental procedure

The root sections were randomly assigned into 6 groups:

- Group I: Nano Biodentine-manual (NBDM) ($n = 20$)
- Group II: ProRoot MTA modified with CaCl_2 -manual (MM) ($n = 20$)
- Group III: Biodentine-manual (BDM) ($n = 20$)
- Group IV: Nano Biodentine-ultrasonic placement (NBUDU) ($n = 20$)
- Group V: ProRoot MTA modified with CaCl_2 -ultrasonic placement (MU) ($n = 20$)
- Group VI: Biodentine ultrasonic placement (BDU) ($n = 20$).

Manipulation

MTA (DentsplyMaillefer, Ballaigues, Switzerland) and Biodentine (Septodont, Saint Maur des Fosses, France) were manipulated according to the manufacturer's instructions. To achieve uniformity while manipulation all the materials used in present

study has been mixed using a capsule attached to a mechanical activator (Ultramat-2). MTA and 5% Calcium chloride were mixed in a 3:1 proportion as previously suggested.^[1] Nano Biodentine (NBD), obtained by Ball milling of Biodentine in a zirconia ball-mill machine (Gold Belt Global) for 24 h. The particle size of final product has been evaluated and confirmed to be in the range of 2.3–5 nm by Dynamic light scattering (Department of Biochemistry, Osmania University, Hyderabad). To enable the condensation of the materials, the specimens were placed over a tinfoil, which was stabilized on a customized elastomeric block [Figure 1].

Measurement of setting time

All the materials were estimated for setting time as it might be the influencing factor on the bond strength, in plastic cylindrical templates by using stop watch/tachometer till the disappearance of surface gloss.

Manual condensation

The materials were then delivered into the lumen of the root canal of each specimen in groups I (NBDM), II (MM) and III (BDM) with a MTA Endo gun (DentsplyMaillefer). The specimens were obturated using a stainless endodontic plugger of size #5 which is subjected to 3.22 MPa vertical compression which is frequently evaluated and corrected by a pressure sensitive device (Department of Mechanical Engineering, AcharyaNagarjuna University, Guntur).

Indirect ultrasonic activation

After condensation of each specimen in Groups IV (NBDU), V (MU) and VI (BDU) with hand plugger, the end of the plugger remained in contact with the material while it is indirectly activated for 5 seconds with ultrasonic tip CPR 1 (Dentsply, Tulsa, United States) attached to a P5 NewtronXS™ unit (SATELEC, 4th Gen. Aceton, North America) set on its medium power setting i.e., with frequency value adjusted at 5 [Figure 2]. Excess unset material was removed gently from the surface of the specimens using the scalpel blade. The samples were wrapped in wet pieces of gauze and kept in incubator at 37° and 100% humidity for 96 h to ensure complete setting of the material.

Push out test

Ten samples from each group were evaluated for bond strength of the materials using an Universal Testing Machine (Instron Universal Testing Machine, Model 8801). The samples were mounted on a metal slab with a 1.5-mm central aperture. A cylindrical

stainless steel plunger of 1-mm diameter and operating at a speed of 1 mm/min⁻¹ was used to apply force on materials inside root slices [Figure 3]. The load applied to the material at the time of displacement was recorded in Newton and later converted to megapascals (MPa).

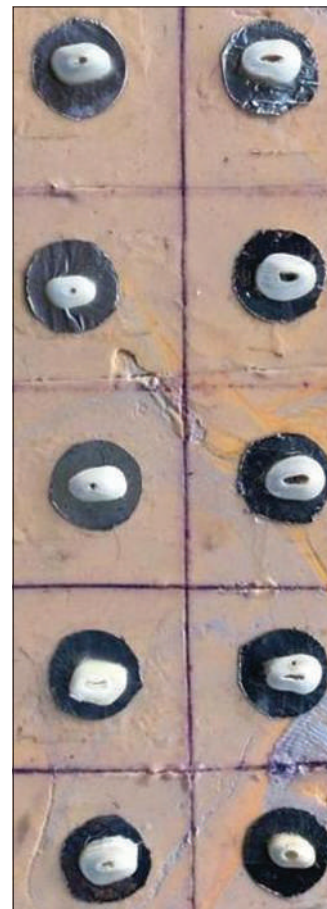


Figure 1: Customized elastomeric block.

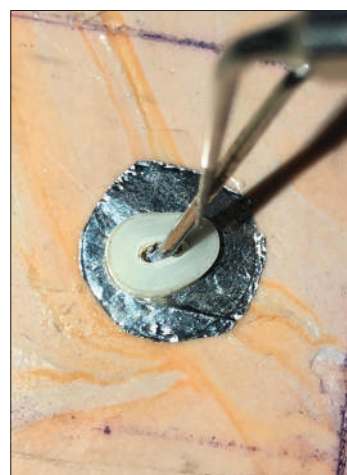


Figure 2: Indirect ultrasonic activation against hand plugger remained in contact with the material.

Evaluation of mode of failure

Mode of failure was evaluated by observing under stereomicroscope at 10 X magnification. Mode of failures was identified as cohesive (within either of the filling material, dentin), adhesive (between filling material and dentin) and mixed (both cohesive and adhesive failures) [Figure 4].

Evaluation under confocal laser scanning microscope

Remaining ten samples from each group were evaluated under confocal laser scanning microscope (LSM 880, Zeiss Microscopes, Germany) to assess the extension of root filling material into dentinal tubules [Figure 5]. In order to facilitate analysis, the filling materials were labeled with Rhodamine B (Sigma Aldrich, St. Louis, MO, USA) to an approximate concentration of 0.1%. Samples were examined at 2.5 X (total magnification of 50 X) and 10 X (total magnification of 100 X) magnifications to evaluate the penetration as well as maximum depth of penetration in micrometers and images were analyzed by Ziess LSM 880 image examiner software (ZEN Blue version). For depth of penetration the point of deepest penetration has been considered and recorded in micrometers [Table 1].

Statistical analysis

Mean pushout bond strength values [Table 2] are obtained and subjected to statistical analysis using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA test was used for evaluation of overall significance ($P < 0.05$) [Table 3] and Pair wise comparison of the groups by Tukey's multiple *post hoc* test [Table 4].

RESULTS

Box plot diagram represents distribution of

Table 1: Mean depth of penetration of bioceramic materials

Groups (n=10)	Mean depth of penetration (µm)	P
Group I (NBDM)	130.8727	<0.001
Group II (MM)	70.2865	<0.001
Group III (BDM)	190.4952	<0.001
Group IV (NBDU)	382.2743	<0.001
Group V (MU)	256.4572	<0.001
Group VI (BDU)	310.5746	<0.001

One way ANOVA was done to compare the mean depth of penetration between the groups. BDM: Biodentine manual, NBDM: Nano BDM, MTA: Mineral trioxide aggregate, MM: ProRootMTA-manual, MU: ProRootMTA-ultrasonic, BDU: Biodentine-ultrasonic, NBDU: Nano BDU



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push out bond strength values with standard deviation [Figure 6]. Push out bond strength was higher in Group VI (BDU), and least with Group II (MM). Intergroup comparison revealed a Statistically significant difference among most of the groups ($P < 0.05$) except between Group II versus III, between Group III versus V and between Group I versus II, III, IV, V. Mean push out bond strength values had suggested the bond strength values in the following order:

Group VI (BDU) > Group IV (NBDU) > Group I (NBDM) > Group V (MU) > Group III (BDM) > Group II (MM).



Figure 3: Universal Testing Machine containing cylindrical stainless steel plunger of 1-mm diameter and operating at a speed of 1 mm/min⁻¹ used to apply force on materials, inside root slices.

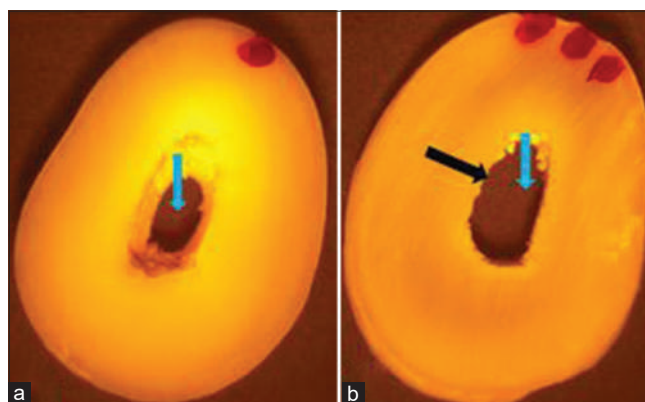


Figure 4: Mode of failures under stereomicroscope. Inspection of samples under a stereomicroscope at ×10: (a) Cohesive failure showing fracture within the obturating material. (b) Mixed mode of failure with cohesive failure observed within the material at some areas and black arrow indicates the adhesive failures between obturating material and tooth interface.

Table 2: Mean, standard deviation, standard error and confidence intervals for bond strength (Mpa) in six groups (1, 2, 3, 4, 5, 6)

Groups	Minimum	Maximum	Mean	SD	SE	95% CI for mean	
						Lower bound	Upper bound
Group 1	6.63	56.09	24.25	16.89	5.34	12.17	36.33
Group 2	6.97	24.88	14.14	5.04	1.59	10.53	17.75
Group 3	18.82	25.48	21.74	2.43	0.77	20.01	23.48
Group 4	14.70	64.22	37.12	14.76	4.67	26.56	47.68
Group 5	2.25	45.65	22.62	13.01	4.11	13.32	31.93
Group 6	70.84	74.96	73.47	1.29	0.41	72.54	74.39

SD: Standard deviation, SE: Standard error, CI: Confidence interval

Table 3: Comparison of six groups (1, 2, 3, 4, 5, 6) with bond strength (Mpa) by one way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	P
Between groups	5	23,174.22	4634.84	39.4355	0.0001*
Within groups	54	6346.61	117.53		
Total	59	29,520.82			

*P<0.05

Table 4: Pairwise comparison of six groups (1, 2, 3, 4, 5, 6) with bond strength (Mpa) by Tukeys multiple *post hoc* procedures

Groups	Mean difference	SE	P	95% CI	
				Lower bound	Upper bound
Group 1-Group 2	10.11	4.85	0.3100	-4.21	24.44
Group 1-Group 3	2.51	4.85	0.9950	-11.81	16.84
Group 1-Group 4	-12.87	4.85	0.1020	-27.19	1.46
Group 1-Group 5	1.63	4.85	0.9990	-12.69	15.96
Group 1-Group 6	-49.21	4.85	0.0001*	-63.54	-34.89
Group 2-Group 3	-7.60	4.85	0.6230	-21.93	6.72
Group 2-Group 4	-22.98	4.85	0.0001*	-37.31	-8.66
Group 2-Group 5	-8.48	4.85	0.5060	-22.81	5.84
Group 2-Group 6	-59.33	4.85	0.0001*	-73.65	-45.00
Group 3-Group 4	-15.38	4.85	0.0290*	-29.70	-1.05
Group 3-Group 5	-0.88	4.85	1.0000	-15.20	13.45
Group 3-Group 6	-51.72	4.85	0.0001*	-66.05	-37.40
Group 4-Group 5	14.50	4.85	0.0460*	0.18	28.82
Group 4-Group 6	-36.34	4.85	0.0001*	-50.67	-22.02
Group 5-Group 6	-50.84	4.85	0.0001*	-65.17	-36.52

*P<0.05. SE: Standard error, CI: Confidence interval

DISCUSSION

In the present study, the dentin discs of 2 mm thickness were used for the purpose of push out test measurement. Over estimation of the bond strength may occur if thickness of discs is increased due to increase the area of friction.^[7] The chemical and physical properties play a major role in the clinical outcome of root canal filling materials which can be affected by factors such as mixing and placement techniques, delivery system, exposure to various

clinical environments, storage conditions and the ratio of the constituent components.^[8] To fill voids and spaces between the obturant and the tooth structure and to form a fluid tight seal, the root canal filling materials are used with the semisolid or solid core filling material to provide the required adhesion.^[9] In contemporary endodontics the concept of monoblock has increased the potential of creating good adhesion in root canal walls.^[10,11] In the present study all the materials have only one interface that extends circumferentially between the material and the root canal wall which is a primary monoblock.

Hydraulic cements are finely ground materials (powders), yielding complex hydrated products when mixed with water or specific water based component. Any interference in hydration reaction might influence the biological, chemical, and physical properties of the resulting product with altered clinical performance.^[12]

The conventional Proroot MTA and Bio dentine have been modified in present study by the addition of CaCl₂ and Nanoparticulation respectively.

The addition of CaCl₂ at the concentrations of 2%–15% improves physicochemical properties of MTA and shortens the setting time. However, the shortcomings of this modified material include reduced expansion, coherence which might influence the bond strength with radicular dentin. However, considering the advantages of addition of calcium chloride such as shortened setting time and increased biomineralization,^[13] altering the mode of placement



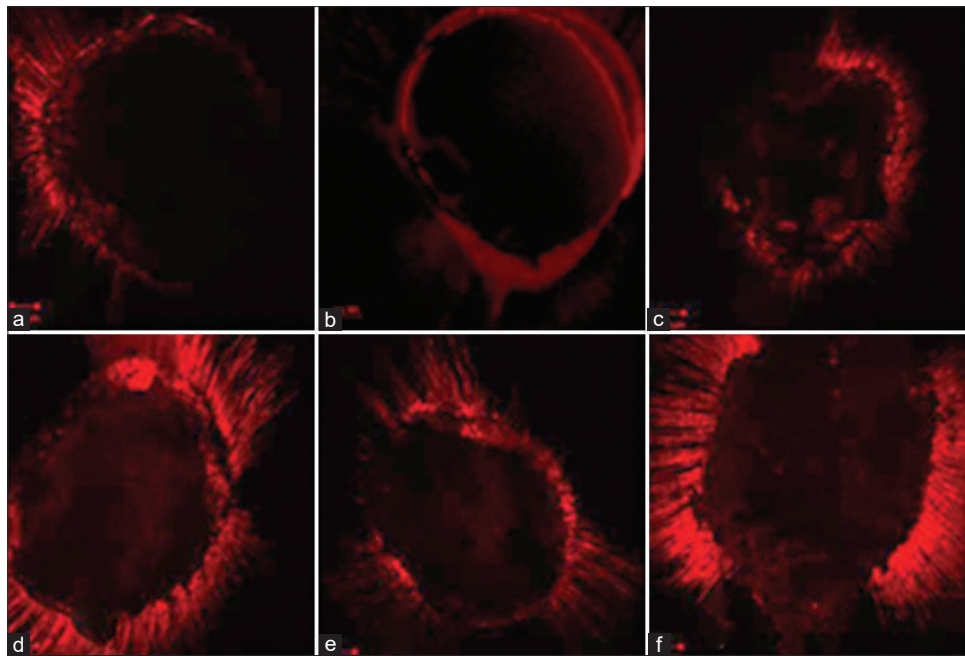


Figure 5: Tubular penetration under confocal laser scanning microscope. Images showing lateral extension of bioceramic material in the root sections when observed under Confocal Laser scanning microscope (LSM 880, zeiss microscopes, Germany) under 10X zoom (total magnification of 100X). (a) NBDM; (b) MM; (c) BDM; (d) NBDU; (e) MU; (f) BDU. BDM: Biodentine-manual, NBDM: Nanobiodentine manual, MTA: Mineral trioxide aggregate, MM: ProRootMTA-manual, MU: ProRootMTA-ultrasonic, BDU: Biodentine-ultrasonic, NBDU: Nano biodentine-ultrasonic.

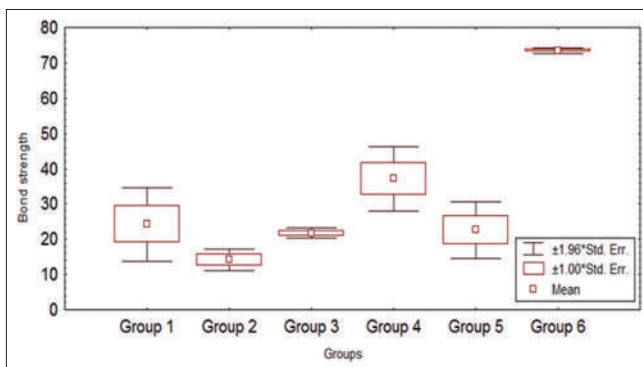


Figure 6: Box plot diagram represents distribution of push out bond strength values with standard deviation.

might aid in compensating the reduced bond strength with root canal dentin. Being a viable tool in endodontics, indirect ultrasonic agitation reduces the size of cement particles and consequently increases the total reactive surface by de-clustering the particles that are clogged to each other^[14] resulting in increased flow, setting, and compaction of the material.^[15] In the current study, Ultrasonic activation of CaCl₂ modified MTA resulted in better bond strength even superior to conventional MTA in accordance with the previous study. Besides ultrasonic placement, presence of nanoparticles also has a positive impact on the penetration of material into the dentinal

tubules. There are other factors that might be able to influence the capacity of dentinal tubule penetration of the endodontic filling material: The surface activity of the sealers, the contact angle between sealer and the dentinal walls, the diameter of the opened dentinal tubules and the employed obturation technique.^[16]

Materials with Nano particles have better workability, fluidity and positive influence on hydration process resulting in efficient filling of root canal. In present study, NBD demonstrated increased chemical reactivity is due to higher solubility and surface area as observed by shortened setting time compared to conventional Biodentine. This is substantiated by a fact that a given mass of material in nanoparticle form is much more reactive than the same mass of material made up of larger particles.^[17] It was demonstrated that the more the material is soluble, the higher OH⁻ and Ca²⁺ release which is correlated to the higher solubility recorded for BD. This had led to more Ca²⁺ from nanomaterials.^[18] Setting accelerator effect of the nanoparticles acts as seeds and stimulates the nucleation of calcium silicate, accelerating the hydration process (sowing effect), also helps in accelerating the setting time and hydration process.^[19]

In the present study, the indirect ultrasonic mode of placement had a better bond strength value than

manual with a statistically significant difference. It was observed that Group VI (BDU), has shown highest bond strength values among all other groups. The effective combination of the unaltered hydration which enables the extension of material into the dentinal tubules with indirect ultrasonic activation has resulted in this superior bond strength. However the tubular penetration is lower than that of nanobiodentine with ultrasonic activation which might be due to less particle size of nanobiodentine than Biodentine thus enabling greater penetration. The reduced bond strength of NanoBiodentine compared to Biodentine could be due to the altered density, particle scattering leading to altered hydration characteristics. In the present study, the mean push out bond strength of Group III (BDM) and Group V (MU) had shown similar results with no statistically significant difference, suggestive of ultrasonic activation of MTA could achieve similar results in terms of bond strength even though there is difference in tubular penetration. Except for Groups IV (NBU) and VI (BDU) tubular penetration values are proportionate to push out bond strength values in all the groups of the present study. The greater tubular penetration of Group IV (NBDU) might be due to smaller particle size of nanobiodentine which is further enhanced by indirect ultrasonic activation. However, as previously described it altered the hydration properties resulting in lower bond strength. It was observed that majority of the failures associated with all the groups are of cohesive nature which suggests that the interface between filled material and root dentin was more durable than core material itself. At present the available literature is not sufficient to ensure the long term performance of the bioceramic materials used in the present study. Further research is needed to evaluate the clinical longevity of these modified bioceramic materials.

CONCLUSION

Within the practical limitations of the current *in vitro* study, it can be concluded that indirect ultrasonic activation has resulted in superior interfacial bond strength and tubular penetration than the manual compaction. A combination of Biodentine with indirect ultrasonic activation has resulted in superior bond strength compared to all other groups. On the other hand, manual condensation of MTA has demonstrated the lowest bond strength values. Having greater tubular penetration, Nanobiodentine appears to be a promising root canal filling material.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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REVIEW ARTICLE

Coronavirus Disease and Cardiovascular Disease: A Literature Review

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ABSTRACT

Background and Aim: Although severe acute respiratory syndrome coronavirus 2 primarily affects the respiratory system, involvement of cardiovascular system is not uncommon and a range of cardiac manifestations among Coronavirus Disease (COVID-19) patients were reported in the literature. Furthermore, it is evident from scientific literature that the incidence of deaths and hospitalizations has been increasingly more among COVID-19 subjects with pre-existing cardiovascular disease (CVD). Various pathophysiological mechanisms have been proposed to explain the cardiovascular involvement in COVID-19. Another emerging significant concern is the varying presentations of COVID-19 and side effects due to the medication used in the management of COVID-19 patients. This review attempts to provide a comprehensive overview of the existing literature on the possible association between CVD and COVID-19 with emphasis on the pathophysiological mechanisms, cardiac manifestations, and impact of medications used for COVID-19 on cardiovascular health. Based on the available literature, we conclude that though CVD could not be reckoned as an independent risk factor for COVID-19 infection, it is evident that pre-existing CVD has an influence on the severity of COVID-19 infection and associated mortality.

Relevance for Patients: Literature suggests that people with pre-existing CVD are at increased risk for COVID-19 and associated severity. Consequently, it becomes important to thoroughly gain insights into the possible pathophysiological mechanisms, cardiac manifestations in COVID-19, and the impact of COVID-19 treatment on the cardiovascular system.

1. Introduction

According to the World Health Organization reports, 79.2 million confirmed Coronavirus Disease (COVID-19) cases were detected and nearly 1.75 million deaths were registered across the world as on December 27, 2020 [1]. As there has been a consistent daily increase in the number of COVID-19 confirmed cases in many countries, these numbers may worsen soon and the disease may continue to impose significant burden on the health-care delivery systems worldwide. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing COVID-19, primarily affects the respiratory system though the involvement of other organs and systems is not uncommon. The clinical presentations of the disease are too heterogeneous ranging from mild fatigue to severe hypoxia with respiratory failure [2]. This resulted in the categorization of the severity of COVID-19 by Wu and McGoogan [3] and the Society of Pediatrics, Chinese Medical Association [4].

SARS-CoV-2 is an enveloped, single-stranded RNA virus belonging to Coronaviridae. HKU, 229E5, and hCoV-0C43 are among the mild common cold causing coronaviruses affecting human beings. However, SARS-CoV and Middle East respiratory syndrome

coronavirus MERS-CoV are the recently emerged pathogenic coronaviruses, infecting humans, with 8000 and 2500 reported cases worldwide, respectively [5,6]. The major route of transmission of SARS-CoV-2 is through respiratory droplets. The secondary attack rate for COVID-19 ranged from 0.5 to 5% [7,8]. Incubation periods for the disease did not differ significantly between people with the 95% confidence interval of the median incubation period for SARS-CoV-2 being 4.5–5.8 days and a vast majority of the affected demonstrating symptoms within 12 days from the time of exposure [9]. Shi *et al.* reported that <20% of COVID-19 affected subjects had significant symptoms such as dyspnea, tachypnea, and hypoxemia. Symptoms of critical COVID-19 infections include respiratory failure, multi-organ failure, and sepsis induced hypotension [10]. Development of cardiovascular disorders was reported in some COVID-19 cases and the literature suggests that people with pre-existing cardiovascular disease (CVD) are at increased risk for COVID-19 and associated severity [11]. With this background, the objective of this review is to provide a comprehensive overview of the association between COVID-19 and CVD.

2. CVD and Coronavirus: an epidemiological perspective

CVD has been identified as a common comorbidity among patients infected with viruses belonging to the Coronavirus family. The prevalence of CVD among SARS patients was 8% and is reported to be a significant contributor for the increased risk of death among those affected with SARS [12,13]. With regard to MERS, hypertension and CVD were prevalent among nearly 50% and 30% of the affected subjects, respectively [14]. This association is apparently evident in the context of COVID-19 as well with presence of cardiovascular comorbidities among the COVID-19 affected at an increased frequency. It was hypertension as a comorbidity and 17% of COVID-19 confirmed cases had coronary heart disease [15]. Yang *et al.* conducted a meta-analysis to document the prevalence of comorbidities among COVID-19 patients and found that hypertension, diabetes mellitus, and CVD are among the common comorbidities among the infected patients. It was reported that the 95% confidence interval for the prevalence of CVD was 4–7% [16]. The presence of underlying cardiovascular comorbidities was also found to be associated with increased mortality among COVID-19 infected. In a study conducted by Wang *et al.* in Wuhan, China, 31% of the COVID-19 cases had hypertension and this percentage increased to 58% when patients admitted in ICU were exclusively considered [17]. Similarly, the prevalence of hypertension and coronary heart disease was 30% and 8% in another study conducted by Zhou *et al.* in China [18]. Although the exact mechanism leading to these observations is not known, the interference of age as a confounding factor in the association between CVD and COVID-19 cannot be ignored, with older people demonstrating high prevalence of CVD and increased susceptibility of a symptomatic COVID-19 infection due to a relatively weaker immune system among the elderly.

3. Pathophysiological mechanisms

The elevated expression of several cardiovascular biomarkers has been reported in severe COVID-19 cases [17-19]. These changes support involvement of the cardiovascular system, which can be explained by the combined effects of several mechanisms.

3.1. Renin-angiotensin system (RAS) imbalance

Angiotensinogen is converted to angiotensin I (Ang-I) by the protease rennin, which consequently gets converted by Angiotensin Converting Enzyme (ACE) to angiotensin II (Ang-II). ACE2 facilitates the conversion of Ang-II to angiotensin-(1–7), by removal of carboxy-terminal phenylalanine, which on binding with the MAS receptor results in vasodilation and anti-inflammation. However, it is scientifically established that to facilitate the fusion with host cells, SARS-CoV binds to human ACE 2 with the amino terminal of its spike protein [4]. SARS-CoV2 being a coronavirus that belongs to the β genus may also have ACE2 as its binding receptor. The expression of ACE2 on cells is downregulated in SARS-CoV infection leading to disruption in the physiological balance between ACE/ACE2 and Ang-II/angiotensin-(1–7). This marked downregulation of ACE2 and the corresponding upregulation of Ang-II may aggravate perpetuate cardiac injuries [20]. High Ang II levels in plasma among COVID-19 patients adds strength to the supposition that SARS-CoV2 binds to ACE2 resulting in the elevated production of Ang II through RAS. This increases burden on the cardiovascular system as the heart load increases leading to cardiomyocyte hypertrophy and hypertension [21].

3.2. Overactivation of the immune system

Replication of virus following the host cell fusion and this invasion of alveolar surfaces would result in alveolitis and the invasion of the cardiac muscle cells results in edema and lysis of the cardiomyocytes, which further lead to release of pro-inflammatory cytokines [22]. The deposition of pro-inflammatory cytokines and the filtration of inflammatory cells at the site of injury causes “inflammatory storms” which warrants an overactivation of the immune system. This leads to damage of cardiomyocytes and decreases the adherence of the atherosclerotic plaques in coronary vessels. All these mechanisms predispose to cardiovascular events [22]. Huang *et al.* reported the cytokine profiles of COVID-19 infected subjects with elevated Interleukin (IL)-2, IL-7, interferon- γ , and tumor necrosis factor α [11]. Virus associated was reported to be associated with increased COVID-19 fatality in a multicentric study among infected subjects in Wuhan, China, where significant differences in ferritin levels and IL-6 were between survivors and non-survivors [19]. In autopsy findings, cytokine storm induced by the inflammatory response syndrome to COVID-19 was discussed to have resulted in cardiac interstitial mononuclear inflammatory infiltrates [23,24].

3.3. Decreased partial pressure of oxygen in blood

Damage to alveolar cells in COVID-19 patients results in hypoxemia. As the partial pressure and saturation of oxygen falls

consistently there is deposition of oxygen free radicals, which on circulation across the body damages the cardiomyocytes. Another mechanism is the activation of NADPH oxidase enzymatic complex by the downregulation of ACE2 and corresponding upregulation of Ang-II in COVID-19. Ang-II binds to Angiotensin Type I Receptor (AT1R) which stimulates the activity of NADPH oxidase that reduces oxygen to superoxide. While clear underlying mechanisms for stimulation of NADPH oxidase are complex, it is known to occur at the genetic, transcriptional, and post-transcriptional levels involving multitude of signaling molecules and scaffolding proteins [25]. ACE2 deficiency was shown to increase NADPH oxidase activity and resulted in oxidative stress in the study conducted by Wysocki *et al.* [26]. To accommodate for the metabolic demands, blood flow intensifies increasing the individual's risk for heart failure. Furthermore, hypoxemia is an independent predecessor for inflammatory response that promotes "inflammatory storm" and contribute toward incidence of cardiovascular events [27].

3.4. Elevated catecholamine levels in plasma

An initial response to viral invasion is the activation of immune system, and the interaction between viral and host cells is a complex phenomenon. Besides, stimulation of α 1-adrenoceptors by catecholamines can reinforce the vasoconstriction caused by excess Ang-II as a result of downregulation of ACE2 and could contribute toward a significant increase in vascular resistance. Moreover, catecholamines stimulate β 1-adrenoceptors in the kidney to increase renin secretion that consequently increases Ang-II resulting in further deterioration of cardiovascular health. Response from the emotional perspective such as anxiety, fear also lead to increased production of catecholamines the concentration in plasma of which is directly related to hypertension, coronary artery disease, and cardiac failure. Increased level of catecholamine levels in plasma also has the implication of myocardial toxicity that could result in circulatory disturbances and arrhythmias [26]. Although a direct study of catecholamines among COVID-19 subjects could offer useful insights, the scope for these observations is hampered by severe illness such as respiratory failure, compromised cardiovascular health, and shock and more so among those on adrenaline infusion.

4. Cardiovascular manifestations in COVID-19

Although it is known that SARS-CoV 2 primarily affects the respiratory system, the negative impact COVID-19 has on cardiovascular health cannot be overstated. It has been thoroughly established in literature that cardiac manifestations contribute significantly towards COVID-19 case fatality rates [27-29]. The following are the most common cardiac manifestations observed among COVID-19 patients.

4.1. Myocardial injury

Regardless of the presence or absence of respiratory symptoms, it has been observed that ischemic and non-ischemic myocardial injury is one of the significant outcomes of COVID-19

infection [30,31]. The incidence of myocardial injury as a COVID-19 manifestation varied between 7% and 28% among hospitalized COVID-19 patients in the scientific literature [32]. High spikes in troponin levels were identified in patients with severe disease. In a cohort study conducted by Shi *et al.*, nearly 20% of the hospitalized patients demonstrated cardiac injury. This finding gains significance in light of the fact that COVID-19 fatality rate is 10 times higher among patients with cardiac injury compared to those who do not have myocardial injury [33]. Zhou *et al.* reported in their multicentric study that the proportion of myocardial injury among COVID-19 non-survivors was 46% as compared to 1% among survivors [18].

4.2. Heart failure

Among COVID-19 patients with previous history of cardiovascular problems and undiagnosed heart diseases, heart failure may occur [34]. It is imperative to focus on the findings reported by Zhou *et al.*, where the proportion of heart failure was 52% among non-survivors as compared to 12% among survivors [18]. These findings corroborated with those reported by Chen *et al.*, where heart failure was noticed among 49% and 3% of the non-survivors and survivors, respectively [35]. It is also reported in the literature that COVID-19 patients with the previous heart failure could possibly experience an acute decompensation [36].

4.3. Cardiac arrhythmias

Arrhythmias are another common manifestation among COVID-19 patients. While progressive arrhythmias among infected individuals could be reckoned as an indication for cardiac involvement, it is not uncommon for arrhythmias to be the initial presentation of COVID-19. It was postulated by Guo *et al.* that high TnT levels have an increased incidence of malignant arrhythmias [37]. Palpitations were reported to be among the most common clinical presentations of COVID-19 in a study conducted by Liu *et al.* in Wuhan, China [38]. Although the reasons for palpitations and the nature of arrhythmias were not mentioned by Wang *et al.*, they reported increased frequency of arrhythmias among COVID-19 patients in ICUs compared to those who did not require intensive care [17].

4.4. Thromboembolism

It has been thoroughly established in the literature that COVID-19 activates the coagulation cascade. In a study conducted by Han *et al.*, a significant reduction in circulating antithrombin III, substantial increases in the fibrinogen and D-dimer levels were reported among COVID-19 patients as compared to healthy controls. Severe inflammation in COVID-19 is also marked by higher platelet counts compared to non-COVID-19 patients with pneumonia [39]. Tang *et al.* reported higher incidence of disseminated intravascular coagulation (DIC) among the deceased compared to the survivors, and a short duration of 1–12 days was identified between the time of hospitalization and the onset of DIC [40]. Literature suggests that almost a quarter of COVID-19 patients had deep venous thrombosis [41]. The rationale for

this observation could be elevated levels of D-dimer, fibrinogen among COVID-19 affected [39]. An association between severity of COVID-19 and the levels of these coagulation markers was also reported by Xiong *et al.* in a meta-analysis [42]. Any level of D-dimer more than 1µg/ml was reported to be a contributing factor for in-hospital deaths by Zhou *et al.* [18]. The incidence of venous thromboembolism as a COVID-19 manifestation varied between 20% and 43% among COVID-19 patients requiring intensive care in spite of preventive anticoagulation. To prevent incident thrombophlebitis among COVID-19 patients, heparin is recommended by the WHO, due to its pleiotropic properties, for hospitalized COVID-19 patients [43]. Yin *et al.* reported improved prognosis with heparin use among hospitalized COVID-19 patients with elevated D-dimer levels [44].

5. Cardiac consequences of COVID-19 treatment

Varying presentations of COVID-19 and side effects due to the medication used in the management of COVID-19 patients emerged as a significant challenge for physicians providing services for these patients. Among various pharmacological management strategies of COVID-19, the use of antiviral drugs, anti-malarial drugs, immunomodulatory medicines, glucocorticoids, and plasma from COVID-19 recovered patients is the common [45-47]. Chloroquine and hydroxychloroquine result in alkalization of the intracellular phagolysosome. This leads to under-glycosylation of ACE2 receptors thus limiting the fusion of SARS-CoV 2 and the host cells [45]. Regardless of the concomitant use of COVID-19, hydroxychloroquine was found to be decreasing the viral carriage among the in-hospital COVID-19 affected [48]. However, these drugs prolong the QT interval and may result in fatal arrhythmias, especially in cases with electrolyte abnormalities [49]. Furthermore, thorough monitoring for hypotension and bradycardia is warranted among COVID-19 patients using chloroquine as the drug holds the potential for inhibiting CYP2D6 resulting in elevated levels of beta blockers [50]. The antiviral drugs used in the management of COVID-19 patients may result in bradycardia, prolongation of QT and PR interval [49,51,52] and may have an influence on the warfarin dosing [53]. This possibility of drug-drug interaction between antiviral medication and the anticoagulants is an area of concern. The side effects of remdesivir, which is established to be contributory toward faster recovery from COVID-19 infection [54], are increase in liver enzymes and respiratory failure [55]. Monitoring ECG parameters is extremely important in the context of COVID-19 pandemic in light of the fact that the medications used in the management of this disease and also the possible hypokalemia subsequent to diarrhea, which is one of the clinical features in COVID-19 affected, may prolong the QT interval increasing the risk for torsade Despointes.

Although there is uncertainty whether RAAS inhibition have any effect on the ACE2 expression [56,57], use of RAAS inhibitors emerged as an area of concern as ACE2 protein is used by SARS-CoV2 for cellular entry [58]. However, the general recommendation amidst uncertainty is to continue the use of

RAAS inhibitors among stable COVID-19 patients [59]. The negative impact of cytokine release syndrome, among COVID-19 affected, on the cardiopulmonary system prompted the use of corticosteroids to curtail the overactivation of the immune system [60]. Nevertheless, use of corticosteroids is routinely avoided to decrease the risk of exacerbation of the prevailing lung damage [61]. Therefore, it has been recommended that screening of COVID-19 patients for ESR, platelet count, serum ferritin levels and obtaining a hemophagocytic response (H Score) be done in the identification of COVID-19 patients where immunosuppression could be necessary.

6. Conclusion

The notion of increased risk of COVID-19 infection among subjects with pre-existing CVD remains equivocal as the numbers suggest a comparable prevalence of CVD among COVID-19 infected and the general source population. Nevertheless, CVD apparently is associated with increased hospitalizations and death among COVID-19 cases. It is evident that pre-existing CVD may aggravate the COVID-19 disease course with the presentation of aforementioned cardiac manifestations. Moreover, the pathophysiological mechanisms involved in COVID-19 may lead to cardiomyocyte hypertrophy and damage, circulatory disturbances, and arrhythmias. Therefore, close monitoring of COVID-19 subjects for cardiovascular health is warranted and it is advisable for subjects with pre-existing CVD to be more careful in practicing the precautionary measures such as maintaining social distancing, avoiding unnecessary travel, following respiratory hygiene/cough etiquette to avoid COVID-19 infection.

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Conflict of interest

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Accuracy of digital intraoral periapical radiography and cone-beam computed tomography in the measurement of intrabony defects: A comparative study

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Abstract:

Background: Periodontal disease is an inflammatory process resulting in clinical attachment loss (CAL), pocket depth (PD), and resulting in the loss of alveolar bone. Diagnostic imaging provides an adjunctive guidelines to assess the alveolar bone height in addition to clinical parameters such as PD and CAL. **Aims and Objectives:** The objectives of the study are to determine whether the digital intraoral periapical (IOPA) radiographs can be reliably used as an alternative to cone-beam computed tomography (CBCT) in the diagnosis of intrabony defects. **Materials and Methods:** A total of 25 patients with the presence of intrabony defects were included in the study. All the radiographic parameters were recorded using digital IOPA and CBCT. Various intrabony defect morphological characteristics such as height, depth, width, and angle were measured and compared between digital IOPA and CBCT. **Statistics:** The data was subjected to statistical analysis. Mann-Whitney *U*-test was performed for interexaminer comparison and independent *t*-test for intergroup comparison. **Results:** The mean intergroup comparison values between digital IOPA and CBCT in relation to defect width were 3.22 ± 1.10 and 3.20 ± 1.16 , respectively ($P = 0.970$), in relation to defect depth were 7.71 ± 2.3 and 7.91 ± 2.4 , respectively ($P = 0.769$), in relation to defect height were 3.80 ± 1.20 and 3.90 ± 1.2 , respectively ($P = 0.794$), and in relation to defect angle were 34.82 ± 8.4 and $35.28 \pm 0.8.6$, respectively ($P = 0.851$). **Conclusion:** With the drawbacks of such as high radiation exposure, unavailability, and high financial cost, digital IOPA with digital software can be used as an alternative to CBCT for measuring intrabony defect morphological characteristics in periodontitis patients.

Key words:

Cone-beam computed tomography, digital radiographs, intrabony defects, periodontitis

INTRODUCTION

Periodontal disease is an inflammatory process resulting in clinical attachment loss (CAL), pocket depth (PD), and resulting in the loss of alveolar bone. One of the characteristic symptoms of destructive periodontal disease is the alveolar bone loss and this bone destruction pattern is divided into horizontal (even) and oblique (vertical/angular) bone defects. The bone destruction in vertical patterns does not proceed in a symmetrical pattern. The magnitude of bone destruction varies in different sites around the tooth, which explains why the crest of the alveolus does not correspond to the cemento-enamel junction (CEJ), and it is not parallel to it. Hence for the diagnosis, treatment planning, and prognosis of intrabony defects evaluation of the defect morphology is crucial.^[1]

Diagnostic imaging provides an adjunctive guidelines to assess the alveolar bone height as well as the occurrence of intrabony defects in addition to clinical parameters such as PD and CAL.^[2] The precise methods for analyzing the morphological

characteristics of the intrabony defects were clinical observation and topography.^[3]

In the diagnosis of periodontal diseases, radiographs play a crucial role by depicting the nature and amount of injury that occurred to the alveolar tissues. For the diagnosis of the amount of alveolar bone loss and intrabony defect morphology, various intraoral and extraoral oral imaging modalities were available.^[3]

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Among the two dimensional (2D) intraoral imaging modalities, digital intraoral periapical (IOPA) radiographs and bitewing radiographs can be cheap, easily acquired, and provide high resolution images.^[4,5] However despite of these advantages, 2 Dimensional imaging modalities are having limitations such as overlapping of anatomical structure and difficulties in standardization.^[6-8]

To overcome the disadvantages of 2D imaging, cone-beam computed tomography (CBCT) (3D imaging) provides 3D analysis and rapid volumetric image in the field of diagnostic imaging, which include the elimination of distortions and the ability to visualize structures in all three orthogonal planes.^[9-11]

According to the literature, the use of digital IOPA had showed better results close to intrasurgical analysis, whereas 3D imaging showed an 80%–100% sensitivity in the detection of intrabony defects.^[12]

Hence, the purpose of the study is to determine whether the digital periapical radiographs can be reliably used as an alternative to CBCT in the diagnosis of intrabony defects.

MATERIALS AND METHODS

The present study was conducted on 25 subjects, selected from the Department of Periodontology and Implantology, Teaching Institution. The Study Protocol was approved by the Institutional Ethical Committee. After explaining the procedure, written and informed consent was obtained from patients who are willing to participate in the study. A total of 25 patients diagnosed with chronic and aggressive periodontitis with the presence of intrabony defects were included in the study. All the radiographic parameters were recorded using digital IOPA and CBCT.

Study design

The patients were selected according to the following criteria:

Inclusion criteria

1. Patients should be in between 18 and 56 years of age
2. Patients diagnosed with chronic and aggressive periodontitis (localized/generalized)
3. Transgingival probing PD \geq 6 mm and CAL \geq 7 mm.

Exclusion criteria

1. Patients who were systemically compromised
2. Pregnant and lactating females
3. Patients who received periodontal treatment in the past 6 months.

Clinical examination was performed by postgraduates under the supervision of senior periodontist. Clinical attachment level (CAL) and trans gingival probing depth were taken.

IOPA was performed using the Scanora digital software (2.2) using the long-cone paralleling technique with a bite registration positioning device. The exposure setting was 70 kVp with 12–25 mA. The millimetric grid used for digital intraoral radiographs was composed of 1-mm copper squares. These squares were used to calibrate the measurement tool of the viewing software to compensate for the elongation and foreshortening of the image.

A CBCT imaging technique was performed on each patient (9300 CBCT, carestream dental). The exposure settings were 80 kV and 10 mA. The scanning parameters selected were 90 μ m voxel size, 12 s acquisition time, and 5 cm \times 5 cm field of view. The CS imaging software module (version 2.2, CS 9300, Carestream Health, Inc, 150 verona street, Rochester street, NY, USA, 14608) was used to reconstruct and reformat the volume and resulting slices and complete all measurements. The axial slices in CBCT were used to verify the presence of combined bony defects, according to the classification of Goldman and Cohen.

Radiological parameters

1. The height of the defect: Distance from the CEJ to the alveolar crest (AC)
2. Depth of the defect: Distance from CEJ to the base of the defect
3. Width of the defect: Highest point of the AC to the root adjacent to the defect
4. Defect angle: Two lines that represent the root surface of the involved tooth and the bone defect surface.^[13]

Imaging evaluation

The images were analyzed by two examiners: a radiologist with 2 years of experience and examiner 2 was a third-year postgraduate student in periodontology.

Radiographic assessment was done by using Image Assistant software for periapical radiographs and CBCT according to Misch *et al.* At each site, three measurements were taken, they are as follows: (1) the height of the AC, measured from the CEJ to the AC, (2) the depth of the defect, measured from the CEJ to the bottom of the defect (BD), and (3) the width of the defect, measured from the highest point of the AC to the dental root adjacent to the defect. If restorations were present, the apical margin of the restoration replaced the CEJ as a fixed reference point. Defect angle was measured from the line drawing from the CEJ to the BD, and the other line extends from the BD to the lateral margin of the defect [Figures 1a-d and 2a-d].^[7]

Interexaminer agreement was evaluated as the standard error of the mean difference of the measurements performed by each of the two examiners. These were <1 . Ninety percent of the first examiner measurements were within the range of $\pm 3^\circ$ relative to the values obtained by the second examiner. Images were interpreted by an experienced orofacial radiologist.

Statistical analysis

The data was subjected to statistical analysis. Mann–Whitney *U*-test was done for interexaminer comparison and independent *t*-test for intergroup comparison. $P < 0.05$ was considered as statistically significant.

RESULTS

The mean interexaminer comparison values for CBCT in relation to defect width between the two examiners were 25.40 and 25.60, respectively ($P = 0.961$). The mean interexaminer comparison values for CBCT in relation to defect depth between the two examiners were 25.58 and 25.42, respectively ($P = 0.969$). The mean interexaminer comparison values for CBCT in relation to defect height between the two examiners were 25.74 and 25.26, respectively ($P = 0.907$). The

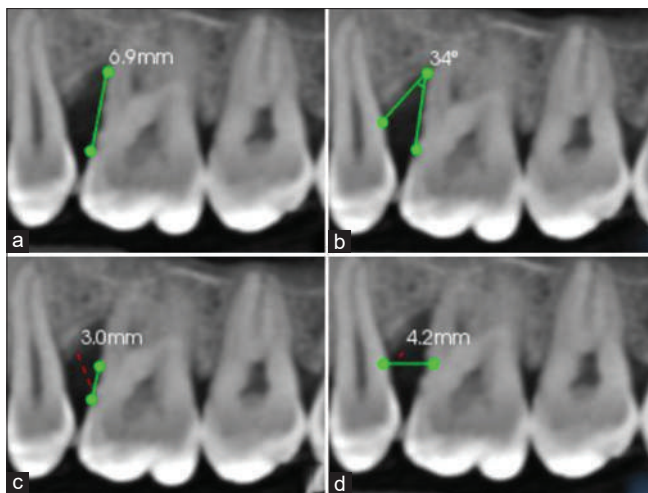


Figure 1: (a) Width of the defect on the mesial surface of 26 in cone-beam computed tomography; (b) Height of the defect on the mesial surface of 26 in cone-beam computed tomography; (c) Depth of the defect on the mesial surface of 26 in cone-beam computed tomography; (d) Defect angle on the mesial surface of 26 in cone-beam computed tomography

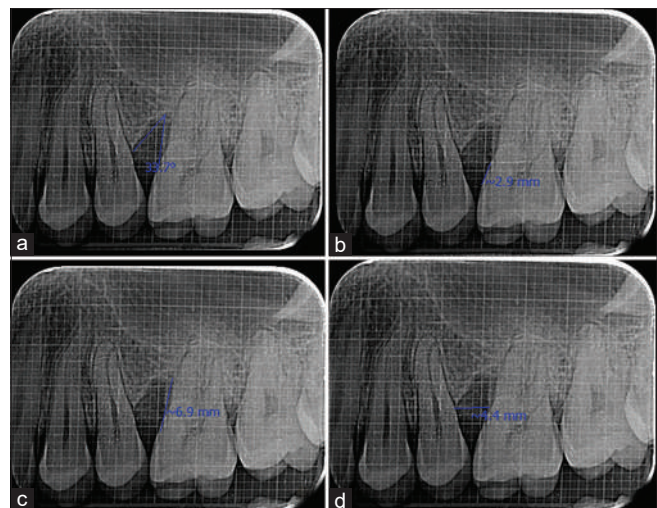


Figure 2: (a) Width of the defect on the mesial surface of 26 in digital intraoral peri-apical; (b) Height of the defect on the mesial surface of 26 in digital intraoral peri-apical; (c) Depth of the defect on the mesial surface of 26 in digital intraoral peri-apical; (d) Defect angle on the mesial surface of 26 in digital intraoral peri-apical

mean interexaminer comparison values for CBCT in relation to defect angle between the two examiners were 25.46 and 25.54, respectively ($P = 0.984$) [Table 1].

The mean interexaminer comparison values for digital IOPA in relation to defect width between the two examiners were 25.54 and 25.46, respectively ($P = 0.984$). The mean interexaminer comparison values for digital IOPA in relation to defect depth between the two examiners were 25.52 and 25.48, respectively ($P = 0.992$). The mean interexaminer comparison values for digital IOPA in relation to defect height between the two examiners were 25.32 and 25.68, respectively ($P = 0.930$). The mean interexaminer comparison values for digital IOPA in relation to defect angle between the two examiners were 25.52 and 25.48, respectively ($P = 0.992$) [Table 2].

The mean intergroup comparison values between digital IOPA and CBCT in relation to defect width were 3.22 ± 1.10 and 3.20 ± 1.16 , respectively ($P = 0.970$). The mean intergroup comparison values between digital IOPA and CBCT in relation to defect depth were 7.71 ± 2.3 and 7.91 ± 2.4 , respectively ($P = 0.769$). The mean intergroup comparison values between digital IOPA and CBCT in relation to defect height were 3.80 ± 1.20 and 3.90 ± 1.2 respectively ($P = 0.794$). The mean intergroup comparison values between digital IOPA and CBCT in relation to defect angle were 34.82 ± 8.4 and $35.28 \pm 0.8.6$, respectively ($P = 0.851$) [Table 3].

DISCUSSION

To our knowledge, this was the first study to compare the intrabony morphological parameters between CBCT and digital IOPA techniques. The mean age of the patients included in this study was 35 years and there was an equal distribution of genders between the two groups.

The normal bone height in relation to CEJ ranges from 1 mm to 3 mm in patients without periodontal disease. The mean alveolar bone height in relation to CEJ For adults with mean age

Table 1: Interexaminer comparison of cone-beam computed tomography

Examiner	n	Mean rank	Mann-Whitney U	P
Width				
1	25	25.40	310.0	0.961
2	25	25.60		
Total	50			
Depth				
1	25	25.58	310.5	0.969
2	25	25.42		
Total	50			
Height				
1	25	25.74	306.5	0.907
2	25	25.26		
Total	50			
Angle				
1	25	25.46	311.5	0.984
2	25	25.54		
Total	50			

Mann-Whitney U-test. $P < 0.05$. n – Number of subjects; P – Probability value

of 35 years was 1.4mm and it was in accordance with previous literature. Therefore, the mean alveolar bone height above 1.4 mm from the CEJ was considered as the level for the presence of periodontal disease.^[14]

The results of the Mann-Whitney U-test for comparison between the measurements made by the two examiners showed $P < 0.05$ for all the measurements indicating that the scores were nonvariant for data collected by the examiners. The results demonstrate a high agreement between the two examiners, revealing good calibration and reliability of the results of the study.

In detection of the bone loss patterns, no statistical difference was seen between the two groups. Buccal and palatal/lingual surface measurements in axial section measurements were not compared due to consideration with limitations of periapical images. The agreement of the absolute measurement evaluation was made between the two examiners, $P < 0.05$ depicts that for

Table 2: Interexaminer comparison of intraoral periapical

Examiner	n	Mean rank	Mann-Whitney U	P
Width				
1	25	25.54	311.5	0.984
2	25	25.46		
Total	50			
Depth				
1	25	25.52	312.0	0.992
2	25	25.48		
Total	50			
Height				
1	25	25.32	308.0	0.930
2	25	25.68		
Total	50			
Angle				
1	25	25.52	312.0	0.992
2	25	25.48		
Total	50			

Mann-Whitney U-test. $P < 0.05$. n – Number of subjects; P – Probability value**Table 3: Comparison of various parameters between cone-beam computed tomography and intraoral periapical techniques**

Group	Mean±SD	P
Width		
CBCT	3.2080±1.16937	0.970
IOPA	3.2200±1.10980	
Depth		
CBCT	7.9160±2.40289	0.769
IOPA	7.7160±2.38357	
Height		
CBCT	3.9000±1.26820	0.794
IOPA	3.8080±1.20897	
Angle		
CBCT	35.2800±8.64831	0.851
IOPA	34.8240±8.45204	

Independent t-test. $P < 0.05$. SD – Standard deviation; IOPA – Intraoral periapical; CBCT – Cone-beam computed tomography; P – Probability value

the assessment of bone loss in both buccal and lingual/palatal surfaces, reliably cross-sectional slices can be used. An agreement in identifying vertical bone loss in 95% of the cases was present between the two examiners.

The results demonstrate a similar accuracy of periodontal bone level measurements when compared to Vasconcelos study.^[13]

The delineation of lamina dura, bone quality, and contrast rating remains better for the digital intraoral images, which contain a higher resolution compared with CBCT.^[12] The present study shows that there were no statistically significant differences observed in assessing bone level measurements in CBCT and digital intraoral radiographic techniques which were similar to the study conducted by Vandenberghe *et al.*^[12]

With respect to width and depth of the defect, no statistically significant difference was observed, which is similar to Misch *et al.*'s study.^[7] In contradiction to the present study, Mol and Balasundaram conducted a study on human skulls where they demonstrated that CBCT was slightly more accurate than intraoral radiographic techniques.^[6]

Various authors had emphasized the importance of defect angle in the establishment of prognosis and calculating the amount of

bone fill after intrabony defect treatment. For the determination of CAL gain in intrabony defects, radiographic defect angle can be used as a presurgical parameter when Guided Tissue Regeneration (GTR) was used for the treatment according to Cortellini and Tonetti.^[15] Small defect angles (0–45) have greater defect fill potential in periodontal surgery when compared to wide angles (45–90) in the analysis done before the treatment phase.^[16,17] In the present study, the mean radiographic defect angles compared between CBCT and Digital IOPA were 35.28 ± 8.64 and 34.80 ± 8.4 , respectively. No statistically significant difference between the two groups in preoperative assessment of defect angle was observed.

In this study, we found that the measurements of height, depth, and defect angle of vertical bone defect obtained by the two techniques were very close, and the difference in the measurements was not significant. The studies used defect angle at baseline with end treatment results in periodontal surgery by enamel matrix derivative and by using a DIA tool.^[18,19]

The limitations of the present study were small sample size and less duration of the study. As with CBCT, the presence of metallic restorations and high resolution is required for the study of intrabony defect morphological characteristics in periodontal diseases.

CONCLUSION

Radiographic defect angle plays a prominent role in assessing the prognosis of the intrabony defects in periodontal disease. Hence, presurgical measurement of these parameters is important and with the drawbacks of such as high radiation exposure, unavailability, and high financial cost, digital IOPA with digital software can be used as an alternative to CBCT for measuring intrabony defect morphological characteristics in periodontitis patients.

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Conflicts of interest

There are no conflicts of interest.

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Influence of Female Sex Hormones in Different Stages of Women on Periodontium

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ABSTRACT

The primary etiologic factor for periodontal diseases is “Dental plaque.” Although pathogenic bacteria in dental plaque are required for the incidence of periodontal disease, a susceptible host is also very important. The susceptibility of the host can be modified by many systemic factors with hormones level being one. The periodontium shows an exaggerated inflammatory response to plaque modified by female sex hormones during puberty, pregnancy, in women taking oral contraceptives, and at the postmenopausal stage. This review provides an in detail analysis of how periodontium is influenced by the fluctuation in sex steroid hormones of females during different phases of their lifetime and to discuss how much the same hormone at different ages and stages shows an exaggerated gingival response to plaque.

KEYWORDS: *Female sex hormones, menopause, periodontium, pregnancy, puberty*

INTRODUCTION

The periodontium, defined as the tissues that surround and support the teeth, includes the gingivae, bone, cementum, and periodontal ligament. Periodontitis is the result of an inflammatory response by the host to a bacterial infection of periodontal tissues. Numerous species of pathogenic bacteria present in subgingival plaque can interrupt the routine homeostatic processes, attack the surfaces of the tooth, infiltrate periodontal tissues, and organize themselves in the form of biofilm.^[1] The host’s immune response, triggered by microorganisms and their metabolites, stimulates the synthesis and release of cytokines, inflammation mediators, and metalloproteinases of the matrix, leading to the damage of the tissues.^[2] The disease progression and severity depend on the aggressiveness of the subgingival biofilm which is countered by the host’s immune response^[3,4] and further modulated by the genetic and epigenetic context and also environmental factors such as gender, age, smoking, and oral hygiene.^[5,6] It is clearer that, due to bacteremia and the systemic release of endotoxins, the presence of pathogenic bacteria in periodontal lesions is connected to various systemic disorders, including cancer, diabetes mellitus, rheumatoid arthritis, cardiovascular disease, infertility, and adverse birth outcomes.^[7] Female periodontium

undergoes developmental changes beginning at puberty and progressing through menstruation, pregnancy, and menopause depending on the levels of sex hormones in the body.

SEX HORMONES AND PERIODONTAL CHANGES

Ovaries secreting estrogen is responsible for the maintenance of secondary sex characteristic and uterine growth while progesterone influences the second half of the menstrual cycle and pregnancy. Progesterone influences the vascularity of the gingival and periodontal tissues through the receptors present in them.^[8,9]

This leads to the production of prostaglandins and the movement of polymorphonuclear leukocytes further leading to increases inflammation while estrogen increases the inflammatory component of gingiva without deposition of bacterial plaque.^[9]

Raised hormonal content is act as a feeder and growth factor for black-pigmented bacteria including

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Bacteroides, *Prevotella* *intermedia*, and the *Capnocytophaga* species. Studies supporting this hypothesis have shown that preeclamptic women are infected in higher proportion as compared to nonpreeclamptic or normal pregnant women.^[10]

Hormonal fluctuations during a female's lifetime may produce an exaggerated inflammatory response to dental plaque, resulting in gingivitis. Dental plaque deposition and high inflammatory gingiva (bleeding and redness) are typical features of hormone-associated gingivitis. Symptoms vary according to individuals' responses to hormonal changes. Susceptibility to infections (e.g., periodontal infection) increases due to variations in the immune system and can be elucidated by the hormonal changes and suppression of T-cell activity, decreased neutrophil chemotaxis and phagocytosis, altered lymphocyte response and depressed antibody production, chronic maternal stress, and even nutritional deficiency concomitant with increased nutritional demand by both the mother and the fetus. The amount of dental plaque and inflammation is not dose dependent; thus, even in a small quantity of irritants can induce heightened response depending on the individuals' immune reaction.

Hormone-associated gingivitis is reversible following puberty or pregnancy, and no radiographic bone loss is observed.^[11] The incidence of periodontitis can be as high as 23% in adult women, but half of the women with periodontitis above the age group of 55 years still have healthy teeth.^[12]

As a woman's health changes throughout her lifetime from prepuberty to puberty to postmenopausal, the body undergoes continuous changes in hormonal levels throughout the life cycle, thus leading to change in the body environment with specific oral health implications. Furthermore, menopausal and postmenopausal women have low levels of sex hormone levels, which also have some oral health problems associated with gingival.^[13]

PUBERTY

Puberty is associated with increased levels of progesterone and estrogen levels. These hormones act as a growth factor for the periodontis causing bacteria to grow and dwell in this specific age group; thus, periodontis is more common in the pubertal age group as compared to children in their prepubertal stage. Gingivitis associated with hormonal presence is highly sensitive to their presence and they return to normal condition in the circumpubertal stage. Most females with healthy gingivae likely will not develop significant periodontal changes. A higher population of bacteria in subgingival pockets is present during puberty, which may selectively accumulate estradiol and

progesterone.^[9] Meticulous oral home care consisting of regular brushing and flossing and routine dental visits may help prevent the development of gingivitis.

MENSTRUATION

Many women report an increase in gingival inflammation and discomfort associated with their menstrual cycle. "Gingival inflammation was lower during menstruation than during ovulation and premenstruation. "This may be attributed to the hormone known as serum estradiol, which is a natural form of estrogen that peaks and drops during ovulation and premenstruation. During the luteal phase of the menstrual cycle, levels of progesterone peak leading to elevated inflammatory changes in gingiva and periodontium. It is at its peak at the beginning of the cycle which eventually subsides with time. This can be hypothesized due to changes in bacterial flora.^[14]

PREGNANCY

Maternal periodontal disease strongly influences pregnancy outcome is a well-known fact.^[15] Researchers consider that hematogenous transport of bacteria and/or pro-inflammatory mediators from sites of periodontal infection into the placenta, fetal membranes, and amniotic cavity induces pathological processes that lead to these adverse outcomes. An exaggerated gingival inflammatory response to dental plaque is the primary cause of gingivitis, which usually starts around the 2nd month of pregnancy and generally resolves following parturition. In addition, pregnancy may accelerate the development of periodontitis (deep pockets and bone loss around teeth).^[16] There may also be a link between periodontitis and adverse pregnancy outcomes, including preterm delivery and low birth weight babies.^[17]

Periodontal treatment of the pregnant patient has been controversial. Findings from one clinical study reported that periodontal treatment during the second and third trimesters of pregnancy is safe, but the risk of adverse effects, such as preterm delivery (birth occurring before 37 weeks of pregnancy), low birth weight, fetal growth restriction, or preeclampsia, is not reduced.^[18,19] On the other hand, data from four clinical trials found that periodontal treatment in pregnant women may lower the incidence of preterm delivery and low birth weight babies. Maternal and fetal exposures to Gram-negative periodontal bacteria may trigger inflammatory events in both the mother and the fetus, which may stimulate the early rupture of membranes and parturition.^[20]

USE OF ORAL CONTRACEPTIVES

Oral contraceptives enhance periodontal break down reducing the resistance to dental plaque and can

induce gingival enlargement in otherwise healthy females.^[21] The long-term use of oral contraceptives may cause clinical attachment loss, increased gingival inflammation, and gingival enlargement. Therefore, it is expected that the same gingival changes seen during pregnancy will also be seen in women taking oral contraceptives. Women using contraceptives have poor periodontal and gingival health. Currently, there are newer oral contraceptive formulations in the market which contain a lower concentration of hormones, resulting in a milder inflammatory response of the gingival to dental plaque.^[22]

MENOPAUSE

Menopause is associated with important systemic and oral changes. The sudden decrease in estrogen levels that happens in menopause is considered to be the main cause of primary osteoporosis, which also affects jawbones. It has been suggested that this reduction in bone mineral density could contribute to the progression of periodontal disease. Besides their effect on bone, estrogens also interfere with other periodontal tissues (gingiva and periodontal ligament) and influence host immune-inflammatory responses. Change in salivary viscosity, especially in postmenopausal women, also leads to a change in the microbial environment of the periodontium.

The relationship between menopause and periodontal disease is difficult to establish due to the multitude of factors involved. If any relationship is found, it will always be less significant comparing to other well-known risk factors of periodontal disease.^[23]

Bisphosphonate-associated osteonecrosis of the jaws is characterized by pain or swelling in the affected jaw, an irregular mucosal ulceration with exposed bone in the mandible or maxilla, and infection. The pharmacist and dentist need to be aware that the patient will be or currently is taking a bisphosphonate. Commonly prescribed bisphosphonates include zoledronic acid (Zometa), pamidronate (Aredia), alendronate (Fosamax), ibandronate (Boniva), risedronate (Actonel), tiludronate (Skelid), clodronate (Bonafos), and etidronate (Didronel).^[24,25]

Additional oral symptoms experienced by postmenopausal women include burning oral sensations – especially on the tongue – altered taste sensations, and a decrease in salivary flow. If xerostomia is present, dental plaque may accumulate secondary to reduced salivary function/flow, leading to caries and, possibly, gingivitis.^[26]

CONCLUSION

Female sex hormone influences the bacterial flora further leading to onset and progression of the gingivitis and periodontal disease during puberty, menstruation, and pregnancy.

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Original Article

Cleaning efficacy of various rotary endodontic file systems in primary dentition

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ABSTRACT

Aim: The aim of this study was to compare the cleaning efficacy of three different rotary instrumentation systems in root canal preparation of primary anterior teeth.

Materials and Methods: Thirty primary anterior teeth were freshly extracted and divided into three groups of ten teeth each. In Group I, root canals were instrumented with ProTaper, Group II with K3, and Group III with Hero 642 rotary file systems. After canal preparation, the teeth were sectioned longitudinally and visualized under standard error of the mean at coronal, middle, and apical thirds. Debris and smear layer removal efficacy was evaluated, and the data obtained were subjected to Kruskal–Wallis ANOVA test and Mann–Whitney *U*-test.

Results: Statistically significant difference was noted in debris and smear layer scores at apical thirds ($P < 0.05$) between three groups with the highest efficacy for ProTaper followed by K3 and Hero 642 rotary systems. At coronal and middle thirds, K3 performed better than ProTaper file system.

Conclusion: All the three groups showed decreased smear layer removal at the apical third. However, ProTaper and K3 rotary files showed significantly better cleaning efficacy at the apical third when compared to Hero 642 rotary files.

Keywords: Debris removal, Hero 642, K3 files, ProTaper rotary files, smear layer

INTRODUCTION

Pediatric endodontics is a branch of dentistry that deals with the management of pulpal pathology in children. Early loss of primary teeth leads to loss of space, so as to maintain the tooth in its normal physiological function in the dental arch till its normal exfoliation pulpal procedures like pulpectomy are carried out in the teeth with infected pulp tissue.^[1]

Till date, root canal preparation has been made with a variety of instruments and techniques. In 1962, Nickel–titanium (NiTi) alloys were first developed, and in 1988, Walia announced that root canal files were made of this NiTi alloy which consists of 55% (w/w) nickel and 45% (w/w) titanium.

NiTi files have 2–3 times higher elastic flexibility in bending and torsion as well as superior resistance to corrosion when compared with stainless steel files. In the present era, several rotary NiTi endodontic systems have been familiarized in the market. The rotary system should be of the right choice as the individual designs and features affect the performance of the rotary instruments.^[2]

Smear layer is formed on the walls of the canal during biomechanical preparation, which is composed of inorganic and organic particles, bacteria, and tissue remnants. The successful endodontic treatment concedes the removal of

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debris and smear layer from the root canal system. If not done appropriately and the smear layer removal is left incomplete, the antimicrobial agents are prevented from gaining access to the infected dentinal tubules. The usage of rotary files in primary teeth was first reported by Barr *et al.* in 1999. Literature available on the use of rotary files in primary teeth has primarily evaluated cutting efficiency, instrumentation time, and shape of the prepared canals.^[3]

Ample studies were carried out on permanent dentition to evaluate the cleaning efficacy of these rotary file systems. However, very few studies were reported in the literature regarding their usage in primary teeth.^[1,3] To rationalize the use of rotary files in pediatric endodontics, the present *in vitro* study was conducted to compare the cleaning efficacy of ProTaper, K3, and Hero 642 rotary file systems in deciduous teeth at coronal, middle, and apical thirds in terms of debris and smear layer removal using a scanning electron microscope.

MATERIALS AND METHODS

After obtaining the patient informed consent, thirty freshly extracted human primary anterior teeth were selected for the study. Teeth with at least two-thirds of intact roots and no evident developmental defects/anomalous morphology were included in the study. These teeth were extracted from healthy children as they were retained beyond the normal time of exfoliation, whereas the teeth showing radiographic evidence of internal resorption or root canal obliteration and grossly decayed teeth were excluded from the study.

After attaining the institutional ethical committee approval (Pr. 34/IEC/SIBAR/2015) regarding the study design, the selected teeth were divided into three groups of ten teeth each according to the file systems used: Group I – ProTaper (Dentsply Maillefer, Asia), Group II – K3 (SybronEndo, Mexico), and Group III – Hero Shaper (MicroMega, France), respectively.

The selected teeth were mounted vertically in impression compound up to the cervical region. The coronal access cavity was prepared using a round diamond bur, and the canal patency was evaluated using #15 hand K-file. Working length was determined using radiovisiography for individual teeth.

In Group I, samples were instrumented with ProTaper in the following sequence: SX – for coronal flaring and S1, S2 – shaping the canals up to the estimated working length and finished with F1 and F2 files. Group II samples were instrumented with K3 SX in the following sequence: 0.10 – orifice shaper, 0.08 – one-third of working length, and 0.06 up to the estimated working length, whereas in

Group III samples, Hero Shaper 642 file systems were used in the following sequence: 0.06–20 for coronal enlargement, 0.04–20 – two-third of working length, and 0.06–25 up to the predetermined working length.

For all the three file systems, a standard diameter of 0.06–25 was used for apical enlargement. Biomechanical preparation was done with predetermined rotary file systems with EDTA gel lubrication following the manufacturer's instructions for each file system in crown-down technique. Later, irrigation of the canals was done with copious amount of saline and 5.25% of NaOCl following the use of each file and finally dried with paper points. Each of these instruments was discarded after usage for five subsequent times.

The teeth were sectioned at the coronal portion using a diamond disc. Two longitudinal grooves were prepared on the midline of palatal/lingual and buccal surfaces of each root using high-speed handpiece with a diamond disk to facilitate vertical splitting. Later, they were separated into two halves using chisel and mallet. The section with the most visible part of the apex was conserved for examination under the scanning electron microscope. The sections were then allowed to air dry in a desiccator at room temperature and attached to coded metal stubs and sputter with 10-nm/m gold–palladium alloys. These sections were further examined under scanning electron microscope, and photomicrographs were taken at $\times 200$ and $\times 2000$ magnification at the coronal, middle, and apical thirds. The geometric center of each third was observed and separate evaluations were recorded for debris and smear layer removal by means of the Numerical Evaluation Scale which was put forth by Hülsmann *et al.*, 1997.

The whole procedure and scoring was performed by a single operator. To ensure intra-examiner consistency, the photomicrographs were randomly evaluated by a second investigator who was blinded to the groups and was trained prior to the recording. As there was no statistically significant variation in the values between the two examiners ($P < 0.05$), the scores recorded by the first operator were only considered and the values obtained were tabulated and statistically analyzed using SPSS (version 18). Kruskal–Wallis ANOVA test and Mann–Whitney *U*-test were used to determine the inter- and intragroup difference, with a significance level of $P < 0.05$.

RESULTS

Results revealed that less amount of debris scores were detected at the apical third of root canals in all three groups compared to coronal and middle thirds, which is highly

significant [$P < 0.01$; Table 1]. The ProTaper and K3 (2.6) instruments showed less debris score at apical third of root canals which is statistically significant when compared to Hero 642 (3.6) rotary instruments [$P < 0.05$; Table 2].

In terms of smear layer, no significant difference ($P = 0.19$) was apparent in coronal, middle, and apical thirds of Group I, but significant difference was noted in Group II and III [$P < 0.05$; Table 3]. Smear layer removal efficacy at the apical third of root canal was superior in Groups I and II (3.70) compared to Group III samples (5.0), which was statistically significant. In the middle third, Hero 642 showed a lesser smear layer score (2.0) when compared to Groups I and II, which is highly significant statistically ($P < 0.01$), whereas no significant difference was noted between the three groups in the coronal third [Table 4].

DISCUSSION

The complexity of root canals in primary teeth makes it a challenge and time-consuming in children, especially during the preparation of the canal. Thus, rotary files are considered more convenient and appropriate to facilitate their use in root canal treatment in children. Canal preparation is done to debride the walls and is considered imperative for primary root canal treatment. Even though the principle behind the mechanical cleaning and shaping of root canals is analogous for both primary and permanent teeth, careful handling is required for narrow and fine canals of primary teeth. Anterior primary teeth with relatively straight canal morphology were selected for standardization in the present study.^[3]

Vaudt et al.^[5] reviewed that individual design features affect the performance of NiTi rotary instruments. Various instrument designs with noncutting tips, different cross-sections, radial lands, and tapers are now available to improve the working efficiency.^[6] Among them, ProTaper, Hero Shaper, and K3 systems have been selected for this study because they have different cross-sectional configurations and variable cutting efficiency and all the three systems were made of the same material (NiTi).

For the preparation of root canal, crown-down technique was used as it reduces friction between instruments and forms a smooth funnel shape that allows deeper penetration of needles and irrigating solutions during instrumentation and minimizes the risk of instrument separation.^[7]

All endodontic instruments create dentin debris and a smear layer as a consequence of their action on root canal walls.^[8,9] Debris is demarcated as dentin chips and the residual vital or necrotic pulp tissue attached to the root canal wall is

Table 1: Intragroup comparison of mean debris scores at coronal, middle, and apical thirds

Groups	Parameter	Mean	SD	P	Inference
I	Coronal one-third	4.80	0.42	<0.01	HS
	Middle one-third	3.80	0.63		
	Apical one-third	2.60	0.52		
II	Coronal one-third	4.20	1.14	<0.01	HS
	Middle one-third	3.50	0.71		
	Apical one-third	2.70	1.06		
III	Coronal one-third	4.60	0.70	<0.01	HS
	Middle one-third	4.20	0.63		
	Apical one-third	3.60	0.84		

$P < 0.01$ HS. SD: Standard deviation, HS: Highly significant

Table 2: Intergroup comparison of mean debris score at coronal, middle, and apical thirds

Parameter	Groups	Mean	SD	P	Inference
Coronal one-third	I	4.80	0.42	0.26	NS
	II	4.20	1.14		
	III	4.60	0.70		
Middle one-third	I	3.80	0.63	0.08	NS
	II	3.50	0.71		
	III	4.20	0.63		
Apical one-third	I	2.60	0.52	<0.05	S
	II	2.70	1.06		
	III	3.60	0.84		

$P < 0.05$ statistically S, $P > 0.05$ NS. S: Significant, NS: Not significant, SD: Standard deviation

Table 3: Intragroup comparison of mean smear layer removal scores at three different areas of the root canal

Groups	Parameter	Mean	SD	P	Inference
I	Coronal one-third	3.70	1.25	0.19	NS
	Middle one-third	4.40	0.97		
	Apical one-third	3.70	0.48		
II	Coronal one-third	3.30	0.48	<0.05	S
	Middle one-third	2.60	0.52		
	Apical one-third	3.70	1.25		
III	Coronal one-third	3.00	0.00	<0.05	S
	Middle one-third	2.00	0.00		
	Apical one-third	5.00	0.00		

$P < 0.05$ statistically S, $P > 0.05$ NS. S: Significant, NS: Not significant, SD: Standard deviation

Table 4: Intergroup comparison of mean smear layer removal scores at coronal, middle, and apical thirds

Parameter	Groups	Mean	SD	P	Inference
Coronal one-third	I	3.70	1.25	0.15	NS
	II	3.30	0.48		
	III	3.00	0.00		
Middle one-third	I	4.40	0.97	<0.01	HS
	II	2.60	0.52		
	III	2.00	0.00		
Apical one-third	I	3.70	0.48	<0.01	HS
	II	3.70	1.25		
	III	5.00	0.00		

$P < 0.01$ HS, $P > 0.05$ NS. HS: Highly significant, NS: Not significant, SD: Standard deviation

infected in most of the cases. Therefore, the risk for bacterial contamination increases due to the presence of debris that ultimately leads to endodontic failure.^[10] Smear layer is a thick surface film of 1–2 μm containing dentin debris, bacteria, and residual pulp tissue that remains on the dentinal walls during root canal instrumentation. Complete removal of smear layer and debris facilitates the diffusion of the irrigants/medications to the root canal system, thereby improving the adaptation of the filling materials to the root canal dentin which in turn reduces the apical and coronal microleakage.^[11]

Under the scanning electron microscopy, $\times 200$ magnification was employed for scoring debris as it offers a wider view, thus permitting us to detect large fragments of dentinal debris. Whereas $\times 2000$ magnification was used for scoring the smear layer as higher magnification covers very small surface and gives accurate information, it also allows proper visualization of the dentinal tubule openings.^[12]

It was observed that irrespective of the file system used for instrumentation, the prepared root canals exhibited maximum debris and smear layer score of 5 in few examined areas, indicating that cleanliness was not thorough due to self-centering and superelasticity of rotary instruments. Irrespectively, as the instrument remains in the center of the canal due to its rotating movement, all the areas are not being instrumented equally.^[13,14]

Comparatively less debris scores were obtained at the apical third of prepared root canal with the three selected systems of rotary files compared to coronal and middle thirds. Taha *et al.*,^[15] Rahimi *et al.*,^[16] and Júnior *et al.*^[17] also reported less debris scores at an apical third of root canals. The probable explanation for this could be the oval shape of the root canal in the coronal, middle third and a round shape as it proceeds apically.^[18] The dentin particles removed from the canal wall are carried coronally by flutes of the file, due to the use of rotary files with round cross-section. This removal was apparently less effective when the canal is oval in cross-section. The oval-shaped canal space hampers the working file being in touch with all the surfaces of root dentin. In such conditions, the debris that is carried coronally or being contained and packed in the file's flute space, gets packed actively into the area with the least resistance. It is conceivable to hypothesize that dentin particles were actively packed into soft-tissue remnants in unprepared areas that are usually resistant to the irrigation with syringe and needle.^[4] In contrary to the present study, previous standard error of the mean studies reported that the amount of smear layer and debris is greater at the apical third than compared to the middle and

coronal thirds of the canals.^[19] Kadhom and Al-Hashimi^[18] reported that ProTaper rotary file system shaped a clean canal at the coronal and middle thirds but was incapable of removing the debris at a similar rate at the apical third of the canal. However, studies that are correlated to primary teeth found no significant difference in the cleaning capacity of rotary instrumentation techniques at all the three areas of root canal based on dye penetration method.^[19,20] The difference in the outcome among various studies could be due to the difference in tooth selection, rotary instruments used, technique followed, irrigation solutions used, and the operator's performance.^[20]

Pertaining to the smear layer removal efficacy, the present study showed a significant difference in the middle third between the three groups. The reasons for removal of more amount of smear layer with Hero 642 and K3 NiTi files include triangular blade design with sharper edge and the usage of more number of instruments with chelator (EDTA) for canal preparation.^[21]

At the apical third, significant difference was observed between the three groups. ProTaper and K3 showed a significant increase in cleaning efficacy both in terms of smear layer and debris removal when compared to Hero 642. This might be attributed to cross-sectional design with a slight positive rake angle and increased helical angle from tip to handle in K3 file system.^[22] ProTaper instruments exhibit a unique variable taper design with a triangular cross-section and reduced radial lands that might allow the file to move freely within the canal and contribute to more aggressive and unconstrained cutting.^[21]

On the contrary, a study conducted by Amritha and Sureshchandra^[22] showed that K3 instruments left more debris and smear layer compared to Hero 642 instruments. The explanations for the result might be due to individual variations within the instruments and "to the core" adherence with manufacturer's instructions.

The factors to be considered while selecting rotary file systems for primary teeth include appropriate rotary instrumentation with lesser cutting efficiency and proper irrigation. A thorough knowledge and clinical expertise of the rotary system is also essential to avoid perforation of thin dentinal walls and apical extension. Further studies are recommended using different instrumentation techniques together with irrigants in the root canals of primary teeth.

CONCLUSION

- All the three file systems showed comparatively less debris scores at the apical third portion when compared to middle and coronal thirds. However, ProTaper and K3 file systems showed better debris removal compared to Hero 642 at the apical third
- In all the three groups, coronal and middle third areas showed less smear layer scores when compared to apical third. Hero 642 and K3 NiTi files displayed better smear layer removal at the middle third when compared to ProTaper NiTi files
- ProTaper and K3 files exhibited significantly better cleaning efficacy in terms of smear layer and debris removal in the apical third when compared to Hero 642 rotary file system.

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Conflicts of interest

There are no conflicts of interest.

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EVALUATION OF BITE MARKS ON SKIN AND AN IN ANIMATE OBJECTS: AN ORIGINAL RESEARCH

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ABSTRACT

Aim: The objective of the study is to compare the accuracy of bite marks on an inanimate substance (fruit) and a living tissue (skin) using digital analysis.

Methodology: A cross-sectional study was conducted involving 15 volunteers. The registered bites of individuals on inanimate object (fruit) and living tissue (skin of forearm) were photographed with the American Board of Forensic Odontology scale No. 2 in the view field immediately after the production of bite marks. Dental casts of the individuals were obtained and photographed out of which computer-assisted overlays were generated, and analysis was carried out digitally using Adobe Photoshop version 7. Statistical analysis was performed using IBM SPSS software, version 22.

Results: Skin had a comparable accuracy to that of an inanimate object which is statistically attested.

Conclusion: The source of bite marks, the substrate onto which they are generated and the technique of lifting the bite imprints serve as important tools in analysis.

Keywords American Board of Forensic Odontology guidelines, bite marks, Forensic odontology.

I. INTRODUCTION

Bite marks are often observed at crime scenes on various parts of the human body, although they are more common in certain parts of the body.¹ In addition, no body part is immune to bite marks.² These marks are not uncommonly observed in physical assault cases³ but are more common in sexual assault cases⁴ and are observed in both homosexual and heterosexual cases. These pieces of evidence have been used successfully to prosecute offenders.⁵⁻⁷ Bite marks have also been observed on various edible leftovers at the crime scenes,⁸ and these bite marks have also been used as evidence for identifying the criminals. Bite marks on inedible objects have also been reported,⁹ including on soap¹⁰ and bullets.¹¹ Bite marks have also been studied on clay,¹² and one case has been solved based on bite marks on clay.¹³ According to Pretty and Turnbull, the central dogma of bite mark analysis is based on two assumptions.¹⁴ The first is that human teeth are unique, and the second is that sufficient

detail of the uniqueness is rendered during the biting process to enable identification. Various experimental studies have been conducted on various food items, human skin and animal skin to determine similarities and dissimilarities of the bite marks and the teeth of the volunteers. Skins of dogs, pigs and sheep have been used to study bite marks.¹⁵⁻¹⁷ Studies have also been performed on wax,¹⁵ chewing gum⁷ and clay^{12,13} to develop better methods. Computers have also been used for this comparison,¹⁸ and 3D studies have been conducted¹⁹ using scanning electron microscopy.²⁰ Several studies have even used radiographs, including computed tomography (CT) scans, to compare bite marks.^{21,22} Bite marks are lifted using various photography techniques and other materials.²³ According to West et al. photographs of bite marks should be obtained as early as possible because of changes due to vital reactions.²⁴ According to McNamee and Sweet, the use of digital cameras has the advantage that it could provide instant preview and does not require scanning of the photographs.²⁵ Reflected ultraviolet imaging techniques may be the future of recording of evidence²⁶ and may be used for bite marks, as well. Casts of the volunteers, suspects and offenders have been made using standard techniques, and the casts have been compared using various methods with different types of overlays and varying success rates. Different techniques for preparing the transparent overlays are used to determine the usefulness of various methods. The data available have been questioned because they have been obtained using animal skin or skin from a small number of volunteers.^{15,17,27,28} West et al. felt that bite marks on human skin can be experimentally created to a level that permits comparison to bites delivered in combative or life threatening situations, and more research is needed using living subjects to explore a variety of experimental situations.²⁴ Although clay may not be observed to have bite marks in actual cases, the American Board of Forensic Odontology advises to use clay as the test bite media.²⁹ With various advantages and disadvantages, bite marks appear to be useful evidence for the identification of the perpetrator.³⁰

AIM OF THE STUDY

This study aims at the evaluation of the bite marks for analysis on a living tissue like skin and an inanimate object like fruit. The objective of the study being the appraisal of the accuracy of registered bite marks on respective substrates using digital analysis.

II. METHODOLOGY

Fifteen volunteers participated in the study and informed consent was obtained. The individuals participating in the study were asked to bite on an inanimate substance such as fruit with a pressure sufficient for the bite imprints to be generated with a care that the food substance (fruit) is not sheared. The bite marks thus imprinted on the surface of fruit were recorded using a camera immediately with a care that the registered bite marks were neither distorted nor deteriorated placing the American Board of Forensic Odontology (ABFO) scale No. 2 in the view field immediately after the production of bite marks to generate life-size images for analysis. The volunteers were then made to bite on their forearm after disinfecting the site. The registered bites were photographed in a similar procedure placing ABFO scale No. 2 in the view field immediately after the production of bite marks. Dental casts of the individuals were obtained using alginate impression material and dental stone. They were photographed and all the life-size images were collected with the reference scale in the field of view. The images were then subjected for digital analysis using Adobe Photoshop (Version 7). The overlays obtained from bite marks on living tissue (hand) and the inanimate object (the fruit) were compared individually with the overlays of dental casts by superimposition method.[4] When the overlays of incisal edges and cuspal tips of all the anterior teeth were apparently superimposed on to the ones generated from bite mark overlays with the alignment of each tooth and anterior arch as a whole, a Grade A was given. When the overlays were comparatively superimposed but not accurate in terms of alignment, a Grade B was assigned. No single tooth in an arch among the registered teeth in a bite mark overlay is superimposed onto the ones on dental cast; a Grade C was allocated. The grades obtained separately on living tissue and an inanimate object were compared for the accuracy of the objects for bite marks production and their analysis. The collected data were analyzed with IBM SPSS statistics software 22.0 Version. To describe the data descriptive statistics frequency analysis, percentage analysis was used. To find the significance in categorical data, Chi-square test was used. In the above statistical tools, the $P < 0.05$ is considered as significant level.

III. RESULTS

In the present study, evaluation of the maxillary bite imprints on skin asserts that the percentage of overlays with Grade A superimposition was 20%, Grade B accounts to be 24%, and Grade C was found to be 56%, whereas on fruit (inanimate object), the percentage of overlays with Grade A superimposition was 56%, with Grade B the percentage was 24%, and Grade C accounted to be 20%. Evaluation of the mandibular bite imprints on skin

asserts that the percentage of overlays with Grade A superimposition was 20%, Grade B accounts to be 32%, and Grade C was found to be 48% [Table 1, 3], whereas on fruit (inanimate object), the percentage of overlays with Grade A superimposition was 56%, with Grade B the percentage was 36%, and Grade C accounted to be 8% [Table 2, 4]. Although clinically, the percentage of distribution asserts that the fruit has a better accuracy in registration and analysis of bite marks, the statistical analysis was not in accordance with the same ($P > 0.05$).

Table 1- The percentage of frequency among three different grades of maxillary and mandibular bite imprints on skin

Grade	N (%)
Maxillary	
Grade A	5 (20.0)
Grade B	6 (24.0)
Grade C	14 (56.0)
Mandibular	
Grade A	5 (20.0)
Grade B	8 (32.0)
Grade C	12 (48.0)

Table 2- The percentage of frequency among three different grades of maxillary and mandibular bite imprints on fruit

Grade	N (%)
Maxillary	
Grade A	14 (56.0)
Grade B	6 (24.0)
Grade C	5 (20.0)
Mandibular	
Grade A	14 (56.0)
Grade B	9 (36.0)
Grade C	2 (8.0)

IV. DISCUSSION

The exact identification of a living person using individual traits and characteristics of the teeth and jaws is the basis of forensic science.³¹ The bite marks left on a person may be used to identify the perpetrator. Bite mark identification is based on the individuality of a dentition, which is used to match a bite mark to a suspected person. One can exactly match the bite marks to the accused biter's dentition.³² The most important step in bite mark analysis is to recognize a patterned injury as a human bite mark followed by pattern analysis of the bite mark which provide the individual information about the suspect or an offender and relate the person who is involved in the crime. Bite marks with high evidence value that can be used in comparisons with the suspects' teeth will include marks from specific teeth that record different characters. The surface abrasion or sub-surface haemorrhage caused by human bites appears as an arch. They are caused by the incisors, canines and premolars. Contusions are the most common type of bite mark. It can be determined from the type of bleeding under the skin whether the victim was alive or dead at the time the bite mark was delivered.^{33,34} Human tissue has been described as one of the least dependable substances for recording bite marks.³⁵ In a study by Gorea et al., skin of the volunteers was used, and it was observed that a match was possible in 60% of cases. The reason for the higher percentage of identification could be contributed to a different classification from that of the present study and a very small sample size (only 25 cases). Whittaker reported that matching of the bite marks on animal skin corresponded in only 76% of the cases.¹⁵ The percentage of nonsuperimposed overlays on the fruit in maxilla was 20% and on mandible 8%. Although stated in the study by Gorea and Jasujaet al. that exanimate object like clay has a better accuracy, this imprecision of mandibular overlays on fruit can be attributed to the fact that the quality of bite imprint is not only determined by the type and consistency of the substrate but also the biting process as a whole.¹³ The variability in the degree of superimpositions among maxillary and mandibular bite imprints can be attributed to the bite mechanism which is when the teeth apply pressure on the substrate with a varied force.³⁶ It begins with closure of mandible, followed by a negative pressure from the suction of skin or any other substrate, and the tongue thrust from the opposite direction; therefore, there would be projection of teeth edges and palatal surfaces.³⁷ However, in our study, patterns from other mouthparts or alterations in the bite patterns due to the pressure from other mouthparts was not perceived since all the participants were elucidated the kind of bite imprints appropriate for their analysis and the agendum was subjective. When an individual bites something, the

superior teeth stabilize the object, while the inferior teeth try to cut it. The indentation created by the superior teeth is significantly important to obtain information such as dental alignment, size, and shape of dentition.³⁸ The factors which influenced the accuracy of bite mark analysis in our study were physical nature of the foodstuff, the biting force with which bite mark was done. The time lapse in collection of the imprints was negligible which serves as an important factor when perishable substances are used for bite mark analysis.

V. CONCLUSION

The importance of bite marks and their analysis in forensics is well asserted. The source of bite marks from which they are produced, the substrate onto which they are generated and the technique of lifting the bite imprints serve as important tools in analysis and identification. Although inanimate objects serve as a better sources for analyzing bite marks, when collected immediately with an appropriate technique skin has an equivalent accuracy in bite mark analysis.

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Crowns in Paediatric Dentistry – A Review

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ABSTRACT

Over the years, there have been numerous studies regarding the restoration of primary teeth. Some of them recommended the need of a full coverage crown for restoration over the conventional class I, class II and class V restoration, others pointed out over the need of aesthetic restorations for Pediatric patients. As the technology advanced, the level of parental satisfaction with the conventional stainless steel crowns lowered, this made the Pediatric dentists to search for an aesthetic restoration for the decayed primary tooth like zirconia crowns, pediatric jacket crowns, strip crowns, glass tech crowns etc. The advent of such advanced techniques, devices and material helps in creating beautiful restorations which help children and adolescents improve their self image as we know that the child esthetics is the guide to the adult esthetics.

Keywords stainless steel crowns, aesthetic, strength, zirconia, strip crown.

INTRODUCTION

The presence of decayed or deformed teeth leads to a negative impact to infants and adolescents due to rejection by their peer group because of unpleasant dental appearance, which is reflected in their adulthood. Seehraj el al stated that dental appearance of a child influences bullying in school which has its long term and short term psychological impact.¹ Besides that, decayed tooth also leads to development of parafunctional habits like tongue

thrusting, reduced masticatory efficiency, loss of vertical dimension of occlusion and speech problems.²Humphrey in 1950 introduced stainless steel crowns, which proved to be a boon to clinical Paediatric dental practice. The stainless-steel crowns are most frequently used in deciduous dentition than permanent dentition because of the reason that even a small carious lesion can destroy the integrity faster in deciduous dentition than in the permanent teeth. But the lack of aesthetics, unacceptable metal appearance to parents, patient and beholders, limited their use to posterior teeth only.³Due to this Open faced stainless steel crowns evolved, which involves the placement of tooth coloured material by fenestrating the labial aspect of the stainless steel crowns, this combined aesthetics along with durability to stainless steel crowns.⁴ Apart from the above mentioned crowns more aesthetic crowns includes Bonded crowns, this includes Polycarbonate crowns. These are heat bonded acrylic resins mainly used to restore anterior teeth, these are more aesthetic than Stainless steel crowns, easy to trim and can be adjusted using pliers.⁵ Another aesthetic approach is Strip crowns, which constitutes the most commonly used variety of Bonded crowns. These are filled with composite and bonded to tooth this imparts superior aesthetics. However, it is technique sensitive and any lapses in patient selection, isolation, tooth preparation and resin bonding can lead to failure.⁶Pedojackets, new millennium and glass tech are other forms of Bonded crowns. Ongoing researches in the field of material sciences have led to the introduction of preformed All-Ceramic and Zirconia crowns in Paediatric Dentistry. It has similar mechanical properties like metals along with tooth coloured appearance and extreme biocompatibility.⁷

CLASSIFICATION OF CROWNS

Paediatric crowns can be broadly classified as luted or bonded depending upon the mechanism of adhesion to the tooth, in case of luted crowns the adhesion is purely physical or mechanical where as in bonded crowns, the crown is bonded to the etched enamel surface, thus establishing a mechano-chemical adhesion to the tooth surface.⁸ (Figure 1)

STAINLESS STEEL CROWN

Stainless steel crowns (SSC) are considered to be the most durable, economical and reliable for restoring severely carious and fractured primary teeth. They are easy to place, fracture proof, wear resistant and attached firmly to tooth until exfoliation.¹

Types of stainless steel crowns:

Stainless steel crowns type is based on shape:

Untrimmed crowns

The untrimmed crowns are neither trimmed nor contoured, need a lot of adaptation and are time consuming. Example of untrimmed crowns is Rocky mountain.

Pre-trimmed crowns

The pre-trimmed crowns are non-contoured but festooned to follow a line parallel to the gingival crest. They still require contouring and some trimming. Examples of pre-trimmed crowns are Unitek, 3M Co., St.paul, MN

Pre-contoured crowns

The pre-contoured crowns are festooned and are also pre-contoured though a minimal amount of festooning and trimming may be necessary. Examples of pre-contoured crowns include Ni-Chromium.

PRE-VENEERED STAINLESS STEEL CROWNS

Preformed metal crowns (stainless steel / nickel chrome crowns) out-performs the intra coronal restorations in terms of longevity as are reliable and durable. Pre-veneered stainless steel crowns provide full coverage, durability, easy placement and aesthetics. These crowns are nickel chrome crown having an aesthetic facing, mechanically and / or chemically bonded.

OPEN FACED STAINLESS STEEL CROWN

To take advantage of the strength and durability of preformed stainless steel crowns with improved aesthetics Open Faced Stainless Steel Crowns were developed. Here a composite material is bonded on the labial surface of Stainless steel crown. Open faced stainless steel crowns can be used for both anterior and posterior teeth, this involves creation of a window or labial fenestration on the cemented crown, followed by removal of the cement used for crown cementation, to make the underlying tooth tissue visible, in cases where the only little amount of tooth tissue is left and the crown is cemented using glass ionomer cements,⁹ the composite can be bonded directly over the cement after creating a few retentive grooves around the gingival areas of the crown.¹⁰

ZIRCONIA CROWNS

Zirconium oxide crowns have exceptional properties such as high flexural strength and fracture toughness, high hardness, excellent chemical resistance and good conductivity ions. Different oxides, such as Yttrium Oxide (Y₂O₃), Calcium Oxide (CaO) or Magnesium Oxide (MgO), can be added to Zirconia to stabilize it. Cyclical stresses are also well tolerated by this extremely biocompatible material. In this way it can be said that this material has the potential to be used for larger restorations and in the molar area.¹¹

POLYCARBONATE CROWNS

Polycarbonate crowns are heat-molded acrylic resin shells that are adapted to teeth with self cured acrylic resin. Though poor in strength, the polycarbonate crowns provide excellent aesthetics as compared to the conventional stainless steel crowns. They can be good restorations in anterior teeth as it presents much less masticatory load as compared to posterior teeth. If combined with microglass fibres, the microglass fibres improves the impact strength and flexibility of the crown.

STRIP CROWNS

Pediatric Strip Crowns are transparent plastic crown forms used for restoring primary anterior and posterior teeth, these crowns accounts for the most commonly used bonded crown to restore primary anterior teeth. These are the first choice restoration for many clinicians, mainly because of the superior aesthetics and the ease of repair if the crown subsequently chips or fractures.¹² Strip crowns are an easy chair side procedure and final restorations are more compatible with gum tissue than stainless steel crowns.⁴ Presently strip crowns for primary teeth are being marketed by 3M ESPE (only anteriors) and Success Essentials (both anterior and posteriors), Dentsply professionals, Directa, Unitek Strip Crown, Nowak Crowns Nowak Dental Supplies Inc. and Carriere, MS.

PEDO JACKET CROWNS

The Pedo Jacket is a crown form similar to the resin bonded strip crown, the only difference is of the "jacket" which is made up of a tooth-colored co-polyester material and is filled with resin material and left on the tooth after polymerization instead of being removed. These crowns are thin yet have a strong inter-proximal wall which allows an easy placement of

multiple adjacent restorations with a minimum amount of tooth reduction. These crowns are cost effective and are easily sized and trimmed with scissors and can be readily adapted over irregular teeth. Polyester is a synthetic polymer made of purified terephthalic acid (PTA) or its dimethyl ester dimethyl terephthalate (DMT) and monoethylene glycol (MEG).¹³

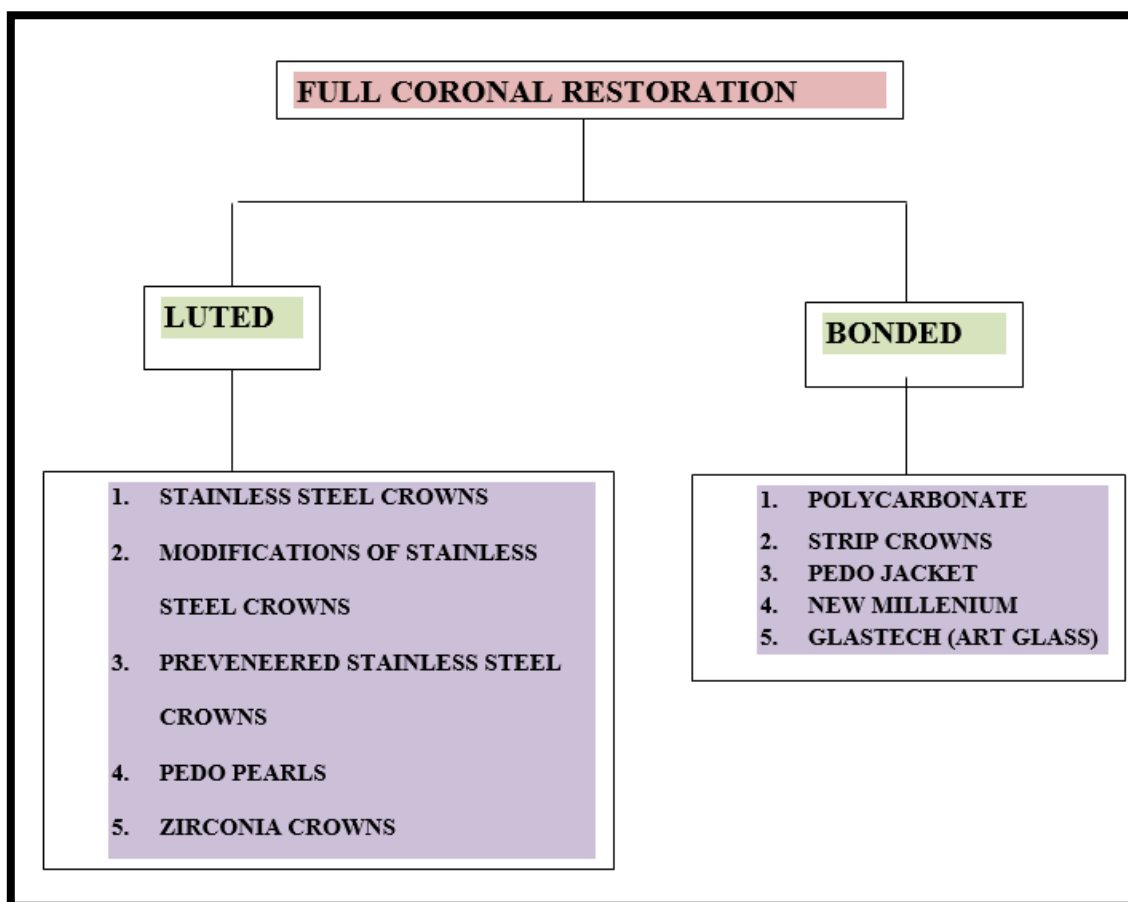
NEW MILLENNIUM CROWNS

This crown is another modification of strip crowns and similar in form to the Pedo Jacket crowns except that it is made of a laboratory-enhanced composite resin material. In contrast to the Pedo jackets these crowns can be easily finished and reshaped with a high-speed bur and a greater degree of aesthetics can be achieved. These Crowns are being marketed by success essentials, space maintainers laboratory and are available for all anterior and posterior teeth.¹⁴

PEDO PEARL CROWNS

It is a metal crown form similar to a stainless steel crown, but it is completely coated with a tooth-colored epoxy powder i.e. Polytetrafluoroethylene (PTFE), a synthetic Fluoropolymer in specific. These crowns are made of heavy gauge aluminum instead of stainless steel because the epoxy coating adheres much better to the aluminum. These crowns are being manufactured by Pedo Pearls, 6111 FM 1960 West Suite 215 Houston, TX 77069, USA, for Anteriors. Its available in sizes 1- 4 and in universal form, for Posterior 5,6.¹

Figure 1- Classification of Paediatric Crowns



GLASS TECH CROWNS

Glass tech crowns are the preformed crowns made of Artglass, which is a polymer glass, it gives a natural feel, bond ability and kindness associated with composite but the esthetics and longevity of porcelain. It is color stable, wear of polymer glass is similar to enamel, kind to opposing dentition, plaque resistant and without any composite interface. The unique filler materials of microglass and silica are proposed to provide greater durability and excellent esthetics than the strip crowns.¹²

RECENT ADVANCES IN PAEDIATRIC CROWNS

Flex crowns

They are white faced, overcome the aesthetics problems associated with plain stainless-steel crowns. These crowns can be manipulated and handled similar to the conventional stainless-steel crowns.¹⁵

Life like paediatric crowns

They are highly durable and aesthetically translucent thus are claimed to deliver natural tooth like appearance to the restored tooth. These crowns have a stable color which does not stain, discolor or fade.¹⁶ Fuks AB et al., assessed the clinical performance of esthetic crowns and compared these to conventional stainless steel crowns, for this purpose they placed twenty two crowns (11 conventional and 11 esthetic) in mandibular primary molars obeying these criterias; the tooth was not mobile; no fistulae were present; the tooth had at least one caries free or properly restored antagonist and had to be in contact with one adjacent tooth mesially, in the case of the primary second molars or distally in the case of the primary first molars and evaluated them clinically and radiographically after 6 months and concluded that the esthetic crowns assessed had several inconveniences, as they resulted in poor gingival health, are very expensive, and, although not measured, are bulky and without a natural appearance.¹⁷

Krämer et al., evaluated the effect of thermo-mechanical loading (TML) on marginal quality and wear of different crown types for primary molars. For this purpose they used eighty extracted human primary molars. After preparation, five groups received different crowns (n=16): preformed metal crowns (3M ESPE) and NuSmile crowns (Orthodontic Technologies Inc.) were inserted as preformed metal crowns; as semi-preformed crowns Protemp crowns (3M ESPE) were luted, the specimens were subjected to 2,500 thermal cycles between 5–55°C and chewing simulation for 100,000 cycles at 50N at a frequency of 0.5 Hz. Before and after thermo-mechanical loading, impressions of the teeth were taken and replicas were made. The replicas received marginal quality evaluation under a SEM at×200 magnification and depending upon their findings they concluded that the different crown types under investigation showed a good performance concerning the evaluated parameters marginal quality and wear.¹⁸

CONCLUSION

The Paediatric dentists have the responsibility and ability to create beautiful smile for young patients. By far numerous literatures are available regarding the use and introduction of various crowns for restoring the Primary teeth but very few studies are available regarding the clinical longevity and success of these restorative technique, making it difficult to recommend any one type of restorative option over the other thus the ultimate choice of

restorative technique depends upon the operator preferences, aesthetic demands by the parents, cost and child's behaviour that affect the final outcome of which ever restorative material chosen.

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A 3d Analysis of Influence of Canal Preparations on the Fracture Resistance of Molars- An Original Research

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Abstract

Introduction: The success of the root canal therapy depends on many factors. Loss of the tooth structure during the canal preparation leads to the fracture of the tooth. The rotary instruments were invented to put less force on the tooth during the preparation.

Hence in the present study we aim to compare the effect of three rotary file systems (Vortex Blue, VTaper, ProTaper,) with dissimilar tapers on fracture resistance of root canal treated teeth by various static and dynamic forces applied to different parts of the coronal part of teeth.

Materials and Methods: 3D printed acrylic maxillary first molar was used. The original geometry was changed and primed for using Spaceclaim software for ANSYS modeling and analysis. The three experimental groups and one control group were created. Group one was ProTaper, group two was Vortex Blue and group three was V-Taper. Young modulus and Poisson ratio of all the materials (Enamel, Dentin, Gutta-Percha, Composite) were used for ANSYS software to recognize these volumes and the constituents that are contained within these individual spaces. In this study seven total contact points were considered on the occlusal surface of the models during the chewing cycle. The Von Mises stress and maximum principal stress on the cervical region were calculated and investigated in four different cross sections of pre cervical dentin.

Results: The highest Von Mises stress was observed in the ProTaper group although V-Taper and Vortex Blue groups showed homogeneous stress distributions in the cervical regions as well. Tensile stress was concentrated on the palatal side of the palatal root and the distal portion of the distobuccal (DB) root in all the experimental groups. However, the control group had the least

amount of stressed area followed by V-Taper group, Vortex Blue and ProTaper.

Conclusions: Taper size of endodontic files seems to affect the distribution of forces along the root structure. Preserving dental hard tissue in precervical dentin significantly reduces the stress concentration in the cervical region and increases the eventual fracture resistance of the tooth.

Keywords: Root canal preparation, root fractures, Taper of the file, Rotary instrumentation

Introduction

Fracture of the Root is the third most common problem for the tooth loss only after caries and periodontitis. (1) For the clinical practice the knowledge of the biomechanics of the fracture is important for the successful treatment. The initiation and the propagation of these fracture lines can be a result of the various types of the forces.(2) The loss of the major portion of the tooth due to the preparation for the root canal may lead to the concentration of the forces and may help in the fracture propagation.(3) To avoid this, removal of the pre-cervical dentin excessively is to be avoided. (4,5)

Various rotary systems apply various types of the file systems that use files of different cross sections, sizes and shapes that have different working patterns once inside the canal. There are many studies that have described the root fractures when these rotary instruments are used. (6–8). In the study of Clark and Khademi, they have suggested that the pre-cervical dentin be protected at all the times to prevent the fracture.(9). Most of the studies done previously are studied invitro. This is because as the human teeth are tough for the standardization. (10)

Hence in our study we used FEA (Finite Element Analysis) to gain a better understanding of the effects of the cleaning and shaping process through endodontic treatment. FEA would also help in knowing the factors for initiation and propagation of cracks and fractures in root canal treated teeth. Thompson et al, stated that during the mastication, the stress at the cervical region of the tooth rises as the taper of the files increases (11). In our study we compared three commonly used files systems. V-Taper, Vortex Blue, and ProTaper are used to understand the fracture resistance after the root canal preparation to the various strains and stresses at different parts of the tooth.

Materials and methods

3D printed acrylic maxillary first molars were used in the study. For the FEA, CAD geometry of the tooth was first prepared. The replica was then scanned by a micro CT. The image was sent to the ANSYS SpaceClaim software for analysis. All the precautions were taken to simulate the original tooth morphology of the molar. We classified the replica into 4 groups. They are Group1- ProTaper, Group 2 - Vortex Blue, Group 3 - V-Taper, Group 4- Control. A conventional access cavity was made with one-degree inward taper to the pulpal floor. Canals were then prepared and designed starting at the pulpal floor and extending to the apex. The apical foramina were enlarged to 0.3 mm, and the working length was set at 0.5 mm coronal to the apical foramen. 16 mm was the length of the canals. Later the canals were filled with the GP obturating materials. Access cavity was restored with composite in the three groups. For the control group the canals were left empty and the access was filled with 2 mm of enamel and the dentin. All the materials were noted for the physical properties. The amount of the loads was

thoroughly registered. Von Mises stress was calculated in each of these elements. All the replicas were subjected to similar stresses and strains that are produced in the routine mastication. All the volumes and the amount of the displacements faced by all the replicas under similar conditions were registered. The obtained values were only compared between the groups.

Results

The Von Mises stress and maximum principal stress on the cervical region of maxillary first molar replica is calculated. In this study, the cervical region was deliberated as a critical zone. The stress distribution of the chewing force was deliberated and analyzed in four separate horizontal cross sections of the cervical region. Three cross sections were located above the pulpal floor and one below the pulpal floor at the level of buccal furcation. Von Mises stress was more at the CEJ level. The highest Von Mises stress was observed in the ProTaper group, although V-Taper and Vortex Blue groups presented homogeneous stress distributions in the cervical regions as well. The stress distributions of the maximum principal stress in the cervical region were analyzed. The ultimate loads that caused failure of dental material was considered to be 50 MPa and any amount of stress higher than that was revealed in red in all the experimental group, and it was considered detrimental to the tooth structure.

In all experimental groups, tensile stress was concentrated on the palatal side of the palatal root and the distal portion of the distobuccal root in the cross sections at the level of CEJ and the pulp chamber floor (Figure 13-14). However, the intensity of stressed areas differed among each experimental group. The control group had the least amount of stressed area in red followed by V-Taper group, Vortex Blue and ProTaper

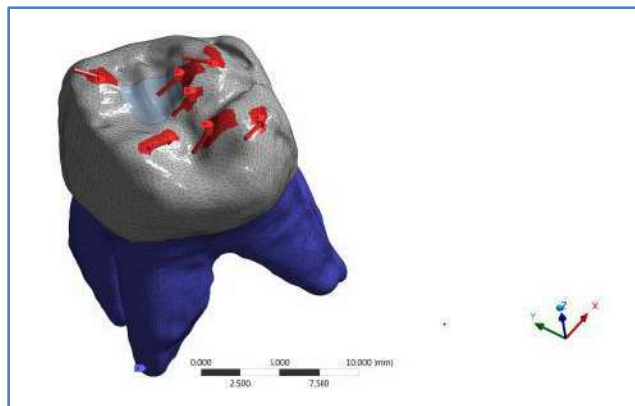


Figure 1: Schematic diagrams of the finite element models and load location. Red areas indicate contact points during chewing cycle. Purple illustrates the location of alveolar bone.


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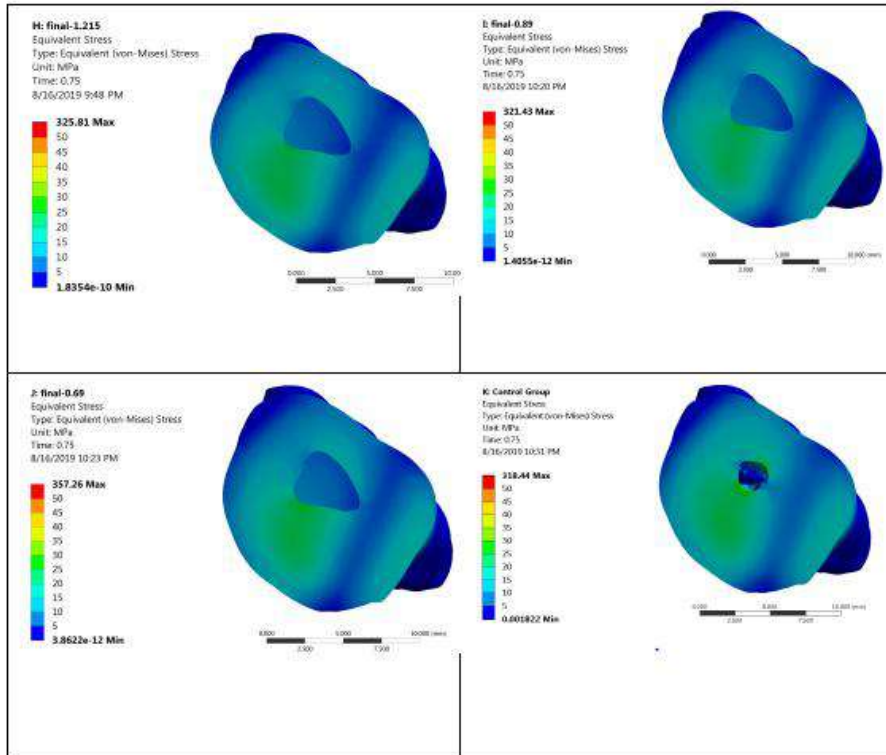


Figure 2: Concentration of the stresses as noted in the FEM.

Table 1: Comparison of the various canal preparation systems

Distance from CEJ	Protaper	V-taper	Vortex blue	Control
2.5 mm above the CEJ	325.81	321.43	357.26	318.44
0.3 mm above the CEJ	325.81	321.43	357.26	318.44
1.3 mm above the CEJ	325.81	321.43	375.26	318.44

Discussion

In the study of Kakehashi et al, they proposed that the microorganisms were the cause for the dental diseases. (12) In the root canal treatment the removal of these microorganisms will help in the healing of the tissues surrounding the periapical region. Many modifications have been made in the treatment modalities of the root canal therapy with the advent of the rotary instruments. Various studies state that the instrumentation by itself can efficiently reduce the microbial load in the root canal system by 90 % (13). But there has been no one particular instrument to fully achieve this sealing ability.

With the usage of the various rotary systems there has been much comparison of how these may impact the strength of the tooth in the long run. It has been stated in the study of Hedge et al, that the preparation of the root canal should follow the anatomy and remove as less tooth structure as possible.(14) In the various studies of Orstavik et al, Peters et al the final preparation sizes and the instrumentations had no significant variations on the outcome.(15,16). The best size of the

file that can bring about an ideal outcome is yet to be determined.

It is imperative to the clinician to not only understand the mechanics of the instruments but also to the impact of these on the strength of the tooth. With the recent rotary systems there has been a noted removal of the tooth dentin in the coronal and the radicular areas. In the study of Gher et al and Kovac et al, they have noted higher ratio of vertical root fracture in endodontically treated teeth (17, 18). Teeth that have been fractured may not be noticed for a long time by the patient and the pain may only be felt under specific conditions. Hence it is challenging for the dentist to identify and diagnose these fractures. Therefore many attempts to minimize the instrumentation as much as possible are done. If the tooth fractures while eating then the effort that was done to endodontically restore it may go in vain.

Hence in the present study we intend to compare the fracture resistance of the tooth while conserving as much as tooth possible. Maxillary first molar CAD design was used to simulate the natural masticatory forces. We selected the rotary instruments with different sizes and tapers.

The FEA was used to measure the amount of stress and strain applied on dentin near the pre-cervical area and alveolar crest of maxillary first molars. It is a critical zone that is roughly 4 mm above the crestal bone and extends 4 mm apical from the crest of the bone.

We selected the pre-cervical area as it has the: 1) The effect of ferrule, 2) the proximity of dentin tubule orifices at the CEJ, 3) the significant reduction in enamel thickness, and 4) the concentration of all masticatory forces in this area (5-11). In this study, a replica of the maxillary first molar that is endodontically treated with three different rotary instruments was generated based on micro CT.

We noted that, Von Mises stress was more on the margin of the palatal root significantly less in the V-Taper group and the control group in contrast to ProTaper and Vortex Blue. With the reduction of dentinal wall removal, the stress concentration also declined.

The ProTaper group showed the most amount of stress concentration surface area in comparison to the other groups at the CEJ level, and with the V-Taper group having the closest stress concentration to intact tooth. The foci of tensile stress were located on the palatal portion of the palatal root, the root furcation, and the distal portion of the distal root. Previous studies have shown that the maximum principal stress in the mesio-buccal root was significantly more than other areas (19).

Nevertheless, in this study, the maximum stress was on dissimilar areas possibly due to the replication of the chewing forces rather than using direct vertical forces.(20-22) The three groups of file sizes in this study had same tip size (0.25 mm), yet, their MFD at D16 are significantly different, with ProTaper being the largest file and V-Taper being the smallest near D16. Hence, within the limits of the present study, it can be stated that saving the dental hard tissue, in particular pre-cervical dentin, in root canal therapy is a feasible method to decrease the maximum principal stress and to decrease the stress concentration near this susceptible area. This was a critical area to scatter stress through the long axis of the teeth.(23-25) V-taper files are intended to decrease the amount of tooth structure removal in pre-cervical dentin to minimize the overall weakening effects of endodontic treatment.

Conclusion

In our study we intended to explain the taper of the instrument and the fracture resistance by using the Finite Element Analysis in the maxillary first molar replica. We can conclude that Taper size may effect to the fracture resistance in the cervical areas by concentration of forces. Conserving dental hard tissue will decrease the concentration of the stress in the cervical region and hence may prevent the tooth fracture. However the simulated FEA method has few limitations. Thus, the clinical implications of results obtained in our study need additional exploration on natural tooth and ideally under masticatory forces in the oral cavity.

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Telepedodontics - Research & Review

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ABSTRACT

Teledentistry in pediatric dentistry - Telepedodontics, a comparatively new field, can modify the dynamics of the dental care delivery system. Maximum of the dentists are ignorant about teledentistry, about its goals and benefits and how to get involved into it. This review demonstrates as to how teledentistry can be an actual solution for dentists and their patients.

Teledentistry gives a likely resolution to many enduring complications in dentistry, but it also faces substantial challenges. Its evolution will be contingent as much, on the struggles of the health authorities as on the combined efforts of the dental professionals.

Telepedodontics can meet the dental care needs of the underserved in the rural areas of India and it can guarantee the good oral health of the children in schools and child care centers. Telepedodontics gives new opportunities for dental education by providing an easy access to primary care professionals for effective consultation, thus serving in piloting effective postgraduate education and continuing dental education programs.

Key Words: Telepedodontics, Teledentistry, Preventive Dentistry, Pedodontics.

INTRODUCTION

Dental care is being continually altered by the opportunities which are delivered by technology and telecommunication [1]. Tele dentistry is a comparatively new field that syndicates telecommunication technology and dental care. Due to the huge development of technological proficiencies, teledentistry owns the potential to basically change the current practice and the face of the dental care [2]. The term “teledentistry” was used in 1997, when Cook defined it as “the practice of using video-conferencing technologies to diagnose and to provide advice about the treatment over a distance” [3]. ‘Teledentistry’ permits a whole new way of giving specialist advice. By the use of telecommunication and computer technologies, it is now conceivable to deliver interactive access to specialist views that are not limited by the limitations of either space or time.

The referring dentist logs into a secure web-server and fills in the patient’s details, the detailed reasons for the consultation, the chief complaints and the provisional diagnosis information and attaches the digital intra-oral images and the scanned digital dental radiographs. The specialist then logs into the secure web server, analyses the case and proposes his diagnosis and treatment plan within a specified time.

TELEPEDODONTICS AND ITS APPLICATION IN DENTAL EDUCATION OF THE PEDODONTISTS

The role of Telepedodontics in education can be divided into two main categories: self-instruction and interactive video-conferencing.

The Web-based, self-instruction educational system contains evidence that has been established and deposited before the user opens the program [4]. The benefit of this system is that the user can regulate the speed of the learning and can read the material many times at convenient times [5]. Johnson and Schleyer [6, 7] considered the Web-based dental continuing education and assessed them on the basis of a set of well-designed guidelines by applying the Design of Educational Software. Spallek and colleagues [8] lead a survey of the applicants in several Web based dental CE courses and found that absence of in person communication with their colleagues and mentors could result in disappointment.

Interactive video-conferencing may be lead via POTS (plain old telephone service), satellite, ISDN, Internet or Intranet. Interactive video-conferencing comprises both a live interactive video-conference with a proper camera set up where the patient's data can be transmitted; and associated information (such as the patient's medical history, radiographs, etc) that can also be referred before or at the similar time (for example, via fax) as the videoconference. The benefit of this type of education system is that the user can receive an instant response [5].

ROLE IN PEDIATRIC AND PREVENTIVE DENTISTRY

Telepedodontics is particularly beneficial for children. Applying teledentistry, children's teeth were checked for early childhood caries, thru which it was reported that all children enjoyed seeing their teeth on the computer screen. Dental imaging was assumed as a game by them, quite than as a dental examination. Furthermore, as no instruments are used and the camera head does not need to be put in far into the child's mouth to obtain dental images, there was less menace of uncooperative behavior by the very young child or toddler than that posed by a routine dental examination. Child comfort is also enhanced when the teledentistry examination is done in a known environment, as compared to a clinic. This lead to in children being more enthusiastic and co-operative at the imaging and recording processes. Furthermore, guardians and children saved the time off work or school for travel, and a child can be tracked effortlessly to conclude whether treatment has been taken, or if an emergency assessment is wanted.

Kopycka-Kedzierawski DT and Billings RJ presented that teledentistry is more or less equal to visual/tactile examinations for dental caries screening in pediatric patients.[9] Kopycka-Kedzierawski DT *et al.* recommended that teledentistry offers a possibly well-organized means of screening high-risk preschool children for signs of early childhood caries.[10] They successfully established a teledentistry project recognized in inner-city child-care centers in Rochester, NY.[11] Amavel R *et al.* detailed that distant diagnosis of children dental problems based on non-invasive photographs set up a valid resource.[12] Kopycka-Kedzierawski DT *et al.* validated that the intraoral camera is a possible and potentially economical substitute to a visual oral examination for caries screening, particularly early childhood caries, in preschool kids attending childcare centers.[13]

TELEPEDODONTICS AND ITS USE IN RURAL AREAS FOR THE PEDIATRIC PATIENTS

In country areas, where there is a scarcity of specialists, the deficiency of comprehensive and sophisticated health care is a problem. Telepedodontics can increase the accessibility of the specialists to the village and underserved groups for their dental needs, also lessening the time and the price which are related with the specialty sessions [14].

Altering the service delivery method may also certainly affect the viability of a rural practice. Separation from peers, specialists, and continuing education opportunities are the undesirable aspects of a rural practice. Providing dental care in a salaried arrangement allows one to meet

the financial obligations while learning to build the competences which are expanded with experience in care delivery without incurring additional debt. It also allows one to model the experience of existing in a rural setting without requiring to a permanent relocation.

TELEPEDODONTICS AND ITS ROLE IN POSTGRADUATE EDUCATION IN THE DEPARTMENT OF PEDODONTICS AND FOR DENTAL PRACTICE

Telepedodontics can serve as a good tool for teaching postgraduate students and for giving current updates for the practicing dentists.

In shared video-conferencing, the patient data is assessed first (with or without the patient's presence), that lets for the communication and feedback among the teacher and the students. The patient cases can be studied thoroughly and at the students' speed. The cases can be deliberated in detail after all the clinical data have been composed and conveyed, without the patient being there at the arranged meeting. This improves the students' enthusiasm and offers new learning prospects for the dental students and the practicing dentists.

THE ROLE OF TELEPEDODONTICS IN SCHOOLS AND CHILD CARE CENTRES

It is the essential to develop models for schools and child care centers in our nation to use Telepedodontics to raise the access to dental care for the kids. Schools and child care centers play an important role in guaranteeing the optimal oral health of the children through:-

- Screening for dental problems beforehand these become emergencies [15].
- Assisting children in handling chronic illnesses.
- Linking children and their families to the required health and social services.
- Giving urgent care.

Pediatric dentists at the University of Rochester use the images of toddlers to classify those with early childhood dental caries. A study of the program found that nearly 40% toddlers had tooth decay. The timely detection of such decay can stop the child from painful and monetary trauma, visits to the emergency treatment room, and eventually, extractions of the teeth [16].

Telepedodontics may serve as a tool to supplement and multiply the capacity of school and child care centers to meet the children's dental care requirements by expending technology to link to the health providers at another location.

SCOPE OF TELEPEDODONTICS IN INDIA

India has opened up to Telepedodontics to address many issues which are being confronted by the healthcare delivery system, like insufficient health organization and clinical services, paucity of competent doctors, the nearly non-availability of specialist care, the late detection of the illness, the deferral in the conveyance of the treatment due to the greater time which is essential for the transport of the patients to city healthcare facilities and the delivery of healthcare by inexperienced primary healthcare service providers [17].

In 1999, the Department of Information Technology, the Ministry of Communications and Information Technology (Government of India) propelled a pilot project which was titled, 'Development of Telemedicine Technology', with the aim of strengthening the national healthcare delivery system [18]. The key terms of the project comprised [19].

- To recognize the suitable technological tools and facilities which are essential to implement telemedicine technology at the three leading hospitals in the northern parts of India, viz., All India Institute of Medical Sciences (AIIMS), New Delhi, the Post Graduate Institute of Medical Education and Research (PGIMER) at Chandigarh and the Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS) at Lucknow (Uttar Pradesh).
- To improve and perform system integration to permit telemedicine technology and for

starting telemedicine services (teleconsultation and teleradiologic services for the specialties of radiology, cardiology and pathology and teleeducation) at three tertiary level hospitals.

- To train clinicians in the use of telemedicine technology.

In India, where a bulk of population lives in countryside areas and where healthcare amenities are inadequate, Telepedodontics can have a significant contribution in linking the gap between the demand and the supply.

FUTURE PROSPECTIVES OF TELEPEDODONTICS

The advances in telecommunication have rightly enabled the dental care to assure many exhilarating modifications during the following few years [20]. Still, like any revolution, it will not be stress-free or effortless. There are definite issues which need resolution for the success of Telepedodontics. These issues comprise inter-state licensure, jurisdiction and malpractice, as well as technological, security and ethical features [21].

Several measures that can be engaged for the effective application of teledentistry are:

- The tutors of the teledentistry education courses need to be well experienced with software knowledge and they should have satisfactory teaching experience [7].
- The practitioners who are involved in teledentistry must have a license in each state in which they practice [22].
- Dentists who are involved in teledentistry must make every effort to confirm the security of their systems, as well as of any data that they may communicate. For example, data encryption, password protection and user access logs can help in discouraging most of the people and in shielding patient confidentiality [21].

SUMMARY

Dentistry, in a synergistic amalgamation with telecommunications technology and the Internet, has produced a relatively new and thrilling field that has limitless potential. Telepedodontics can be used as an appreciated tool for giving dental care in village areas, where there is a scarcity of pedodontists and a lack of complete and sophisticated health care. It can be an answer to the obstacles of dental care like the absence and price of transport, leave from work and school and to save the patient's money. Furthermore, Telepedodontics gives new chances for dental education by providing the primary care professionals with an easy access to effective consultation and by serving in directing postgraduate education and continuing dental education programs.

In spite of few problems which need to be determined, the potential of Telepedodontics is wonderful, which needs to be discovered.

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Level of Knowledge, Understanding, and Impact of the COVID-19 Pandemic on Continuing Fixed Orthodontic Treatment in Adults: A Questionnaire Study

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Abstract

Objectives: To evaluate the level of knowledge, understanding, and impact of the coronavirus disease 2019 (COVID-19) pandemic on adult patients regarding their ongoing fixed orthodontic treatment.

Subjects and Methods: A cross-sectional and descriptive survey was conducted on 108 adult patients undergoing fixed orthodontic treatment at GSL Dental College (GSLDC), India. All the candidates were aged 18 years and above, who were in lockdown due to the COVID-19 pandemic and devoid of treatment.

Results: Among the patients 43.5% were male and 56.5% were female; 22.3% were not at all aware that the COVID-19 virus spreads quickly in a dental setup; 64.8% were definitely willing for their status disclosure and to undergo pretreatment screening; 71.3% were definitely willing to adhere to strict appointment timings for adequate sanitization of the clinical area; 60.2% thought that missed appointments during the COVID-19 pandemic would prolong their overall orthodontic treatment and affect their treatment outcome; and 51.9% were financially affected due to the pandemic.

Conclusions: Not all adult orthodontic patients were in knowledge of COVID-19 cross infection. The majority were in a state of understanding and willing to follow infection-eradicating protocols. Patients' view on overall orthodontic treatment was altered due to the psychological and financial impact of COVID-19.

Keywords

COVID-19 pandemic, adult patients, ongoing fixed orthodontic treatment, lockdown

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Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a significant public health emergency across the world, which has led the World Health Organization (WHO) to declare it as a pandemic.¹ Currently, it is believed that its interpersonal transmission occurs predominantly via respiratory droplets/secretions (cough, sneeze), saliva, and contact transmission where the virus enters the mucous membrane of the mouth, nose, and eyes.² All dental professionals, including orthodontists, are at higher risk of SARS-CoV-2 infection due to dental practices and hospitals' characteristics, which include aerosol generation, handling of sharps, and proximity to patients' oropharyngeal region.^{1,3} If acceptable safety measures are not taken, cross-contamination risk could be extreme among dentists, orthodontists, and patients.

Most countries initiated a regional or national lockdown to restrain the spread of this disease, permitting only medical and dental emergency services to continue. In many such efforts, performing of elective tasks, including orthodontic treatment, is suspended.⁴ Since fixed orthodontic treatment is a long and

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continuous scheduled process, millions of patients who were already undergoing treatment have been affected. The overall treatment time might be affected due to the unknown span of this mandatory suspension. A lot of awareness and understanding of the COVID-19 pandemic needs to be emphasized for orthodontic patients, so that it does not impact their psychological well-being. For instance, most minor to moderate orthodontic problems can be taken care of through teleconsultation. However, addressing orthodontic emergencies in this pandemic might be an extra financial burden, as dental setups need to be maintained with a very high standard of sterilization, infection prevention and control protocol.

The study aims to evaluate the level of knowledge, understanding, and impact of the COVID-19 pandemic on continuing fixed orthodontic treatment in adults.

Subjects and Method

Ethical approval was obtained from the GSL Institutional Ethics Committee and GSL Research Cell, Project ID: 032020/001. A cross-sectional study design was used.

Inclusion Criteria

Adult patients aged 18 years and above who were undergoing fixed orthodontic treatment at GSL Dental College (GSLDC) during the lockdown due to the COVID-19 pandemic were included in the study.

Sample Size

The sample size calculation for the present study was done through a questionnaire-based study that considers the level of knowledge and understanding among people in different regions.⁵

Questionnaire Design

A questionnaire was devised from the key themes and concepts identified with regard to the COVID-19 pandemic (Table 1).

Table 1. Overview of the Results of the 13 Questions

No.	Question	Options	Count (%)
Q1	Are you aware that the COVID-19 virus spreads easily in the dental setup?	(a) Extremely aware (b) Very aware (c) Moderately aware (d) Slightly aware (e) Not at all aware	43 (39.8) 12 (11.2) 10 (9.3) 19 (17.5) 24 (22.3)
Q2	Are you willing to undergo treatment from an orthodontic team who were previously affected with COVID-19?	(a) Definitely (b) Probably (c) Possibly (d) Probably not (e) Definitely not	17 (15.7) 19 (17.6) 16 (14.8) 32 (29.6) 24 (22.2)

(Table 1 Continued)

No.	Question	Options	Count (%)
Q3	Are you willing to disclose your COVID-19 status and undergo pre-treatment screening before every appointment?	(a) Definitely (b) Probably (c) Possibly (d) Probably not (e) Definitely not	70 (64.8) 22 (20.4) 8 (7.4) 6 (5.6) 2 (1.9)
Q4	What is your view on visiting the orthodontic office after the COVID-19 pandemic?	(a) I will visit the orthodontist if proper infection control precautions are followed. (b) I will have some fear or doubt even though the orthodontist follows the infection control precautions. (c) I will not visit even though the appropriate infection control precautions are followed. (d) I will only visit after this COVID-19 pandemic issue is resolved.	68 (63.0) 18 (16.7) 1 (0.9) 21 (19.4)
Q5	Do you adhere to strict appointment timings for the clinical area's adequate sanitization to counter-act COVID-19?	(a) Definitely (b) Probably (c) Possibly (d) Probably not (e) Definitely not	77 (71.3) 27 (25.0) 3 (2.8) 1 (0.9) 0 (0)
Q6	Would you like to adhere to "Strictly no accompanying person" (until and unless specified by the orthodontist) for future appointments?	(a) Definitely (b) Probably (c) Possibly (d) Probably not (e) Definitely not	58 (53.7) 31 (28.7) 8 (7.4) 5 (4.6) 6 (5.6)
Q7	Do you have any pain or discomfort due to missed appointments during the COVID-19 pandemic?	(a) Severe (b) Moderate (c) Mild (d) Very mild (e) None	13 (12.0) 16 (14.8) 22 (20.4) 20 (18.5) 37 (34.3)
Q8	For unexpected orthodontic problems, which of the following options do you choose?	(a) I prefer telecommunication with the dental office. (b) I prefer to visit the dental office. (c) I will wait until my next appointment.	50 (46.3) 24 (22.2) 34 (31.5)

(Table 1 Continued)

(Table 1 Continued)

(Table 1 Continued)

No.	Question	Options	Count (%)
Q9	Do you think the missed appointments will have an effect on your orthodontic treatment during the COVID-19 pandemic?	(a) It will prolong my overall treatment.	65 (60.2)
		(b) It will affect my treatment outcome.	20 (18.5)
		(c) It will not prolong my overall treatment.	7 (6.5)
		(d) It will not affect the treatment outcome.	16 (14.8)
Q10.	Would you like to undergo additional treatment procedures, if indicated by your orthodontist, to compensate for the lost time?	(a) Definitely	50 (46.3)
		(b) Probably	31 (28.7)
		(c) Possibly	18 (16.7)
		(d) Probably not	5 (4.6)
		(e) Definitely not	4 (3.7)
Q11.	Does your current financial situation have any effect on future orthodontic appointments?	(a) My financial situation is not affected, and I can afford further treatment.	38 (35.2)
		(b) My financial situation is affected, but I can afford the treatment.	56 (51.9)
		(c) My financial situation is affected, and I cannot afford further treatment.	14 (13.0)
Q12.	Protective gear and equipment to counteract COVID-19 may increase future treatment costs. What is your opinion?	(a) I understand the situation, and I will bear the extra amount.	23 (21.3)
		(b) I understand the situation, but I cannot bear the amount.	41 (38.0)
		(c) I won't pay the additional amount, as it was not informed before the start of treatment.	14 (13.0)
		(d) I expect the dental institution/clinician/government/insurance companies to bear the extra amount.	30 (27.8)

The questionnaire consisted of 12 closed questions, which the researchers considered to be ideal. The questions cover the fundamental importance of knowledge and the understanding of adult patients about the COVID-19 pandemic, and the financial burden and its impact on ongoing fixed orthodontic treatment. It was mandatory for the patients to answer all 12 questions to send their response. The Likert's response format was used for 7 questions, and the other questions were provided with options that are considered appropriate by the researchers. The initial questionnaire was rated by 6 experts involved in the provision of orthodontic care, and the item-level content validity index was 0.94. Minor changes in the questionnaire were made based on the suggestions received by the experts to make it more readily understandable to the subjects. The Cronbach's alpha of 0.86 demonstrated good internal-consistency reliability of the questionnaire when administered to 30 subjects meeting the inclusion criteria for this study. The questionnaire was also assessed for test-retest reliability through administering it to the same set of participants based on the response of whom Cronbach's alpha was derived. The intraclass correlation coefficient (ICC) of 0.91 between the test and retest responses was indicative of the temporal stability of the questionnaire. The typical time taken for completion of consent and the questionnaire was approximately 5 to 10 minutes.

Questionnaire Distribution and Study Duration

The online questionnaire was posted to 153 adult patients undergoing orthodontic treatment, selected through the random sampling method at GSLDC, India, on May 18, 2020. Google Forms® was used as an online plotting source, and the questionnaire's link was sent via WhatsApp or email to the patients on the contact list in the GSLDC orthodontic database. Online consent was obtained from the participants. All the responses were anonymous and could not be traced by the researchers. This was done to eliminate bias that could arise if patients felt that their answers could influence any aspect of their subsequent treatment. The deadline for the final return of the questionnaire was set for May 23, 2020.

Data Analysis

Of the 153 adult patients undergoing fixed orthodontic treatment, 108 consented to participate in this study. The findings from the questionnaire were analyzed using SPSS Version 20.0 (SPSS Corporation, Chicago, IL).

Results

A total of 153 adult patients undergoing fixed orthodontic treatment were approached to complete the questionnaire, and 108 completed responses were returned. The responses to each question are illustrated in Table 1. The gender distribution

of the study subjects and the differences in responses to the study questions based on gender are illustrated in Tables 2 and 3, respectively.

Discussion

The knowledge, understanding ability, and financial standing of adult patients play an influential role in the progress of fixed orthodontic treatment during the COVID-19 pandemic. In the course of fixed appliance therapy, a higher intensity of pain and discomfort was often reported.⁶ Although it varies based on the age and pain threshold of patients, our questionnaire was confined to adults, as patients below 18 might experience problems with specific complex questions and their characteristics might affect the quality of the data.⁷ After the questionnaire link was sent, there were a total of 108 responses, at a response rate of 70.5%, which seems to be similar to other orthodontic-based questionnaire studies published.⁸ However, the response rate is difficult to compare in light of this new aspect of the COVID-19 pandemic.

During the period of lockdown, patients might be in a state of stressful agony. This factor may well account for the missing responses and have resulted in some degree of pessimism.

Each Question Was Discussed to Benefit the Reader

- *Question 1: Are you aware that the COVID-19 virus spreads easily in the dental setup?*
Of the patients, 17.5% are slightly aware and 22.3% are not at all aware that the COVID-19 virus spreads quickly in a dental setup. Hence, there is still a need to educate and create awareness among patients regarding the COVID-19 pandemic irrespective of gender.
- *Question 2: Are you willing to undergo treatment from an orthodontic team who were previously affected with COVID-19?*
Of the patients, 5.6% are probably not willing to undergo treatment from an orthodontic team previously affected by COVID-19. Most patients believe that they

Table 2. The Number (n) and Proportion (%) of Patients, Based on Gender, to Total Sample (N = 108)

	n	%
Male	47	43.5
Female	61	56.5
Others	0	0
Total sample	108	100

Table 3. The Proportion (%) of Patients, Based on Gender (Male [M] and Female [F]), Who Responded to the Questionnaire (N = 108)

	(a)		(b)		(c)		(d)		(e)		P-value
	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	
Q1	34.9	65.1	41.7	58.3	60.0	40.0	42.1	57.9	54.2	45.8	.473
Q2	35.3	64.7	31.6	68.4	37.5	62.5	65.6	34.4	33.3	66.7	.057
Q3	41.4	58.6	54.5	45.5	25.0	75.0	66.7	33.3	0.0	100.0	.305
Q4	44.1	55.9	33.3	66.7	100.0	0.0	47.6	52.4			.584
Q5	39.0	61.0	55.6	44.4	66.7	33.3	0.0	100.0	0.0	0.0	.271
Q6	51.7	48.3	29.0	71.0	62.5	37.5	20.0	80.0	33.3	66.7	.150
Q7	46.2	53.8	37.5	62.5	63.6	36.4	40.0	60.0	35.1	64.9	.282
Q8	32.0	68.0	70.8	29.2	41.2	58.8					.006*
Q9	43.1	56.9	30.0	70.0	57.1	42.9	56.2	43.8			.385
Q10	50.0	50.0	38.7	61.3	16.7	83.3	80.0	20.0	75.0	25.0	.022*
Q11	42.1	57.9	46.4	53.6	35.7	64.3					.752
Q12	47.8	52.2	41.5	58.5	28.6	71.4	50.0	50.0			.566

Notes: Questions 1, 3, 4, 5, 6, 9, 10 and 11 used Fisher's exact test. Questions 2, 7, 8 and 12 used the chi-square test. Q1 to Q12 are the questions asked, and (a) to (e) are the options provided (see Table 1). P ≤ .05 is considered statistically significant. * Denotes statistical significance.

could get infected via dentists with viral infections.⁹ Moreover, recurrence of COVID-19, with a previously infected person capable of transmitting the virus to other people even after a second negative test, has been reported in the literature.¹⁰ This could be a probable reason why patients tend to avoid treatment from a previously infected orthodontist.

- *Question 3: Are you willing to disclose your COVID-19 status and undergo pretreatment screening before every appointment?*

Of the patients, 64.8% are definitely willing, and the rest are still in a dilemma over disclosing their COVID-19 status and undergoing pretreatment screening before every appointment. This is a dangerous sign and might put many at risk of contracting the virus. Thus, we need to make patients understand the importance of their status disclosure and participation in a pretreatment triage.³

- *Question 4: What is your view on visiting the orthodontic office after the COVID-19 pandemic?*

Of the patients, 63.0% are willing to visit the orthodontic office after the COVID-19 pandemic if proper infection control precautions are followed. However, the rest of the patients fear cross infection in a dental office and do not want to risk it.⁹

- *Question 5: Do you adhere to strict appointment timings for the clinical area's adequate sanitization to counteract COVID-19?*

Of the patients, 71.3% are definitely ready to adhere to strict appointment timings to sanitize the clinical area to counteract COVID-19. More emphasis should be placed on this aspect among orthodontic patients. Additional time is required for proper sanitization of the clinical area, as the virus has been detected on stainless steel, on plastic, and in aerosols for up to 5.6, 6.8, and 3 hours, respectively.^{1,11}

- *Question 6: Would you like to adhere to "Strictly no accompanying person" (until and unless specified by the orthodontist) for future appointments?*

Of the patients, 53.7% would like to adhere to "Strictly no accompanying person" (until and unless specified by the orthodontist) for future appointments. The current unprecedented circumstances put the accompanying person also at risk of COVID-19 infection. Performing pretreatment triages as a routine procedure for accompanying persons adds a physical burden on the dental team.¹

- *Question 7: Do you have any pain or discomfort due to missed appointments during the COVID-19 pandemic?*

Of the patients, 34.3% have no pain or discomfort due to missed appointments during the COVID-19 pandemic. There is a nonlinear relationship between age, gender, psychological state, and cultural background in pain perception following placement of an orthodontic appliance, according to the literature. However, with

fixed orthodontic treatment, mild to moderate pain or discomfort is often experienced by patients.⁶

- *Question 8: For unexpected orthodontic problems, which of the following options do you choose?*

Of the patients, 46.3% preferred telecommunication in case of unexpected orthodontic problems. Gender distinction was observed in the participants' responses ($P = .06$). While most females expressed interest in teleconsultation with the dental office, males demonstrated readiness in visiting the dental office and seeking care. Teledentistry facilities should be encouraged during this pandemic to prevent unnecessary visits to the dental office. This would aid patients in resolving a new orthodontic problem that can be managed at home and help determine which patients need in-person attendance.

- *Question 9: Do you think missed appointments will have an effect on your orthodontic treatment during the COVID-19 pandemic?*

Of the patients, 60.2% think missed appointments due to the COVID-19 pandemic would prolong their overall treatment time. Association of the pandemic with orthodontic treatment time and outcome has not been previously investigated.

- *Question 10: Would you like to undergo additional treatment procedures, if indicated by your orthodontist, to compensate for the lost time?*

Of the patients, 46.3% are definitely willing to undergo additional treatment procedures to make up for the lost time. From a patient's perspective, shorter treatment times impact psychosocial well-being, which contributes to the overall quality of life.^{12,13} Comprehensive orthodontic treatment requires, on average, less than 2 years to be complete.¹⁴ The lost time during the pandemic prolongs the overall treatment duration. Males ($P = .022$) and more qualified participants ($P = .028$) expressed willingness to undergo additional procedures in this regard. However, the type of intervention and financial aspect may affect patients' decision to undergo additive procedures when indicated.

- *Question 11: Does your current financial situation have any effect on future orthodontic appointments?*

The virus has spread throughout the inhabited world and led to unprecedented financial crises. Of the patients, 51.9% opined that their financial situation is affected but they can afford future treatment. However, economic crises depend upon several factors, such as the lockdown period, type of occupation, and socioeconomic status.

- *Question 12: Protective gear and equipment to counteract COVID-19 may increase future treatment costs. What is your opinion?*

Of the patients, 38.0% revealed that while protective gear and equipment might increase future treatment costs, they cannot bear that extra amount, and 27.8%



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expect the dental institution/clinician/government/insurance companies to bear the additional amount.

Limitations

A potential concern in this study is the noninclusion of the study participants' socioeconomic background and geographical location as independent study variables.

Though these parameters were documented at the beginning of the orthodontic treatment, they were not included in the present study's data analysis. A review of patient records suggests little variability concerning these parameters among the study subjects, who share the common characteristics of availing orthodontic treatment at a single facility at substantially lower costs because the facility is provided at a dental teaching institution. However, it would be interesting to see if the study results are replicated in geographical areas where COVID-19 is comparatively more prevalent.

The preliminary nature of the study with a small sample is a limitation.

The present study intended to qualitatively explore patients' attitudes regarding ongoing orthodontic treatment during challenging COVID-19 times. Moreover, the study attempted to include all the eligible study subjects seeking care at the facility, as mentioned earlier, circumventing sampling. The authors opine that the observations made in this study form a concrete basis for future research to more thoroughly understand the attitudes, knowledge, and impact of COVID-19 on patients undergoing orthodontic treatment considering the study's preliminary nature with a small sample.

Conclusions

- Knowledge of cross infection, the importance of status disclosure, and pretreatment screening regarding COVID-19 was not up to mark for adult orthodontic patients.
- The understanding of the adult orthodontic patients regarding the significance of adhering to strict appointment timings and having no person accompany them to the orthodontic office during the COVID-19 pandemic was satisfactory.
- In the case of an unexpected orthodontic problem, most patients preferred telecommunication during this pandemic.
- Most patients think missed appointments due to the COVID-19 pandemic would prolong their overall treatment time, and the majority of them showed a willingness to undergo additional treatment procedures to make up for the lost time.
- The adult orthodontic patients mostly opined that there has been an impact on their financial situation due to

the lockdown but they can afford future treatment. A few cannot bear the extra amount due to protective gear and equipment, and patients expect the dental institution/clinician/government/insurance companies to cover this.

Declaration of Conflicting Interests

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Saliva as a Reliable Diagnostic Tool during the Coronavirus Disease Times: A Focused Review

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also currently known as CoV disease 2019 (COVID-19), has created a global health upheaval. The saliva of an infected person serves as a source for the transmission of CoV. The angiotensin-converting enzyme-2 receptors serve as the host receptor cells for CoV which is expressed in high numbers in salivary glands, oral mucosa, and gingiva. The salivary gland is a potential reservoir for COVID-19 even in asymptomatic but infected carriers. This knowledge could be used to employ salivary sampling as a noninvasive diagnostic method. IgA, IgM antibodies detected in self-collected saliva show more stability than RNA and may aid in the identification of asymptomatic patients. Saliva shows high sensitivity and specificity in the diagnosis of COVID-19 with >90% concordance reported between the saliva and nasopharyngeal swabs. Standardization with respect to sample collection, storage, and transport media can help in validation of diagnostic application of salivary-based testing.

Keywords: Corona virus, reverse transcription-polymerase chain reaction, saliva and coronavirus disease-2019, salivary diagnostics, severe acute respiratory syndrome coronavirus 2

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INTRODUCTION AND BACKGROUND

Declared as a pandemic by the WHO on March 11, 2020, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), also currently known as CoV disease-2019, (COVID-19) has created a global health upheaval.^[1]

The last two decades have witnessed three major global health issues, namely the SARS CoV, Middle East Respiratory Syndrome (MERS) CoV, and the SARS-CoV-2, with a mortality rate of 9.5%, 34.4%, and 2.3%, respectively. Although COVID-19 expresses a much lower mortality rate, it has raised alarm with its capability to spread much more rapidly than MERS-CoV and SARS-CoV.^[2]

TRANSMISSION

Majorly, COVID-19 transmission occurs through the direct and indirect modes. Direct mode of transmission occurs through respiratory droplet nuclei generated by an infected person while talking, coughing, sneezing, and through aerosols generated during the clinical procedures. Body fluids and discharges, for example, feces, saliva, urine, semen, and tears also serve

as a potential transmission route. The exposure and risk of transmission is elevated if the infected person is present within 1 m radius of a susceptible host. Indirect transmission may occur through fomites or through surfaces, objects that have come in contact with the infected.^[3]

TESTS FOR DETECTING CORONAVIRUS DISEASE-2019

Most of the tests used for Covid-19 detection can be grouped under the polymerase chain reaction (PCR) or serological tests. All these necessitate sample collection employing various means to identify the characteristic hallmarks of the SARS-CoV-2 virus.^[4]

Although chest computed tomography scans sound promising with high sensitivity for COVID-19 detection, they cannot be employed for mass screening due to low specificity, huge

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machinery involved, difficulty in sterilization as well as increased radiation exposure.^[5]

Reverse transcription-PCR is being considered as the gold standard for the detection of novel CoV.^[6] However, this requires the need for nasopharyngeal swabbing by skilled medical personnel. In addition, it is known to be less tolerant to patients. Literature documents it to be contraindicated in patients with significant nasal septum deviation, recent facial trauma, patients on anti-coagulant therapy and thrombocytopenic patients; in whom bleeding can be anticipated. Furthermore, nasopharyngeal swabs are not considered to be suitable for serial controlling of viral load.^[6]

All these limitations clearly picturize the need for a simpler sample collection technique which is not only less invasive but with minimal logistic burden.^[7,8]

SALIVA AND ITS POTENTIAL ROLE IN CORONAVIRUS DISEASE-2019 DIAGNOSIS

Sputum is a noninvasive lower respiratory tract specimen which has immense diagnostic value in diseases such as tuberculosis and chronic obstructive pulmonary disease, but Huang *et al.*^[9] in their study stated that only 28% of patients with 2019-nCoV can produce sputum. It also represents a drawback that it is mandatory to collect sputum only before tooth brushing and breakfast, as nasopharyngeal secretions move posteriorly, and bronchopulmonary secretions move by ciliary activity to the posterior oropharyngeal area, whereas the patients are in a supine position during sleep.^[10] Hence, these limitations eliminate sputum as a reliable diagnostic aid in COVID-19.

Few others have suggested the employment of blood specimens for rapid PCR-based assays or immunochromatography-based *in vitro* assays to detect specific antibodies. Although these techniques sound promising in terms of time, their suitability for screening at the large scale is questioned by procurement of blood samples at a medical point-of-care.^[10]

The saliva of an infected person serves as a source for CoV^[3,6] and this knowledge could be further investigated to determine if a noninvasive salivary diagnostic method could be employed for the COVID-19 detection for limiting the spread.^[11]

Asymptomatic patients provide a major challenge in the current diagnosis protocols of many countries. In a study by Azzi *et al.*,^[10] two patients who were monitored showed positive salivary results on the same days that their pharyngeal or bronchoalveolar swabs were negative. This raises the possibility that individuals can be contagious through their saliva even when pharyngeal swabs are negative. This could be a point in favor of employing saliva for virus detection and also further strengthens the view that salivary glands serve as potential reservoir for Covid-19 virus in asymptomatic but infected people.

Diagnostic strategies with high specificity and sensitivity for both asymptomatic and symptomatic potential “super

spreaders” may help combat against this cataclysmic pandemic.^[12]

Different pathways have been put forward to substantiate the presence of COVID-19 in saliva: First, it might be present in the liquid droplets constantly being exchanged in the upper and lower respiratory tract that enters the oral cavity. Second, virus in the blood can reach the oral niche through crevicular fluid. A study of SARS CoV in Rhesus macaques widely documented the susceptibility of epithelial linings of the salivary glands to infection. From the same, it might be suggested that infections of the minor, major salivary glands, and its ducts can serve as an entry portal for COVID-19. Drainage of debris from nasopharyngeal epithelium into the oral cavity serves as another potential route.^[8,11]

Xu *et al.*^[13] have demonstrated that the receptor-binding domain of SARS-CoV-2 spike protein supports strong interactions with the human angiotensin-converting enzyme-2 (ACE-2) receptor seen in taste cells, which might be responsible for infection-related ageusia. ACE-2 protein is also expressed in vascular endothelial cells, alveolar epithelial cells of lungs, enterocytes of the intestine, and myocardial cells. Hence, justifying the higher susceptibility of these organs to get infected by SARS-CoV-2.^[14] Appearance of ACE-2 is higher in minor salivary glands than that in the lungs.^[7] Among other oral sites, epithelial cells of the tongue exhibit the highest expression of ACE-2 followed by the buccal tissues and gingiva. Endothelial cells of the oral mucosa show exaggerated expression of ACE-2 during SARS-CoV-2 infection and may be responsible for the high viral load in saliva.^[15]

Brann *et al.*^[16] interpreted that the COVID-19 virus has damaging effects on nonneuronal olfactory epithelium causing anosmia, and its intimate correlation to the chemosensory senses of taste may be responsible for the resultant ageusia.

Investigations have further confirmed that SARS-CoV RNA is present in the saliva before the emergence of lesions in the lung. The positive rate of COVID-19 in salivary samples can exceed about 92%, and live virus can also be cultivated through the salivary samples.^[7] Whereas certain scenarios have required repeated nasopharyngeal swab sampling before a positive finding has been obtained in otherwise overt COVID-19 patients, suggestive of its compromised reliability.^[10] Many authors have substantiated the promising role of saliva in COVID-19 detection with high sensitivity, specificity, and consistency^[10,15]. More than 90% concordance has been displayed between self-collected saliva and nasopharyngeal swabs collected by health-care workers.^[15]

Various methods have been mentioned in the literature to collect saliva. A simple drooling technique has been suggested by Azzi *et al.*^[10] to collect saliva and the collected specimen was suspended in 2 ml of phosphate-buffered saline. To *et al.*^[17] collected saliva by coughing up and clearing the throat such that the collected sample contained fluid from the posterior oropharynx also. Williams *et al.*^[18] suggested a method in

which prior to collection, patients were asked to pool saliva and then spit 1–2 ml into a collection tube. However, this method of saliva pooling in the mouth might lead to dilution of the viral load in the specimen because of excessive saliva secretion.

TIMING OF SAMPLING

The fact that saliva may depict alteration due to physiological processes taking place throughout the day must be taken into consideration. Most of studies have requested participants to avoid oral stimulation such as eating, drinking, and oral hygiene practices during a certain period before a sample is taken therefore establishing standard sample conditions due to the presence of circadian rhythms in saliva and their effect on its composition.^[8,19]

It is recommended to collect saliva between 8 and 10 a. m. to reduce the influence of circadian rhythms on the results.^[19] Tajima *et al.*^[20] also found that early morning saliva specimens were more likely to show positive 2019-nCoV RNA results than daytime saliva specimens. The positive rate of saliva specimens collected during the early phase of onset defined as within 9 days of symptom onset was 93.4% and 63.0% after 10 days of symptoms.^[20,21]

PROSPECT OF SALIVARY BIOMARKERS IN CORONAVIRUS DISEASE-2019 PROGNOSIS

Coronaviruses delays or decreases interferon (INF) production which activates inflammatory responses triggering severe pulmonary conditions.^[22,23] The unregulated host immunological response and the “cytokine storm” are considered to correlate with poor prognosis and severity of the disease.^[22]

Recent studies have outlined that severity of COVID-19 cases can be correlated with elevated levels of granulocyte colony-stimulating factor, interleukin (IL)-2, IL-6, IL-7, IL-10, INF- γ -inducible protein-10, macrophage inflammatory protein-1A, tumor necrosis factor- α , and macrophage chemotactic protein, suggestive of inflammatory response mediated by cytokine release having a pivotal role in COVID-19 progression.^[9,23]

Saliva provides an opportunity to measure the markers of the inflammatory process such as chemokines and cytokines. Thus, evaluation of inflammation-related biomarkers in saliva can provide us with a platform to establish inflammatory profile assisting in prognosis stratification of COVID-19-infected patient.^[24]

ANTIBODIES AGAINST SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

Supplementary to RT-PCR-based RNA detection of COVID-19, few have documented encouraging results for the detection of IgG and IgM antibodies in the serum/plasma samples of infected patients. Further investigations suggest it may be

justified to hypothesize that human saliva is a source for anti-SARS-CoV-2 antibodies.^[24]

Antibodies such as IgA and IgM have been detected in different biological fluids including self-collected saliva. These being more stable than RNA simplify the sampling process and aid in the identification of asymptomatic patients. Antibody detection tests also assist to track down how competitively the patient’s immune system is fighting against the virus and are potential guide for plasma transfusion therapies.^[24,25]

RUCDR Infinite Biologics at Rutgers University have stated that salivary testing equals in performance to the approved swab-based collection technique.^[26]

The salivary diagnostic technique offers us with an additional advantage of:^[27]

- Real-time diagnostic value
- Any number of samples can be easily collected
- Eliminates the need for specific clinical setup for the collection and screening of samples
- Unlike blood, saliva does not clot
- In comparison to serum, saliva requires less manipulation during the diagnostic procedures.

CONCLUSION

Salivary sampling for COVID-19 detection ensures high sensitivity, efficacy, reliability specificity, and concordance. Saliva offers a promising diagnostic alternative and can minimize the risk of nosocomial transmission. The current article highlights the pivotal role of saliva in COVID-19 detection.

Scope for future research

Insight into diagnostic application of saliva for COVID-19 detection brings with it a strong promise, at the same times opens up immense scope for investigation with regard to optimal temperature for sample collection, storage, and ideal transport media. Standardization of an appropriate assay can help in validation for the diagnostic application of saliva. The reliability of gingival crevicular fluid as a diagnostic source for COVID detection also needs further insight.

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Occlusal stress distribution in the human skull with permanent maxillary first molar extraction: A 3-dimensional finite element study

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Introduction: The objective of this research was to analyze the effect of orthodontic treatment with maxillary permanent first molar extraction on the occlusal stress distribution and displacement in the human skull. **Methods:** A 3-dimensional finite element model was constructed on the basis of a computed tomography scan, and it was used as the pretreatment model. The software used for geometric modeling was Solid Works (Dassault Systèmes, Paris, France). For the extraction model, the maxillary permanent first molar was removed, followed by a repositioning of the anterior and posterior segments to create a space closure model. Stress distribution was evaluated under the simulation of 1000 N for occlusal forces and 400 N for masseter muscle force. **Results:** The highest von Mises stress was observed at the zygomatic process of the temporal bone across all 3 models (25 MPa), whereas stress at the pterygomaxillary suture area was almost 50% less. However, the stress in the pterygomaxillary suture area was lowest in the extraction model (18%) and space closure (30%). Stress in the zygomatic process of the frontal bone and frontal process of the maxilla increased from pretreatment to extraction model followed by space closure model. **Conclusions:** The occlusal forces were transferred through maxillonasal, maxillozygomatic, and maxillopterygoid stress trajectories. The mesial displacement of the molars may weaken the role of maxillopterygoid stress trajectory while strengthening the role of maxillonasal stress trajectory. (Am J Orthod Dentofacial Orthop 2021; ■: ■-■)

The occlusal forces during mastication are transferred through the teeth, periodontal ligaments (PDLs), and the alveolar bone to the supporting structures of the skull.¹ Theoretical fundamentals of biomechanics explain the skull architecture on the basis of bone pillars in the maxilla and trajectories of stress dissipations in the mandible.²

According to Sicher,³ the masticatory forces are dissipated from the alveolar process to the 3 enhanced bone pillars in the maxilla, located at each antimer and bypassed by nasal and orbital cavities. The canine and zygomatic pillars (ZPs) are horizontally connected along

with the supra and infraorbital edges, which act as beams that resist the mechanical stresses. The pterygoid pillar is an enhanced bone arched toward the skull base and hard palate, connecting the pillar systems on each side of the skull.² The nature of strain and stress distribution helps in better understanding of diagnosis and treatment of stomatognathic diseases and reconstruction of masticatory function.⁴

It is generally accepted that the maximal occlusal force is higher in molars than incisors.⁵ There is an inherent relationship between key ridge, infrazygomatic crest, and the position of the first molar.⁶

Recent dental health surveys in the United Kingdom have shown an increase in the incidence of decay associated with the first molar, and it also significantly display enamel hypoplasia.^{7,8} As a general rule, the compensating extraction of a maxillary first permanent molar has been recommended when extraction of the mandibular first permanent molar is required. This is to avoid supraeruption of an unopposed maxillary first permanent molar, which can prevent desirable mesial movement associated with the erupting mandibular

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All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.

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second permanent molar and other occlusal interferences, whereas balancing extraction of sound first permanent molar has been recommended to preserve arch symmetry.^{9,10}

In certain occlusions, when space is not required to reduce overjet or relieve crowding, it may be beneficial to extract the first molar, which favors mesial eruption of the second molar into extraction space.¹¹

In the finite element study by Cattaneo et al,¹² the forward and backward displacements of the maxillary molars from the infrazygomatic crest affected the pattern of load transfer through the maxillary complex. Thus, it becomes important to determine the areas of stress distribution in patients with missing or extracted maxillary first molars.

The finite element analysis (FEA) is the most advanced and precise tool in biomechanical research. It is the ultimate method for modeling complex structures and analyzing their mechanical properties.¹³ It allows establishing the location, magnitude, and direction of an applied force, as it may also assign stress points that can be theoretically measured. Furthermore, it does not affect the physical properties of the analyzed materials.¹⁴

However, to date, there have been no studies dealing with the stress distribution and displacement after orthodontic treatment with maxillary first permanent molar extraction. The purpose of this study is to evaluate and analyze the effect of orthodontic treatment with maxillary permanent first molar extraction on the occlusal stress distribution in the skull by FEA.

MATERIAL AND METHODS

A 3-dimensional (3D) FEA model of a human skull was produced on the basis of computed tomography (CT) scan data of a healthy 15-year-old male patient with no craniofacial anomaly. The CT scan was taken for study with a 3-mm scan increment and a 1-mm slice thickness. The model had a set of all permanent dentition with normally erupted teeth and maxillary permanent first molar with a poor prognosis on the right side. The protocols of this study were approved by the ethics committee of the institute (IEC/2017-18/02). The CT scan was read into image processing software (Mimics, version 14.0; Materialise, Leuven, Belgium). The images were segmented by thresholding to obtain the right skull and maxillary teeth. The 3D reconstruction of these parts was then formed with a surface triangularization technique (Fig 1). This reconstruction formed the basis for the so-called pretreatment model. The triangularized shapes of the bone were subsequently read into image processing software to manipulate the molar position. The 3D models were assumed to have maxillary teeth, PDLs (0.25-mm thick),¹⁵ and cortical and cancellous bone thickness of 1-mm thick.¹⁶ CT scan images of the maxilla, including the skull, are made with a top cutting plane. The physical model of brackets was scanned at Advance CAD Technologies Pvt Ltd (Pune, India), using REXCAN III 3D white light scanner (Solutionix, Seoul, South Korea). The computer-aided designing model with proper details about the brackets and teeth were obtained. The obtained computer-aided designing model was used to construct the geometric model of the teeth with the bonded brackets in Geomagic Design X software (3D Systems Manufacturing, Rock Hill, SC).

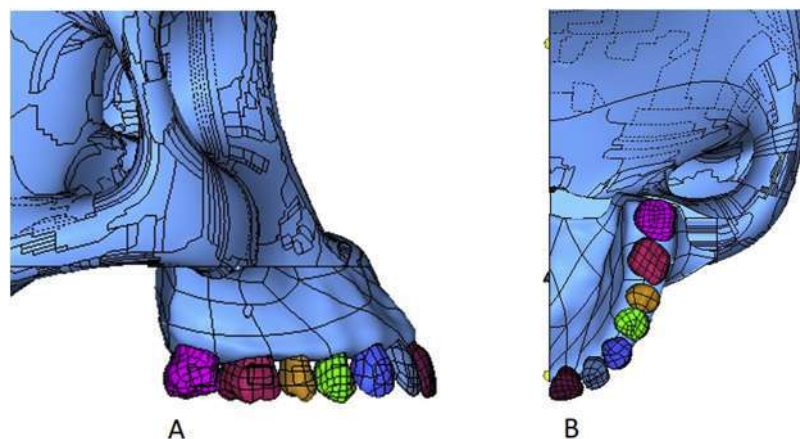



Fig 1. The process for the 3D FEA model: **A**, 3D reconstruction of the CT scans; **B**, 3D FEA model occlusal section.


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The software used for geometric modeling and to import individual CT scan sections and traced was Solid Works (Dassault Systèmes, Paris, France). Individual CT scan sections were imported into the Solid Works software and traced, taking care not to disturb the anatomy of the region. These blocks were imported into HyperWorks software (version 13.0; Altair Engineering, Troy, Mich).

The study aimed to determine the occlusal stress distribution and displacement in the human skull after permanent maxillary first molar extraction. For the boundary conditions, the movement was restricted in the top cutting plane.

The von Mises stress and the normal stress were evaluated under the simulation of 1000 N for the occlusal force on the maxillary right teeth and the simulation of 400 N for the masseter muscle force, which originated at the inferior part of the zygomatic arch and inserted into the lateral surface of the ramus of the mandible, before and after extraction of maxillary permanent first molar¹ (Table I). To produce the extraction model, the permanent maxillary right first molar was removed. Then the anterior segment was repositioned back by 4.1 mm and the posterior segment forward by 6.9 mm on the sagittal plane (Fig 2). Buccolingual minor repositioning and rotation of the anterior and posterior dental segments were done to maintain the continuous curvature of the dental arch. The stress was compared at 4 points within the main stress trajectories: the frontal process of the maxilla (FM), the zygomatic process of the frontal bone (ZF), the zygomatic process of the temporal bone (ZT), and the pterygomaxillary suture area (PM).

The linear FEA method has been used, and all the materials were assumed to be isotropic, homogenous, and linearly elastic. The total number of nodes and elements in the pretreatment model were 207,148 and 1,072,185 for teeth, 98,532 and 145,986 for a PDL, 138,225 and 680,737 for alveolar bone and skull bone. In the case of the extraction model, a total number of nodes and elements were 167,902 and 869,724 for teeth, 92,654 and 134,864 for a PDL, 138,225 and 680,737 for alveolar bone and skull bone, 11,802 and 45,587 for the bracket, 2653 and 980 for archwire whereas in space closure model, 167,902 and 869,724 for teeth, 92,654 and 134,864 for a PDL, 147,231 and 694,632 for alveolar bone and skull bone. The space closure model had a higher number of nodes and elements than the other 2 models. After extraction of the first molar, as the second molar moved anteriorly, these geometric changes in the position of the second molars led to the reconstruction of the space closure model with highly refined mesh. The precision of the FEA calculation increases as

Table I. Loading conditions¹

Force application point	Model		
	Pretreatment	Extraction	Space closure
Central incisor	20	20	20
Lateral incisor	20	20	20
Canine	20	20	20
First premolar	60	120	120
Second premolar	60	140	140
First molar	370	–	–
Second molar	450	680	680
Total occlusal force	1000	1000	1000
Masseter muscle	400	400	400

Note. Values represent force (N).

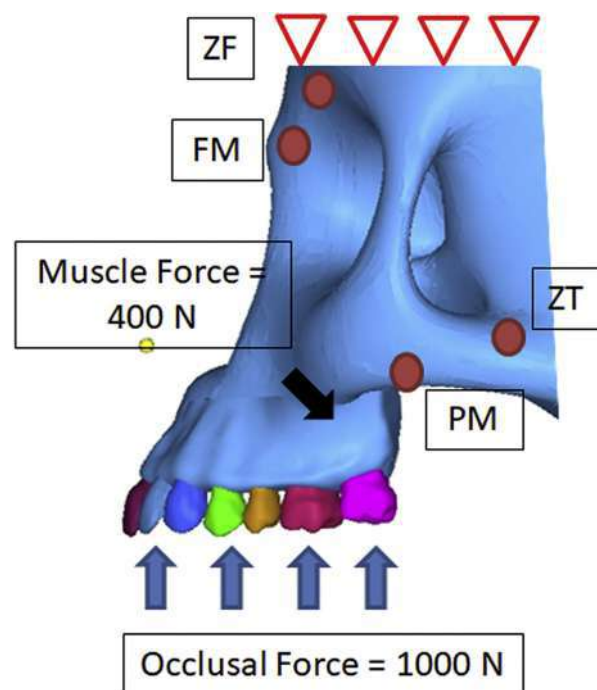


Fig 2. Lateral view of the FEA model. The boundary and external loading conditions are shown. The amounts of stress in both models were compared at 4 points: the FM, ZF, ZT, and PM.

highly refined meshes of the model are used. The highly refined mesh is required in critical areas such as areas with a higher stress concentration and zones of complex and highly curved geometric shapes within the model.⁴ Hence, a mesh convergence study was recommended. The space closure model was reanalyzed using a 10-noded tetrahedral element to ensure convergence in the pattern of stress; therefore, discretization error can be well controlled to get more accurate results with a reasonable model dimension. The material properties

of the bone and teeth in the model were defined according to experimental data from previous studies.^{1,17-19} (Table II).

RESULTS

When occlusal forces were applied to the maxillary dentition, the stresses were observed and distributed throughout the skull (ie, the PDL, alveolar bone, maxilla, zygomatic arch, and reached the frontal and temporal bones in all the 3 models). A high concentration of stresses was present at the zygomatic arch and infrazygomatic crest. The stresses were also concentrated in the pyriform aperture, infraorbital rim, and pterygoid plate areas.

The von Mises stress expresses the overall stress intensity, and its distribution gives an impression of overall load transfer. The von Mises stress was highest at ZT across all 3 models (25 MPa), whereas von Mises stress in PM (13.5 MPa) was almost 50% less than the stress at ZT. Stress in PM was reduced in the extraction model (11.36 MPa) by approximately 18%, whereas 30% reduction in von Mises stress was observed for the space closure model (10.24 MPa). The stress in ZF and FM increased from pretreatment (11.92 MPa and 20.17 MPa) to the extraction model (17.63 MPa and 22.35 MPa) and further in the space closure model (18.91 MPa and 24.82 MPa), respectively (Fig 3; Table III).

The normal stresses were distributed in the x-, y-, and z-axes. In the vertical direction (y-axis), compressive stresses were found at the infrazygomatic crest, PM and FM, whereas tensile stress was found at ZF (Fig 4; Table IV). The reversal of stresses (ie, compressive to tensile stress) was seen at ZT from pretreatment (0.56 MPa) to space closure model (0.16 MPa). The compressive stress at PM (1.03 MPa) was lower in the pretreatment model than the extraction model (1.23 MPa) and space closure model (1.16 MPa), whereas stress at FM was higher in the space closure model (0.21 MPa) than the pretreatment model (0.18 MPa) followed by extraction model (0.16 MPa).

In the anteroposterior direction (z-axis), compressive stresses were found at the zygomatic process of the frontal bone and the infrazygomatic area, whereas tensile stresses were found at the infraorbital rim, alveolar bones near the teeth, and the zygomatic process of the temporal bone in all the 3 models. The compressive stress was highest at PM in the pretreatment model (3.30 MPa), whereas it was slightly reduced in the extraction model (3.06 MPa), and in the space closure model, it was lowest (2.46 MPa). Tensile stress was highest in the pretreatment model (2.20 MPa)

Table II. Material property data representation^{1,17-19}

Material	Young's modulus (MPa)	Poisson's ratio
Teeth	20,000	0.3
Cortical bone	13,700	0.3
Trabecular bone	1370	0.3
Stainless steel archwire	200,000	0.3
PDL	0.13	0.45
Brackets	193,000	0.35
Buccal tube with traction arm	200,000	0.3

at ZT when compared with the extraction model (2.03 MPa) and space closure model (1.91 MPa) (Fig 4; Table IV).

In the lateral direction (x-axis), the compressive stress was found at the infrazygomatic area, frontal process of the maxilla and pterygomaxillary suture area, and the tensile stresses were found at the infraorbital rim and the alveolar bones near the teeth in all the 3 models. The highest compressive stress was seen at PM in the extraction model (6.01 MPa) when compared with its corresponding pretreatment model (5.67 MPa) and space closure model (4.82 MPa). In contrast, the lowest stress was seen at FM in the pretreatment model (0.35 MPa) when compared with the extraction model (0.36 MPa) and space closure model (0.42 MPa), whereas tensile stress was seen at ZT, in which the highest stress was in space closure model (0.78 MPa), and the lowest stress was in the pretreatment model (0.47 MPa) compared with the extraction model (0.53 MPa) (Fig 4; Table IV).

In the x-, y-, and z-axes, the space closure model showed a different stress pattern than the pretreatment model, and the compressive stress decreased at PM lateral (x-axis) and anteroposterior direction (z-axis). In addition, in the ZT area, the stress pattern was changed from compressive to tensile stress in a vertical direction (y-axis) (Fig 4; Table IV).

Maxillary displacement in the pretreatment and extracted model was the same (0.57 mm). However, a different displacement pattern was observed in the space closure model (0.55 mm). The pretreatment and extraction model had maximum displacement seen at the posterior segment with a v-shaped triangular pattern observed in the palatal region. However, the space closure model had different and exactly opposite v-shape triangular patterns that were seen in the palatal aspect (Fig 5; Table V). In the space closure model, the molar moves ahead by 70%, and the anterior segment moves behind by 30% of extraction space.

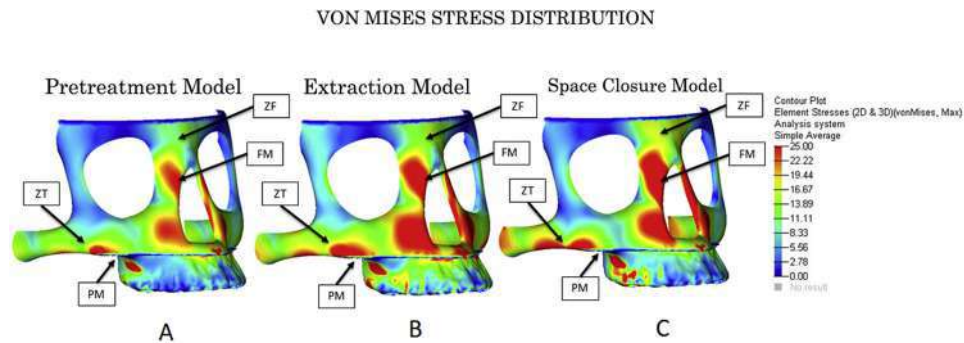


Fig 3. von Mises stress distribution: **A**, pretreatment model; **B**, extraction model; **C**, space closure model.

Table III. von Mises stress in the pretreatment, extraction, and space closure models

Area	Models		
	Pretreatment	Extraction	Space closure
FM	20.17	22.35	11.36
ZF	11.92	17.63	18.91
ZT	25.12	25.34	25.72
PM	13.45	11.36	10.24

Note. Values are presented as MPa.

DISCUSSION

Understanding the pattern of occlusal load distribution is very important as it not only reflects the functional morphology but also prevents bone damage caused by excess masticatory load and orthodontic forces.^{1,2} The pattern of occlusal force distribution observed in our study was consistent with the classical theory of buttresses (ie, the occlusal forces were transferred through the maxillonasal, maxillozygomatic, and maxillopterygoid buttresses), as previously reported.^{20,21}

The maxillary first molar is considered as the key to occlusion and has a favorable anatomic functional relationship with the zygomatic alveolar crest and ZP.² Previous studies by Cattaneo et al¹² and Gross et al²² confirmed that the first molar occlusal relationship could influence the stress distribution to the ZP, which had a significant impact on the success and failure of orthodontic treatment and anchorage implants.

Daugaard-Jensen,²³ suggested that patients with first molar extraction were no more time consuming than patients with 4 premolar extraction as it offers distinct advantages in terms of anchorage management, and this was supported by Williams and Hosila,²⁴ who highlighted the fact that extraction of the first molar will have less effect on the profile and a 90% chance

of successful third molar eruption compared with the approximately 55% chance with patients with premolar extractions.

The von Mises stresses were higher at the zygomaticotemporal suture and nasofrontal areas than at the pterygoid plate and zygomaticofrontal suture areas in all the 3 models, from which it can be inferred that these areas play an important role in bearing and transferring stress (Fig 3; Table III). The findings of the present study were in agreement with Hilloowala and Kanth,²⁵ who concluded that one of the most resistant areas of the skull involved in the distribution of stress of masticatory force is the ZP. However, in the extraction and space closure models, the von Mises stress decreased in the PM and increased in the ZF and FM, compared with the pretreatment model. Thus, the mesial displacement of the molars resulting from orthodontic treatment weakens the role of the maxillopterygoid stress trajectory (PM) while strengthening the role of the maxillonasal stress trajectory (FM). Furthermore, this study was in agreement with the study conducted by Choi et al,¹ in which after extraction of maxillary first premolar showed a similar pattern of stress distribution and concentration of stresses at the same trajectories.

In the vertical direction (y-axis), compressive stresses were found at the infrazygomatic crest, PM, and FM, whereas tensile stress was found at ZF (Fig 4; Table IV). Interestingly, we observed a reversal of stresses (ie, compressive to tensile stress) at ZT from pretreatment to space closure model. This reflects the equilibrium force (ie, compressive stresses created by the upward occlusal force and the tensile stress by the downward masseter muscle force), creating a balance in these areas. The stresses were moved more anteriorly, contributing to the bending of the zygomatic bone downward. Our finding was in agreement with studies by Choi et al,¹ Cattaneo et al,¹² and Prado et al.²⁶

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NORMAL STRESS DISTRIBUTION

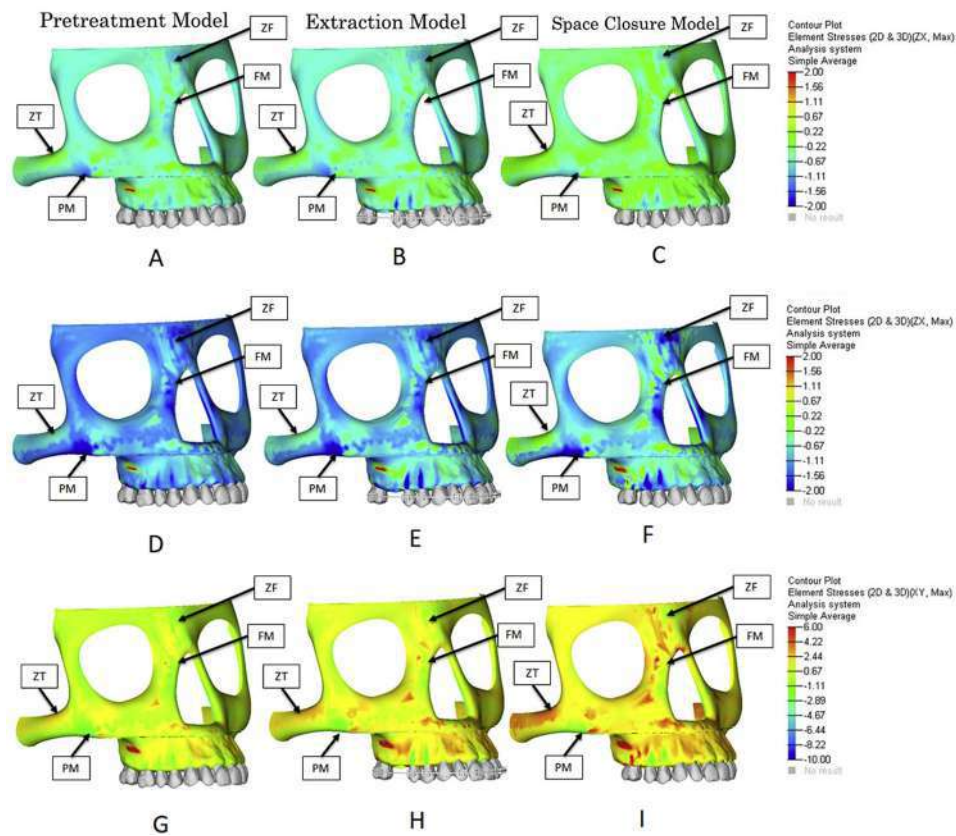


Fig 4. Normal stress distribution along the y-axis (A-C), z-axis (D-F), and x-axis (G-I). Blue color (negative value) represents compressive stress, whereas red color (positive value) represents tensile stress.

Table IV. Normal stresses in the pretreatment, extraction, and space closure models

Area	Models								
	Y-axis			Z-axis			X-axis		
	Pretreatment	Extraction	Space closure	Pretreatment	Extraction	Space closure	Pretreatment	Extraction	Space closure
FM	-0.18	-0.16	-0.21	-1.236	-3.299	-1.362	-0.35	-0.36	-0.42
ZF	0.65	0.62	0.63	-0.807	0.566	-0.390	-2.24	-2.03	-1.67
ZT	-0.56	-0.67	0.16	2.199	2.031	1.907	0.47	0.53	0.78
PM	-1.03	-1.23	-1.16	-3.299	-3.059	-2.462	-5.67	-6.01	-4.82

Note. Values are presented as MPa. Negative values indicate compressive stress, and positive values indicate tensile stress.

In the anteroposterior direction (z-axis), compressive stresses were found at the zygomaticofrontal bone and the infrazygomatic area, whereas tensile stresses were found at the infraorbital rim, alveolar bones near the teeth, and the zygomatic process of the temporal bone in all 3 models. In the pretreatment model, the compressive stress was highest at the PM area, whereas tensile stress was highest at ZT (Fig 4; Table IV). This was because the application of mechanical loading creates

a resultant to the masseter muscle contraction, which showed 2 lines of tensile stress at the maxillary sinus walls.

According to studies by Blaney²⁷ and Blanton and Biggs,²⁸ sinus walls are areas of compact bone located between the skull pillars, which were resistant to the masticatory forces. The low tensile stress on the floor and high tensile stress at the lateral wall confirm that resultant forces of the masseter muscle contraction



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DISPLACEMENT

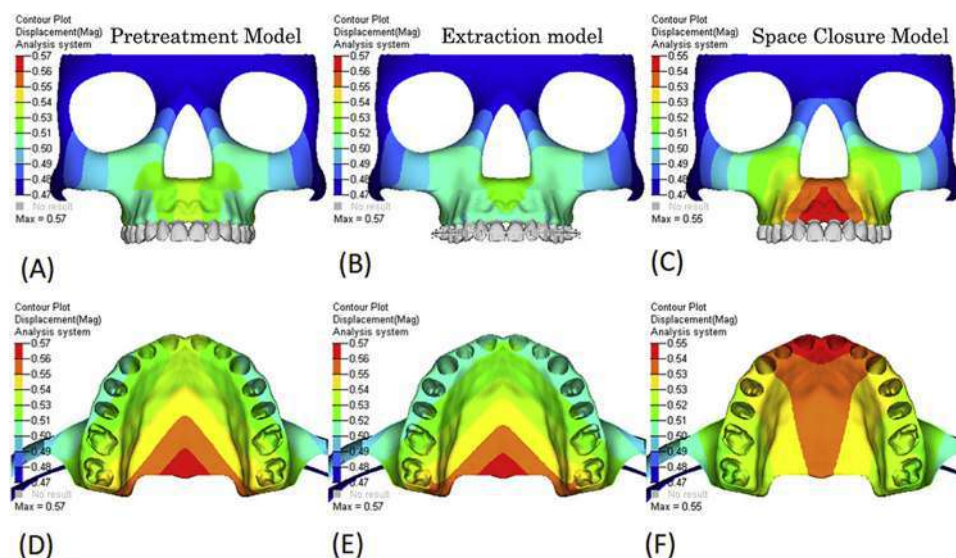


Fig 5. Displacement (frontal view - A-C and occlusal view - D-F) in the pretreatment, extraction, and space closure models. Red color indicates tensile stress (+) and Blue color indicates compressive stress (-).

Table V. Displacement in the pretreatment, extraction, and space closure models

Variable	Pretreatment model	Extraction model	Space closure model
Displacement	0.57 mm	0.57 mm	0.55 mm

were nonuniformly distributed through the ZP.² Tensile stresses were also observed in the alveolar bone near the teeth, and compressive stress was observed in the interdental area between the second premolar and second molars.

According to Frost,²⁹ when bone strains are below a lower threshold range, disuse-mode remodeling can turn on to reduce whole bone strength by removing some amount of trabecular and endocortical bone. If the alveolar bone does not adapt physiologically to the local change in the stress distribution in relation to the mesial displacement of maxillary second molars after extraction of the maxillary first permanent molar, alveolar dehiscences and fenestrations can occur in this area.^{1,12,29}

In the lateral direction (x-axis), the compressive stress was found at the infrazygomatic area, frontal process of the maxilla and pterygomaxillary suture area, and the tensile stresses were found at the infraorbital rim and the alveolar bones near the teeth and the zygomatic

process of the temporal bone in all the 3 models (Fig 4; Table IV). The highest compressive stress was seen at PM in the extraction model, and the lowest stresses were seen at FM in the pretreatment model, whereas the highest tensile stress was seen at ZT in the space closure model. It can be explained that as the occlusal force and masseter muscle force are not on the same line of force, the occlusal force does not disappear when the masseter muscle force appears.

From this stress pattern, it can be speculated that the entire maxillary complex may bend in the frontal plane and sagittal plane, resulting in the compression of the midpalatal suture area. This was in support of studies conducted by Burkes et al³⁰ and Jainkittivong and Langlais,³¹ who concluded that stresses cause the crestal alveolar bone to grow along the opposing vector of occlusal forces. Therefore, an altered function may lead to the development of exostosis in genetically predisposed populations.

Maxillary displacement in pretreatment and extracted models were the same. However, a different displacement pattern was observed in the space closure model. The pretreatment and extraction model had maximum displacement seen at the posterior segment with a v-shaped triangular pattern observed in the palatal region. However, the space closure model had different and exactly opposite v-shape triangular

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patterns seen on the palatal aspect (Fig 5; Table V). From this finding, it can be assumed that this change in displacement pattern might be due to treatment resulting in strengthening nasomaxillary stress trajectories and an increase in cortical bone thickness in this region.

CONCLUSIONS

The present study derived the following conclusions:

1. The occlusal forces were transferred through the maxillonasal, maxillozygomatic, and maxillopterygoid stress trajectories
2. The mesial displacement of the molars resulting from orthodontic treatment may weaken the role of the maxillopterygoid stress trajectory (PM) while strengthening the role of the maxillonasal stress trajectory (FM)
3. Bone adapts physiologically to such local change in stress distribution; if not, dehiscences and fenestrations may occur.

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Review Article

Rapid prototyping: A frontline digital innovation in dentistry

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ABSTRACT

The power, predictability and speed that today's digital solutions offer to human oral health care have gained a rapidly growing foothold in clinical dentistry. One such revolutionary innovation is the rapid prototyping technique. This technique facilitates the fabrication of physical models from computer aided design (CAD) data using 3D printers. This technique enables the dentist and laboratory technician to produce dental-related prosthesis with a greater speed and accuracy, thus making it a reliable alternative to the much labour intensive and time consuming hands on procedure. The present article reviews this current technology, its historical development, methods and applications in dentistry.

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1. Introduction

The necessity of answering geometrical complexity has led to the introduction of Rapid Prototyping into the dental setup. It has the potential to become the next 'state-of-art fabrication technique in modern dentistry.¹ Rapid Prototyping equips us to broaden our vision in this era of digitalization, from conventional two-dimensional (2D) thinking to more informative three-dimensional (3D) thinking.²

Rapid Prototyping (RP) enables quick and automatic construction of a three-dimensional (3D) model of a part/product utilizing stereolithography machines or 3D printers.¹ Initially, slicing of digital models is done, then through automated layer-by-layer construction, transverse sections are produced and these 3D physical structures are known as rapid prototypes.³

RP is also termed as Layered manufacturing, Solid freeform fabrication, or Generative manufacturing.⁴

Digitalization in the form of Rapid Prototyping not only provides a brilliant break-through in various aspects of dentistry like orthodontics, prosthodontics, oral surgery, implantology and operative dentistry etc, but it also has created a great spur on the current time consumed on traditional laboratory design and procedures.²

The pivotal objective of this review is to focus on the types of Rapid Prototyping technique and its applications in the field of dentistry.

It provides fascinating opportunities in various aspects of dentistry like orthodontics, prosthodontics, oral surgery, implantology and operative dentistry etc. The key objective of this review is to focus on recent advancements of RP technology and its applications in dentistry.

2. Timeline

The concept of RP is not new and has seen development at various timelines.^{5,6}

- 1894 - Chuck Hull first introduced the concept of 3DP described it as stereolithography (SLA).

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2. 1957 - World's first CAM software program using a numerical control programming tool named PRONTO by Dr Patrick J. Hanratty.
3. 1971 - Computer-assisted production of dental restorations by Duret.
4. 1988 - Hull and the company 3D system developed the first 3D printer termed "SLA apparatus".
5. 1990 - Scott Crump engineered a technique called "FDM".
6. 1992 - Introduction of SLS.

3. Steps in RP Models Production

Rapid prototyping involves fabrication of a 3-D physical model directly from a computer-aided design model. Either contact or non-contact technique can do data acquisition.⁷ They all start with a 3D computer-aided design (CAD) model of the anatomical area, which usually can be derived from X-ray CT or MRI data.⁷ The steps involved in RP model production are summarised in Table I. Steps have been represented in Figure 1.

Table 1: Steps in RP models production

CAD-CAM steps	CAD-CAM system
Data acquisition	Contact or non-contact methods Example: Optical modeling, laser scanning, CT, MRI, digital photographs
Data processing	Digital data is processed to obtain a CAD model
Model fabrication	Rapid prototyping, CNC milling

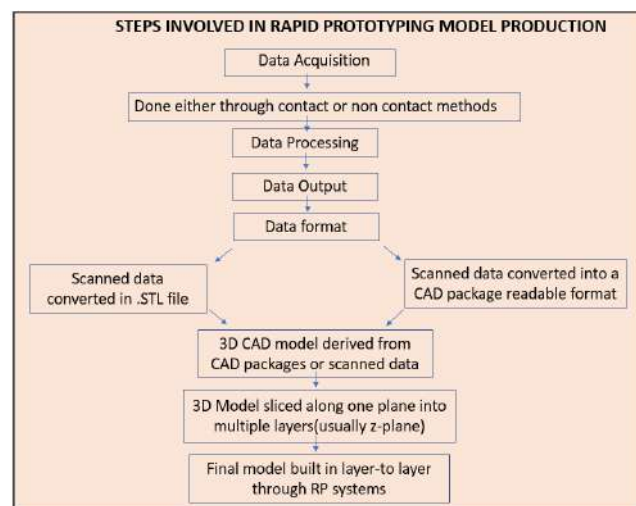


Fig. 1: Schematic flowchart of steps involved in RP model production

4. Digital Approaches for a Physical Prototype

The emergence of advanced digital technology has opened new perspectives in clinical dentistry.⁸ To fabricate a

physical prototype or a scale model through digital technology, two different approaches can be used:

1. Subtractive approach.
2. Additive approach.

Subtractive approach is usually facilitated using computer numerical control machining (CNC milling). The data principally obtained from an optical or contact probe surface digitizer captures the external surface data of the prescribed anatomy and not the internal tissue surface, hence less effective. This approach relies on milling an available larger blank using a CNC machine.⁹ The CAM software automatically translates the designed CAD model into a tool path for the CNC machine. This involves series of procedures, including sequencing, milling tools, and tool motion direction and magnitude. The dental CNC machines are composed of multi-axis milling devices facilitating 3D milling of dental prosthesis.

4.1. 3-axis milling devices

These are the most commonly used. The milling burs move in three axes (x -, y - and z - axes) according to calculated path values. Therefore, 3-axis milling has the advantage of having minimal calculation and cumulative milling time.⁹

4.2. 4-axis milling devices

These machines allow for blank movements in an additional axis, which helps mill a larger blank producing long-span frameworks.⁹

4.3. 5-axis milling devices

The 5th axis in this machine provides a rotating path of the milling tool or the blank. This facilitates the production of very complex geometries and smooth external surfaces such as acrylic denture bases.⁹

Keynote: For dental applications, the quality of the restoration is independent of the number of axes; instead, it reflects the method of processing the workpieces and CAD path of milling.

The Additive approach, on the other hand, can produce arbitrarily complex shapes with cavities such as human anatomical structures with the use of rapid prototyping. Additive manufacturing is defined as joining materials to make objects from 3D model data, usually layer upon layer. Once the CAD design is finalized, it is segmented into multislice images. The machine lays down 5–20 layers for each millimeter of material successively with liquid and powder that fuses to create the final shape. This is followed by workpiece refinement to remove the excess materials and supporting arms.¹⁰

4.4. Types of Rapid Prototyping Techniques:

Rapid Prototyping system commonly engaged in dentistry are:

1. Stereolithography (SLA),
2. Inkjet-based system (3D printing - 3DP),
3. Selective laser sintering (SLS and selective laser melting),
4. Fused deposition modeling (FDM).

Keynote: All the methods share the common work principle that distinguishes them from subtractive manufacturing:

1. Incremental vertical object build-up
2. No material wastage
3. Production of large objects
4. No application of force (passive production)
5. Production of fine details

4.5. Materials used for rapid prototyping

Wax, Plastics, Resins, Ceramics and Metals in the form of powder and liquid, Modeling materials and colors, such as medical-grade ABS, polycarbonates.⁵

4.6. Stereolithography

It creates a three-dimensional model using a computer-controlled moving laser beam to build up the required model from a liquid in a layer-by-layer manner. The first process of this type of RP was patented by Hull (1984).

4.7. Components of this system

A bath of photosensitive liquid resin, a model-building platform, and an ultraviolet (UV) laser for curing the resin.

4.8. Procedure

On the model-building platform, a layer of resin is exposed to UV light. After a resin layer is cured and hardened, the cured resin platform is lowered to the bath by a pre-fixed distance. A Wiper blade is used to wipe a new layer of resin across the previous layer, which is consequently exposed to the UV light and cured. The process of curing and lowering the platform into the resin bath is repeated until the complete model is built. (Figure 2)⁴ The self-adhesive property of the material causes the layers to bond to each other and eventually form a 3D object. The model is then removed from the bath and for then placed in a UV cabinet for sometime.^{11,12} The advantages and disadvantages of this technique are described in Table 2.

5. Inkjet-Based System (3D printing - 3DP)

A measured quantity of powder is dispensed from a supply chamber by incremental upward movement of piston. The

Table 2: Stereolithography

Advantages	Disadvantages
High accuracy	Expensive equipment
Good surface finish	High Material cost
Can be made transparent	Can be used only for polymers
100 percent density possible	Requirement of Post - cure
High-mechanical strength	
Fine building detail	

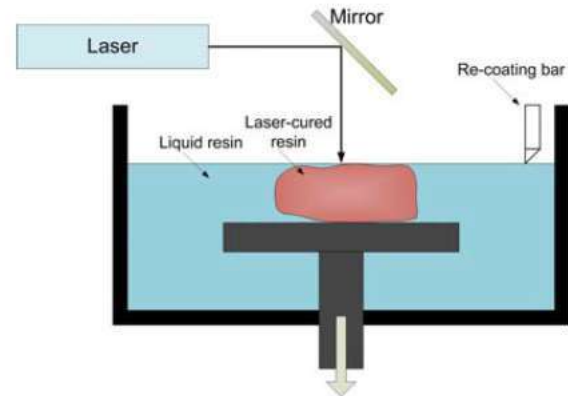


Fig. 2: Schematic diagram of stereolithography

roller then distributes and compresses the powder at the top of the fabrication chamber. Then a multi-channel jetting head subsequently deposits a liquid adhesive in a 2D pattern onto the layer of the powder, which becomes bonded in the areas where the adhesive is deposited to form a layer of the object. Once a layer is finished, the piston that supports the powder bed and the part lowers so that the next powder layer can be spread and selectively joined. This layer-by-layer technique is gradually continued until the prototype is completely built up. (Figure 3)⁴ The unbound powder is swept off with a heat treatment process, leaving the fabricated part intact.¹³ The advantages and disadvantages of this technique are described in Table 3.

Table 3: Inkjet based system

Advantages	Disadvantages
Fast fabrication time	Large tolerance
Low material cost	Lower strength models
The capability of being colored	Rough surface finish
Build models can be used for casting	
Low toxicity	
Relative material variety	

6. Fused Deposition Modeling (FDM)

In this system, a temperature-controlled head extrudes thermoplastic material layer by layer. A filament of a

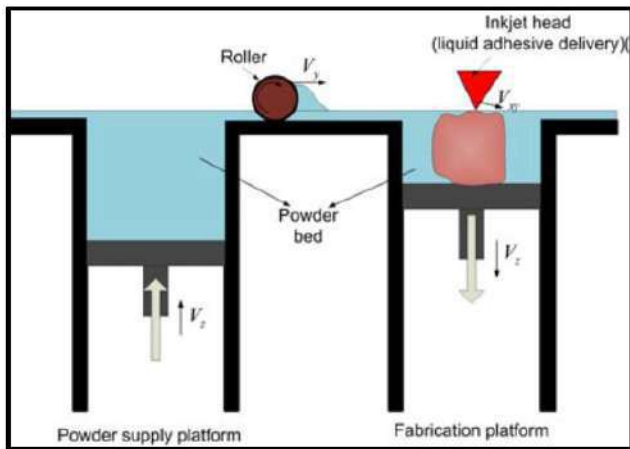


Fig. 3: Schematic diagram of Inkjet-based system

thermoplastic polymer material feeds into the temperature-controlled FDM extrusion nozzle head, where it is heated to a semi liquid state. The motion of the nozzle head is computer controlled and is used to trace and deposit the material in ultra-thin layers onto a fixtureless base. The final model is built up layer by layer, and the material solidifies within 0.1s after being ejected from the nozzle and bonds to the layer beneath. This entire system works within a chamber that is held at a temperature just below the melting point of the proposed material. The supporting structures and overhanging geometries are later removed by cutting them out from the object.¹⁴ The advantages and disadvantages of this technique are described in Table 4.

Table 4: Fused deposition modelling

Advantages	Disadvantages
Direct wax pattern	Support structure must be removed
Multi-color part	Rough surface finish
Speedy procedure	Thermoplastic material only
	Not 100 percent dense

7. Selective Laser Sintering (SLS)

In the SLS method, layers of a particular powder material are fused into a 3D model using a computer-directed laser (Figure 4).⁴ A roller distributes the powdered material over the surface of a build cylinder. The powder is spread layer-by-layer on top of the preceding hardened layer and sintered repeatedly with a laser beam.¹⁵ The advantages and disadvantages of this technique are described in Table 5.

8. Selection of RP System

As discussed above, every RP system has its strengths and limitations. A suitable RP machine needs to be chosen such that it matches up to satisfy maximum requirements. Criteria quoted by Zein et al.¹⁶ and Xiong Z et al.¹⁷ for system

Table 5: Selective laser sintering

Advantages	Disadvantages
Fast fabrication time	Large tolerance
Low material cost	Lower strength models
Capability of being colored	Rough surface finish
Build models can be used for Casting purposes directly	
Low toxicity	
Relative material variety	

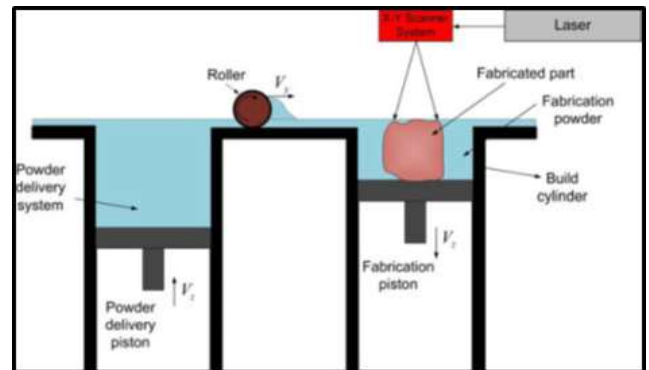


Fig. 4: Schematic diagram of selective laser sintering

selection include: Application purpose, time constraint, cost, materials availability, accuracy, surface finish, and so on.

8.1. For example

While planning a surgery, an RP system, like SLA or FDM, enables us to use different colours to highlight and enhance the visualization of critical structures; hence, be preferable. Similarly, for designing a drilling guide for implants, SLA built model resins have visualization benefits due to their translucent nature.

When building speed is a consideration, the SLA machine is more opted for than the FDM machine. When surface quality and detailing is a significant concern, SLA followed by SLS is preferred. The FDM serves as an economical option.

9. Applications of RP in Dentistry

9.1. Applications in endodontics

The invention of RP has found tremendous applications in endodontics.¹⁸

9.1.1. Guiding the canal

Successful endodontic therapy requires a thorough understanding of root canal anatomy and its variations. In cases of complex root canal anatomies, 3D visualization of the canal through digital reconstruction of tooth model can be simulated through RP technology to achieve the precise

working path of the root canal.¹⁹

9.1.2. Accurate diagnosis of the lesions

In cases of root resorption, clinical and radiographic examination provides only a rough outline of the lesion that may not be continuous with the pulpal cavity. Rapid prototyping in these kinds of scenarios provides three dimensional view of the lesion and assists accurate diagnosis.²⁰

9.1.3. Autotransplantation

Earlier in cases of autotransplantation, the extracted donor tooth is used a guided template for the preparation of the recipient site. But with the advent of rapid prototyping, fabrication of a computer-aided surgical guide for the recipient site is possible. Adopting this new technique has led to a decrease in procedural duration and intraoperative errors.²¹

9.1.4. Endodontic training and research

It can evaluate the efficacy of the newer root canal systems in preparing curved canals of 3d printed tooth models.¹⁸

9.2. Applications in prosthodontics

9.2.1. Wax pattern fabrication

Traditionally, wax pattern making is the most labor-intensive step during the fabrication of crowns and RPD frameworks. With this technology, a new approach to automated wax patterns is now available in the field of prosthodontics. This simplified the traditional age-old technique of hand-made wax patterns. The advantages of automatic wax-up include:

1. High production rate of 150 crowns/hr by dental laboratories
2. Quality control of the wax copings is improved, resulting in the high precision fit of the crowns in the oral cavity.²²

9.2.2. Direct metal prosthesis

Fabrication of high precision metal parts with RP technologies such as selective laser melting (SLM) and SLS technology are used. Dental prostheses processed by employing SLS/SLM technique are appropriate for their complex geometry and their capability to be customized. This procedure eliminates the extensive manual pre and post-processing steps.²²

9.2.3. All ceramic restoration fabrication

The direct inkjet fabrication process has been anticipated using a slurry micro-extrusion process for the fabrication of an all-ceramic crown. This innovative method is a good CAD/RP system with a remarkable ability to produce

high precision fit all-ceramic crowns. The other advantages include cost competence and minimum material intake.²²

Complete dentures:

In complete denture situations, RP technology can be used for:

1. Establishing a 3D graphic database of artificial teeth positioning.
2. Getting 3D data of edentulous models and rims in centric relation.
3. Exploring a CAD route and developing software for complete denture finishing.

9.2.4. Maxillofacial prosthodontics

A wax prototype sculpted on the stone cast by using the free-hand technique can be eliminated using this RP technique. The 3D model of the patient's face (reconstructed with the CT data) can be used, and the technology can be applied in the following areas.²²

9.2.5. Fabrication of obturators, auricular and nasal prosthesis

1. Manufacturing of surgical stents for patients with large tumors scheduled for excision
2. Manufacturing of lead shields to protect healthy tissue during radiotherapy treatment
3. Fabrications of burn stents, where the burned area can be scanned rather than subjecting delicate, sensitive burn tissue to impression-taking procedures.

9.2.6. Implants

Achieving an ideal implant position is one of the key criteria determining implant success. Computer-aided designing and fabrication techniques employing implant simulation software provide a preoperative view of anatomical structures and restorative information, thus improving the procedure's outcome.

Guided implant surgery applies these digital techniques using drill guides processed by stereolithographic rapid prototyping through which implants are positioned with minimal surgical exposure of bone or even with a flapless approach. The advantages of the less invasive flapless surgical procedure include the following:

1. Shorter duration and facilitation of the surgical procedure.
2. Faster and less complicated recovery.
3. Enhanced esthetic results.

9.3. Application in Oral and Maxillofacial Surgery

The length and shape of the grafts to be used in surgical procedures are estimated through the surgical models. Customized plates can be pre-produced to hold the future bone graft, thus facilitating the surgical procedure and



saving intra-operating time. Also, the tumor areas can be coloured using stereolithographic techniques to visually establish their extension and clarify their relationship to the alveolar nerve in the mandible and hard surrounding structures, such as paranasal sinuses, orbit, etc.²³

Prototypes can and should be used in several situations such as: -

1. Evaluation of asymmetrical features
2. Reconstruction of symmetrical structures using mirroring
3. Fracture assessment
4. Modelling rigid internal fixation plates and screw selection
5. Modelling osteogenic distracters
6. Calculation and adaptation of bone grafts
7. Tumor assessment
8. Fabrication of surgical guides

9.4. Applications in Orthodontics and Dentofacial Orthopaedics

1. **Diagnosis and Treatment Planning-** Faber et al. state that RP is especially beneficial in cases with impacted teeth and helps understand the impacted tooth's exact position in relation to its surrounding structures.²⁴
2. **Fabrication of Removable Appliances and aligners** is easier and more accurate using the CAD/CAM technology. Lee et al.²⁵ described a technique where a polyvinyl chloride impression was used and converted into a 3D model in STL format directly using a CBCT image. This method was cheaper, with fewer manual errors when compared to the conventional technique of laser scanning and digitization of human tissues. The prototype is created using the SLA technique, and the aligner is built-in layered fashion using photosensitive liquid resin.
3. **Custom-made Trays-** It assists in the fabrication of custom-made trays in case of indirect bonding. Here, virtual bracket positioning is done on the software-generated digital model. Then using RP technology, trays are created.
4. **Lingual Orthodontics-** Customised lingual brackets can be produced using knowledge of RP technology. This facilitates direct bonding and easier positioning of the brackets onto the tooth surface.
5. **Orthognathic Surgery-** 3D models made using SLA helps understand the accurate positions of anatomical landmarks. Computerized orthognathic surgeries serve special importance in asymmetric cases where discrepancies can be measured and manipulated directly on the models. Mock surgery done on these models can help achieve more efficient post-operative results. Literature also suggests using the RP technique to create a 3D model of the jaws in Distraction

Osteogenesis cases.

10. Bioengineering Research in Dentistry

Tissue engineering is an important genre of treatment when attempting craniofacial reconstruction. RP technique has been proposed for bioprinting of tissue analogs and organ analogs. Features such as the possibility for multicolour printing provide us with added benefits during the arrangement and positioning of multiple cells.⁵

11. Scope of RP

Rapid Prototyping is comparatively a recent advancement with much scope for improvising the exactness, speed, and consistency. Further research needs to be conducted to widen the horizon for materials used in prototype construction. The current cost of the RP system is another area that needs to be dealt with.⁵

12. Conclusion

The ever-growing arena of digital services used for the automated production of dental prosthesis requires knowledge, skill sets and time to master the technology. As dentistry continues to move from analog to digital workflow, it may be time to evaluate state of the art and realize that it is not the computer that makes the decisions-it's only a tool to help clinicians make appropriate decisions and improve their decision with clinical outcomes.

13. Source of Funding

None.

14. Conflict of Interest

None.

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Qualitative evaluation of learning environment in Indian teaching dental institutions from the students' perspective using focus group interviews

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ABSTRACT

INTRODUCTION The perspectives of students towards their learning environment has seldom been in introspect in Indian dental institutions, which are against the advocacy of international dental education working groups. The aim of this study is to document students' perceptions of their learning environment in two of the thirteen teaching dental institutions in the state of Andhra Pradesh, India.

METHODS This qualitative study, involving focus group interviews, was conducted among undergraduate dental students in two teaching dental institutions in the state of Andhra Pradesh. Theoretical sampling was done with concurrent conduct of purposive sampling for focus group interviews and two researchers who participated in the pre-session were involved in moderating the interviews, which consisted of groups of six to eight participants. All the focus

group interviews were audio-taped and transcribed. The transcripts were analyzed using general inductive approach to extract themes.

RESULTS Problems in lectures and in the clinical learning environment, dissatisfaction with evaluation processes and poor academic drive were identified as the major themes responsible for negative learning outcomes. Some of the responses given by the students highlighted their disinclination towards receiving feedback and emphasized the need to move away from the authoritarian approach in teacher–student interactions. Evaluation is a major domain where reforms are necessary, as opined by the students.

CONCLUSIONS The perspectives of students towards the learning environment has seldom been in introspect in Indian dental institutions, which may have a substantial negative impact on students' attitudes and efficiency.

INTRODUCTION

Education is a word that is intriguing, even though it is rather familiar. The meaning assigned or attributed to the word is more belief bound than fact bound. In the rapidly evolving stream of education in healthcare, it appears that transfer of knowledge is the sole purpose of education, with application of this knowledge to real-world scenarios as a potential consequential outcome. However, the learning process does determine the acquisition of abilities that enable an individual to deal with the real world in a constructive way. The relevance of the learning process to socially desirable outcomes only increases for education in healthcare in light of the social roles the healthcare students assume in the future¹.

Initially there was refusal from medical schools to

incorporate dental education in their curricula, which could find its rationale in the opinions of some groups that the independent establishment of dental schools would be more practical as medical schools may be unwilling to offer the requisite physical space and expensive equipment for the greater technical and mechanical training involved in dentistry². From those times, dental education has gone far during the last century to establish dentistry as one of the most sought after and celebrated healthcare professions. However, the quality and recognition of dental education has not been the same worldwide, leading to evolution of the accreditation process for foreign-trained dental graduates in some nations³. International dental education working groups have highlighted the relevance of students'



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perspectives in the quest of improving dental curricula and have urged that students' views be considered in dental education planning and development^{4,5}.

In the Indian context, it is evident that there has been a phenomenal growth in the number of dental colleges over the past couple of decades⁶. Currently, there are 313 functional dental institutions in the country making India one of the countries that produces the largest number of dental graduates every year⁷. In this regard, it is essential to continuously review the way in which the curriculum is being implemented at the institutional level to keep the standards of dental education in India abreast with the global dynamics in dental education. It must also be understood that this responsibility of program evaluation and refinement of curricula, as necessary, must occur both at the institutional and national level. Unfortunately, it appears that most of the dental institutions in India have not focused on this responsibility. Seldom has it been introspect how well the curriculum is being followed and how satisfied the students, the main stakeholders, are with the learning environment and what their problems are in following the curriculum⁸. Regular evaluation and critical appraisal are as cardinal as the design and implementation of these curricula. With this background, the objective of this qualitative study was to document students' perceptions of the learning environment in two of the thirteen teaching dental institutions in the state of Andhra Pradesh.

METHODS

The dental curriculum in India requires that the dental students undergo clinical training in the third and fourth (final) year of the course and, after successful completion, are required to follow a one year paid rotating internship that must be completed for the students to graduate⁹. This qualitative study involved focus group interviews of dental students in two teaching dental institutions in the state of Andhra Pradesh, India. Focus group interviews are well documented in the literature as effective methods for gaining a deeper insight into the construct of interest. In India, undergraduate dental education has a 5-year curriculum, where the first two years are devoted to basic sciences and pre-clinical exercises in dentistry. Clinical training is offered in the third and fourth years of the curriculum; the fifth year of the curriculum requires the student to complete a rotatory internship with scheduled posting in all the specialties before being awarded a Bachelor of Dental Surgery degree (BDS). In this study, each focus group had representation from all clinical years (i.e. third, fourth and internship); all the participating students were given a pseudonym to ensure anonymity. The number of participants in each focus group ranged from 6 to 8, as recommended in the literature. Two researchers were involved in moderating the interviews. The interviews were semi-structured and a loose set of questions were prepared beforehand to facilitate the discussion. Table 1 shows the interview guide used in this study, which is not

Table 1. Interview guide

As a student, what have been your personal experiences over the years with lectures given by faculty members?
You have entered clinical postings in the third year of your BDS program. Since then, have you ever faced problems in clinical learning?
What are the most common issues that preclude your comprehensive learning in clinics?
How satisfied are you with the current student evaluation processes being followed?
Do you feel that there is a need for objective assessment methods for evaluation of students' performance?
Are you really interested to demonstrate excellent academic performance?

exhaustive as other questions emerged from the interaction between the moderator and the study participants. This set of questions was prepared by a group of four experienced dental educators and included the following constructs: teaching skills and attitudes; clinical learning environment; assessment criteria in practice; and challenges in meeting requirements. A pre-session was conducted before the focus group discussions so that the participants and moderator became acquainted. All the focus group interviews were audio-taped and transcribed by two members of the study team. The transcripts were given to two researchers for independent open coding to extract thematic constructs from the discussion. All the themes were reviewed by both researchers and consensus was obtained wherever there were differences in coding.

Theoretical sampling was done with concurrent conduct of purposive sampling for focus group interviews, data collection, and data analysis, which identified if there was a need for further recruitment of participants. Data saturation was considered to have been achieved after seven focus groups were interviewed in Institution A and six in Institution B. The study was conducted between October and November 2019. Ethical approval for the study was obtained from the institutional review board of SIBAR Institute of Dental Sciences and verbal informed consent was obtained from all the participating students with prior information to and approval from the Institutional Review Board (IRB). It was explained to the IRB that the need to obtain written informed consent would limit the rate of participation, given the objectives of the study and that the study participants had yet to complete their course. A script of verbal consent was reviewed and deemed satisfactory by the IRB.

RESULTS

The mean age of the study participants was 22.6±1.71 years, with no significant differences between institutions. The

average duration of the focus group interviews was 64.7±8.2 minutes. Within each of the focus groups, there were more female than male students. The following thematic constructs were developed from the responses to determine negative learning outcomes: 1) problems in lectures; 2) problems in clinical learning; c) dissatisfaction with evaluation processes, and d) poor academic drive.

The following are some of the responses in the students' own words. Some of the responses were translated for convenience in presentation, since both the local language Telugu and English could be used in the focus group interviews. After translation, the responses were reviewed by two bilingual experts for any changes in the meaning of the responses. Few of the responses that were representative of the general opinion expressed by students were presented in a construct sense.

Problems in lectures

The majority of the students expressed concerns about the lectures. While the importance of theory classes as influencing the conceptual foundations in a discipline was well acknowledged by the students, it was the way in which theory classes were being conducted that received criticism. Almost all the theory classes were reported to be power point presentations with nominal use of other teaching aids. The following are some responses obtained from students:

'Some of the teachers read from power point slides. We are asked to take notes from the slides. I never felt engaged in the class'

'We want teachers to be interactive. Theory class must include questions. In classes where the teachers question, I feel more engaged'

'Some topics require video demonstrations in the class for us to better understand. Certain practical works are understood better by videos than reading from a power point'

'Is it mandatory for a lecture to be completely in English? I feel some faculty convey the message better if they have the opportunity to use both the languages, English and Regional'

'Afternoon theory classes are very difficult to concentrate. Either we keep thinking about what had happened in the clinic that day in the morning or we sleep'

Problems in clinical learning

The problems in clinics were reported to be affecting the interest and confidence of students to perform the clinical procedures being taught and practiced. It was reported that the students are not very comfortable at and do not enjoy going to clinics.

'In my opinion, the main problem in clinics is demos. When any demo is given, 13 of us are standing around the professor and not everyone can see the work. Demos should be given for small groups of 4 or 5'

'Sometimes, my instructor does the work for me. At that moment, I feel happy. But if the same work has to be done under a different instructor in my second posting, I regret

about why I had not learnt that work in the previous posting'

'We don't want our work to be done by the instructors. We want the instructor to stay beside and help us through'

'My fear is that my instructor would belittle me if I ask a stupid doubt. There was an occasion when I was made fun of in front of the patient after which the patient refused to get the examination done and walked out'

'Feedback is very important for us to improve. When I joined here, I was very enthusiastic about interacting with staff and seek feedback. The scenario has changed now. I don't know whether it is because of a legacy that is transferred from the previous batch or due to some bitter experiences we had, but I am not keen in receiving feedback now'

'I think we should be allowed to go to clinics to complete the unfinished quotas whenever we find time. My professor says "you don't have to sacrifice theory class from another department and come here". But no one will be there in the clinic after the theory class in the afternoon. How can I ever finish my quota like this?'

Dissatisfaction with evaluation processes

Evaluation processes adopted by the faculty were opined by the students to be far from objective. It was reported that comparison instantly comes where competition exist, and it is discouraging to see comparatively better grades being given for under-performing students.

'There is definitely partiality in evaluation. We bother more about who is the internal for the practical examination than what all topics to be prepared for'

'Some students move closely with faculty. I feel it can be inconvenient both for fellow staff and the students as well. But I don't know whose mistake is it more'

'You can compare marks in pre-clinical examination and final year examinations. The huge difference in the marks of a student is because of the difference in examiners on the two occasions'

'If everyone is getting highest marks, the students who are really interested to perform will lose that enthusiasm. I know this has happened'

'If you see the theory marks of the students who got highest marks in practical, they are less. There must be something wrong, no!'

Poor academic drive

At the end of each focus group interview, the students were asked if they were really interested to do well and perform academically.

'Most of us are not very keen. Many students want to just get through'

'Yes, obviously everyone wants to perform well. I don't really understand the question'

'I know why this question is being asked. There are things which the students need to change as well. It is not right to give suggestions for others to improve when you yourself are not prepared enough to excel'



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'I have written so many internals. Every time I feel that I should write this internal well. But I have never prepared for an internal and never passed one. There is a notion that we will get at least 5 marks for the 10 marks allotted for internals. I don't know how true it is.'

'As far as internals are concerned, we just need to attempt all of them. There is no time to prepare and study, and what I know is we don't really have to pass.'

DISCUSSION

The changing scenario of education in healthcare, globally, shifted the focus from preparing trainees to developing an institutional culture, with administration, faculty, and the students being recognized as accountable for the latter¹⁰. Literature suggests that faculty development programs (FDPs) in dental education are almost nonexistent in India¹¹. A Master's in Dental Surgery (MDS) is a clinical training program wherein the necessary skills for educating and training students are not fully imparted, so if one is interested in teaching, the faculty needs to learn pedagogical techniques through FDP or another way. Therefore, FDPs are essential and must be observed as part of the institutional curriculum. Most of the problems faced by students relating to classroom teaching in this study could be comprehended as being consequential to the lack of initiatives such as FDP. Engaging the students in the class translates to facilitating active learning and allowing for intuitive appreciation of instantaneous feedback.

Since there were no large differences in the opinions of the students, and it was not the intention of this study to compare institutions but to try and develop an understanding of the learning environment from the students' perspective, the responses were not categorized based on the institution. This choice was made since these institutions follow the same curriculum and offer admission to students predominantly from the state of Andhra Pradesh, India. Moreover, initial review of the participant responses did not show marked differences in opinions relating to different aspects of the learning environment. Few incidents were identified in the study where students were reportedly mistreated in the clinics. It was recognized that the students did not report these incidents before and opined that it was senseless to report the incidents. Similar findings were reported by the Association of American Medical Colleges, where students refrain from reporting mistreatment thinking that the incident does not carry enough importance, fearing reprisals and believing that the incidents would be ignored¹². A study on students' perceptions of the clinical learning environment in New Zealand noted that students were not satisfied with the feedback received¹³. Consistent with the above observations, students in this study expressed disinclination towards receiving feedback.

It is imperative for both faculty and students to understand that misinterpretations and uncertainties are inevitable in clinical practice as the presentation of a

clinical condition differs from individual to individual and so does the provision of care. It was suggested by Perry that empathetic teachers in a clinical environment who admit their uncertainty in a given clinical situation helps students in the much-needed transition from right-wrong dichotomy to acceptance of uncertainties¹⁴. Furthermore, the authoritarian attitude of teachers towards students has the unwanted consequence of the attitude being adopted by students towards their patients. It is well documented in the literature that students who face public humiliation and considered unworthy do not empathize with patients¹⁵. In this regard, it becomes the responsibility of any healthcare institution to focus on student-centered teaching which translates to patient-centered care being observed by the students or trainees.

Globally, assessment of students' academic performance is an area of professional education that has undergone a multitude of changes over the past few decades¹⁶. However, the assessment criteria in most dental educational institutions in India continue to be the same, with no objective methods in place to evaluate the clinical skills demonstrated by the students. It is essential to discern that assessment constructively navigates learning. It is only when the influential role of assessment in motivating the students to learn is recognized that the need for appropriate assessment criteria relevant to the domain to be assessed is understood. The dissatisfaction expressed by the students with evaluation in the present study emphasizes the need to institute more objective assessment criteria. International experience suggests that students show better interest and report better learning when marking and grading are not in concomitance with the learning process^{13,17}. Inclusion of Objective Structured Clinical Examination (OSCE) in the dental curricula would be beneficial in eliminating prejudice and allows everyone to be assessed by the same criteria¹⁸. Furthermore, use of portfolios facilitating students to reflect upon their own performance would facilitate better learning¹⁹. OSCEs are widely used in undergraduate dental education in European²⁰, American²¹, and Canadian dental education²². A study focusing on the integrated aspects of team-based learning with peer-to-peer teaching at the Harvard School of Dentistry reported that weaker students performed considerably better with less faculty involvement and more self-directed learning²³. These findings highlight the need for preparing customized teaching plans to stimulate students' quest for learning. Student Evaluation of Teaching (SET) is known to be an effective way for assessing faculty performance by the recipients themselves. When constructed and delivered in the right manner, SETs are acknowledged to be valid and reliable measures for assessment of faculty performance, and should be linked with annual salary increment or promotion of faculty members²⁴. The relevance of SETs in dental curricula has been widely discussed over the years, and can be considered for inclusion in the Indian dental educational context^{25,26}.

Strengths and limitations

The results from this qualitative study highlight the fact that students face challenges in the learning environment, which lead to negative learning outcomes, especially when the students demonstrate poor academic drive. Nevertheless, the temporal association between perception of problems in the learning environment and poor academic drive cannot be inferred from the results of this study. However, it is certain that the responses given by some students demonstrating their mediocre interest to perform well academically may not entirely be attributed to the learning environment but could be circumstantial, especially in light of the growing debate for the future of the dental profession in India.

The findings of this study provide some solutions and offer a framework for the conduct of future research to better understand the guiding factors for students' perceptions of the learning environment based on what was documented here. The study also highlights the need for regular institutional reviews of curricula in dental education. Though it cannot be claimed that the findings of this study can be generalized to all teaching dental institutions in India, it is most likely that they apply across dental institutions.

CONCLUSIONS

In countries like India where the burden of oral diseases is very high, it is pertinent that technically well equipped, socially empowered, and cognitively empathetic dental professionals be prepared to meet the ever-increasing need for dental healthcare. Conducive learning environments are necessary for the preparation of such professionals, which demands commitment from educators, administrators and students. Regular introspection of the strength and implementation of the curricula and documentation of the perceptions of the stakeholders demonstrate the need for this commitment and provide an opportunity for better dental education. In a competitive world where mediocrity is not entertained, striving for excellence has become the norm. In this context, it is recommended that all teaching dental institutions develop institution-specific models for assessment of the learning environment and councils regulate the learning environment by instituting fundamental common norms across the dental institutions in the country.

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CONFLICTS OF INTEREST

The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none was reported.

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Management of Extracted Human Teeth by Dental Students in East Coastal Region of India

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Abstract

Background: Dental students practice on extracted human teeth (EHT) in their preclinical to learn professional and technical skills before performing dental procedures on patients. **Aim:** The aim of the present investigation was to assess the management of EHT among dental students in the east coastal region of India. **Materials and Methods:** A cross-sectional study was conducted on II, III, and IV Bachelor of Dental Surgery (BDS) students from five dental colleges in India's east coastal region, which were chosen using the fishbowl technique. Data was collected using a pretested, validated self-administered questionnaire and analysed using the Chi-square test, Mann-Whitney test, Kruskal-Wallis test, and linear regression. **Results:** A total of 649 students participated from five dental colleges, among them, 43.6% (283) were II BDS, 29.6% (192) were III BDS, and 26.8% (174) were IV BDS. About 66% of the participants do not know that the EHT are a source of infection; 22% of them noted that there was an incidence of infection with the use of EHT; 81.5% do not know that guidelines have to be given by the regulatory body/institution to handle EHT. Only 2% of the people think it was necessary to disinfect/sterilize EHT before use, while 33% of the study population felt that there was a need to wear mouth masks and gloves while working on EHT. In the study group, only 43% know that formalin was used as a storage/disinfecting medium for EHT and 80% of them experienced hazards due to chemicals used for storage/disinfecting medium of EHT. **Conclusion:** The results of this investigation stipulated that awareness, attitude, and practices of undergraduate students in relation to the handling of EHT were poor. However, deficiencies were observed in relation to teaching the materials and methods suitable for the management of EHT which reveals the need to incorporate the guidelines in their curriculum.

Keywords: Awareness, dental student, disinfection extracted human teeth, dental, practice management.

INTRODUCTION

Unblemished extracted human teeth (EHT) are utilized by dental graduates to create and hone their clinical abilities for treating patients. EHT are utilized for different purposes, for instance, like preparing ground sections to study histology of teeth, for learning endodontic procedures such as cavity preparation and root canal treatment, for learning crown preparation, and to evaluate microleakage of the restorative material and marginal integrity in crown preparation techniques.^[1]

Nowadays, typodont models are used as practical learning aids for dental students for dental students, permitting them to exercise certain dental procedures on the plastic teeth of a

model before really playing out the systems on live patients. Working on the extracted teeth results in achieving the same tactile sensation as like operating on the live case scenario. In middle- and low-income nations like India where the per capita income is less, extricated human teeth serve a low spending substitute to typodont that permits the students to utilize them for training.^[2]

Individuals handling EHT for preclinical training or clinical assignments must be conscious of the potential exposure to

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the blood-borne pathogens acquired from the extricated human teeth. Therefore, individuals who collect extracted teeth must decontaminate before using them. Various solutions have been used to store and transport extracted teeth. Of all the media, the most common ones are formalin, saline, hydrogen peroxide, and sodium hypochlorite. Since these liquids are easily available in dental clinics, they have become the obvious choice to store extracted teeth.^[3]

The Occupational Safety and Health Administration Blood-borne Pathogens Standard considers human teeth used for research and teaching purposes as a potential source of blood-borne pathogens.^[4] Since extracted teeth form a part of the human body before they are extracted, they are an obvious source of infection, and the Center for Disease Control (CDC) also recommends that the extracted teeth be treated as potential blood-borne pathogens. The CDC and prevention have adopted guidelines for infection control of extracted teeth used for research and teaching, requiring that teeth be sterilized before use, to minimize the risk of transmission of blood-borne pathogens.^[5,6]

While there are studies on the effectiveness of disinfectants, assessment of knowledge, attitude, and practice regarding the handling of extracted teeth among dental students are limited. Hence, this study was conceived with the intention to assess the knowledge, attitude, and practice regarding the handling of extracted teeth among the students of the dental colleges in the east coastal region of newly formed state of India.

MATERIALS AND METHODS

A cross-sectional study was carried out on the II, III, and IV bachelor of dental surgery (BDS) dental students among five dental colleges located in the east coastal region of Andhra Pradesh, India [Image 1]. There are 16 dental colleges in the state of Andhra Pradesh out of which 13 colleges are located in the east coast region where five colleges were selected randomly using the fishbowl technique.

A pretested, validated, self-administered questionnaire which was taken from the previous study was used to test the

knowledge, attitudes, and practices of students regarding the handling of EHT.^[3] Questionnaires were anonymous and filling the questionnaire was completely voluntary where consent was taken prior to the start of the study. The questionnaire was distributed and collected by a single investigator. The goal of the study was explained and students were left alone to fill the questionnaire. Ethical approval was obtained from the Institutional Review Board (IRB) with reference number 39/IRB/SIBAR/2019.

The questionnaire consisted of four sections, where the first section consists of demographic details and II, III, and IV sections include knowledge, attitude, and practice-based questions on the management of EHT.

Data were collected and statistically analyzed using Statistical Package for the Social Sciences (SPSS) version 25 (IBM, Chicago Inc., IL, USA). Descriptives, Chi-square test, Mann–Whitney test, Kruskal–Wallis test, and linear regression were done where $P \leq 0.05$ was considered as statistically significant.

RESULTS

Figure 1 shows the distribution of study participants in accordance to their academic year. Total study population were 649 students, among them 43.6% (283) were II BDS, 29.6% (192) were III BDS, and 26.8% (174) were IV BDS. Table 1 describes all knowledge, attitude, and practices in handling of EHT. Most of the study participants (66%) do not know that the EHT are a source of infection. About 22% of the participants noted that there was an incidence of infections such as respiratory problems, eye and skin irritation was reported with the use of EHT for educational purpose. Almost 81.5% do not know that guidelines are necessary to be given by regulatory body/institution to handle EHT. Only 36% of the students are aware of guidelines by a regulatory body regarding the handling of EHT and only 2% of the people think it was necessary to disinfect/sterilize EHT before use. Nearly 33% of the study population felt that there was a need to wear mouth mask and gloves while working on EHT. In the study group,

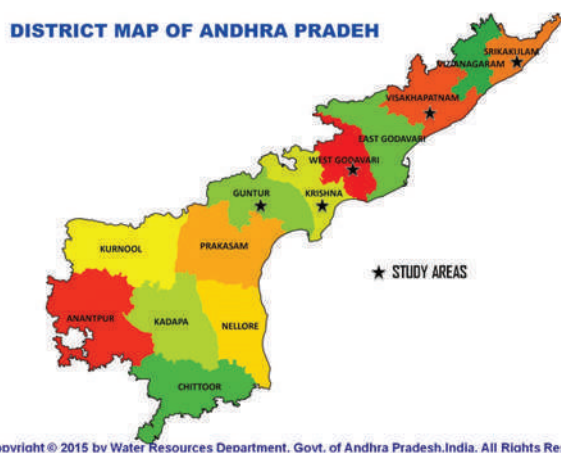


Image 1: Illustrating the areas of the study in the state of Andhra Pradesh

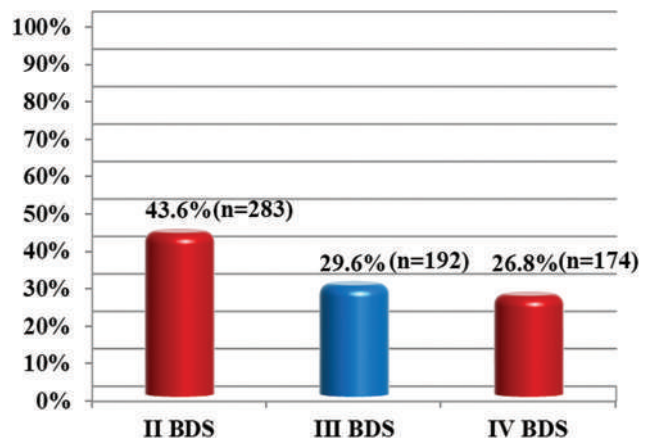


Figure 1: Distribution of participants according to their academic year

Table 1: Perceptions of the individuals regarding handling of extracted human teeth

Questions	Yes (%)	No (%)	Do not know (%)
Do you consider extracted human teeth as a source of infection?	6.5	27.5	66
Is there any incidence of infection noted with the use of extracted human teeth for educational purpose?	22	33	45
Do you think it is necessary to have guidelines by regulatory bodies/institutions to handle extracted human teeth	5.5	13	81.5
Are you aware of any guidelines by a regulatory body regarding handling of extracted human teeth?	36	64	-
Do you think it is necessary to disinfect/sterilize extracted teeth before use?	2	4	94
Is there a need to wear mouth masks and gloves while working on extracted human teeth for educational purpose/s?	33	10.5	56.5
Is the use of Formalin for storage/disinfection of extracted teeth hazardous?	43	34.5	22.5
Have you experienced any hazard due to the use of chemicals for storage/disinfection of extracted teeth?	80	20	--
Have you referred to any published scientific literature regarding the use of an appropriate medium for storage/disinfection of extracted human teeth?	11.5	88.5	--
Have you noticed any change in surface characteristics of the extracted teeth samples after storage in a disinfectant medium?	52.5	47.5	--

Questions	Dust bin (%)	Red color bag (%)	Yellow color bag (%)
How do you dispose of extracted human teeth after its use for educational purpose/s?	17.9	66.1	16

only 43% know that formalin was used as storage/disinfecting medium for EHT and 80% of them experienced hazards due to chemicals used for storage/disinfecting medium of EHT. Merely 11.5% of the participants referred to any published scientific literature regarding the use of an appropriate medium for storage/disinfection of EHT and 52.5% have noticed some changes in surface characteristics such as a change of color and surface texture of the EHT samples after storage in a disinfectant medium.

Figure 2 explains that majority of the students (56%) used hydrogen peroxide (56%) followed by usage of both formalin and H₂O₂ (11%), while 9% of them are using formalin as a storage medium to store EHT. Table 2 depicts the knowledge and attitudes of dental students in handling EHT according to their academic year. Second BDS (60.3%) and IV BDS (88.5%) students reported that they do not know that the EHT as a source of infection, while III BDS (78.2%) students did not consider them as an infectious source ($P = 0.001$). Second BDS (67.2%) and IV BDS (95.3%) students have disclosed that they were not aware of any guidelines by a regulatory body regarding the handling of EHT ($P = 0.001$); similarly, they have also proclaimed that they do not know whether it is necessary to disinfect/sterilize extracted teeth before use (72.4% and 100%, respectively, $P = 0.001$). Almost all the students of II BDS, III BDS, and IV BDS opted red color disposal bag to dispose EHT ($P = 0.42$).

The highest percentage of the students among all the 3 year use hydrogen peroxide to store EHT and it is statistically significant ($P = 0.005$) [Figure 3]. Table 3 elucidates that the mean rank of knowledge was more for females (310.07), while the mean rank of attitude (312.11) and practice (319.25) in participants were higher in males. Statistically significant results are seen in attitude ($P = 0.004$) between genders, but

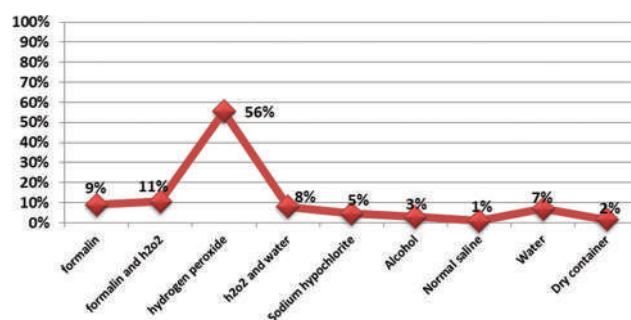


Figure 2: Usage of various storage medium for extracted human teeth by the dental students

there was no statistical significance in knowledge ($P = 0.194$) and practice ($P = 0.208$). The mean rank of knowledge was higher in III BDS students (356.39) when compared to II BDS (294.94) and IV BDS (339.26) ($P = 0.001$), where the mean rank of attitude was observed to be more in II BDS students (357.60) followed by IV BDS (324.40) and III BDS (227.50) ($P = 0.001$), and the mean rank of practice score was more in IV BDS (348.02) compared to III BDS (335.36) and II BDS (303.82) which was statistically significant ($P = 0.032$).

Knowledge and practice scores in dealing with EHT increase as the academic year progresses ($B = 0.181, P = 0.004$ and $B = 0.589, P = 0.004$, respectively), but attitude score showed an inverse relationship ($B = -0.213, P = 0.002$) [Table 4].

DISCUSSION

Extracted teeth contain virulent microorganisms capable of causing diseases such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV).^[4,7,8] Even after the complete

Table 2: Awareness on handling of extracted human teeth by the dental students according to their academic years

	Second BDS (%)			Third BDS (%)			Fourth BDS (%)			P
	Yes	No	Do not know	Yes	No	Do not know	Yes	No	Do not know	
Do you consider extracted human teeth as a source of infection?	10.3	29.5	60.3	21.8	78.2	0	2.6	9.0	88.5	0.001*
Are you aware of any guidelines by a regulatory body regarding handling of extracted human teeth?	1.6	31.2	67.2	46.9	53.1	0	3.1	1.6	95.3	0.001*
Do you think it is necessary to disinfect/sterilize extracted teeth before use?	6.9	20.7	72.4	43.1	56.9	0	0	0	100.0	0.001*
How do you dispose of extracted human teeth after its use for educational purpose/s?	Dust bin (%) 21.8	Red color disposal bag (%) 56.4	Yellow color disposal bag (%) 21.8	Dust bin (%) 14.1	Red color disposal bag (%) 79.7	Yellow color disposal bag (%) 6.2	Dust bin (%) 15.5	Red color disposal bag (%) 69.0	Yellow color disposal bag (%) 15.5	0.042*

Chi-square test, *Statistically significant

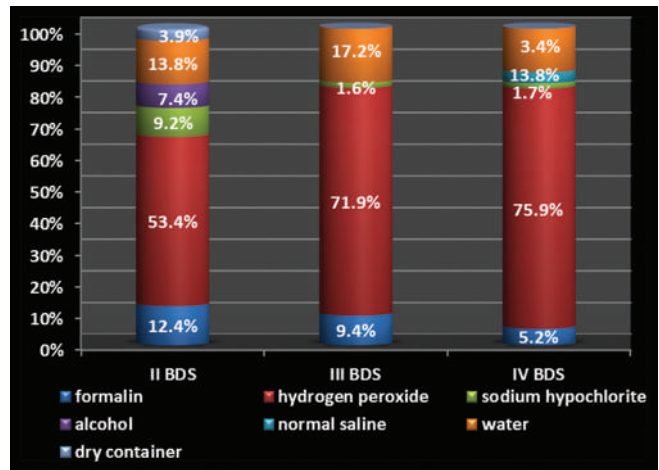


Figure 3: Choice of storage medium for extracted human teeth according to academic year of the participants

removal of saliva and blood, HBV might remain contagious up to 6 months, while under the similar conditions, HIV can remain infectious for 4–8 weeks.^[7,9] Since EHT may harbor potential pathogens, proper handling of EHT in the preclinical’s is important for educators and the students.

Developed countries such as Australia and the UK have legislation to limit the usage of EHT for the purpose of educational use with the help of the patient/legal guardians written consent, whereas in India, we do not have such legislation and acts to abide, while EHT were used for the educational purpose by honing their clinical skills rather disposing in the clinical waste.^[10]

In the present study, 66% of the study participants do not know that EHT are a source of infection which is lower when compared to the other studies done by Kumar *et al.*^[11] (97%), Hashemipour *et al.*^[12] (95%), Deogade *et al.*^[13] (90.2%), and Amith *et al.*^[14] (88%), whereas it was higher when compared to studies done by Omar OM *et al.*^[15] (55.6%) and Sethi *et al.*^[16] (57.4%). This could be due to the fact that usage and handling of EHT was considered as a conventional practice while they are easily accessible, affordable while there were no guidelines framed in the curriculum on the management and utilization of EHT.

In the current study, 81.5% do not know that guidelines are necessary by regulatory body/institution to handle EHT which is in contrast with the study done by Smitha *et al.*^[3] (84.7%) and Sethi *et al.*^[16] (83.3%), who reported that it is necessary to have guidelines by regulatory body/institution. In contrast to Smitha *et al.*^[3] study, only a meager current study participants (36%) are aware of the guidelines on handling of EHT. (80.6%). In this study, 94% of the study participants do not know that it is necessary to disinfect the teeth which is in contrast with the studies done by Amith *et al.*^[14] (93.2%), Deogade *et al.*^[13] (91.5%), Smitha *et al.*^[3] (90%), and Omar OM *et al.*^[15] (87.9%) who reported that they know that it is necessary to disinfect the teeth.

Table 3: Mean rank comparison of knowledge, attitude, and practice scores according to student's gender and academic year

	Mean rank		
	Knowledge score	Attitude score	Practice Score
Gender ^a			
Male	310.07	358.08	339.74
Female	330.82	312.11	319.25
<i>P</i>	0.194	0.004*	0.208
Academic year of the students ^b			
Second BDS	294.94	357.60	303.82
Third BDS	356.39	277.50	335.36
Fourth BDS	339.26	324.40	348.02
<i>P</i>	0.001*	0.001*	0.032*

^aMann-Whitney, ^bKruskal-Wallis test, *Statistically significant

Table 4: Simple linear regression between the academic year of the dental students and knowledge, attitude, and practice scores in handling of extracted human teeth

Model Academic year of the students	Unstandardized coefficients		Significant	95% CI for B	
	<i>B</i>	SE		Lower bound	Upper bound
Knowledge score ^a	0.181	0.062	0.004*	0.060	0.303
Attitude score ^a	-0.213	0.069	0.002*	-0.348	-0.077
Practice score ^a	0.589	0.206	0.004*	0.185	0.994

^aDependent variable, *Statistically significant ($P \leq 0.05$). CI: Confidence interval, SE: Standard error

About 56.5% of the current study participants do not know that there was a need to wear mouth masks and gloves while working on EHT where dissimilar results were observed in the studies done by Amith *et al.*^[14] (90.7%), Deogade *et al.*^[13] (95.3%), and Smitha *et al.*^[3] (91.7%) which reveal that the study participants are had not kept themselves updated about the latest guidelines for safety. About 52.5% of the participants in the study have noticed some changes in surface characteristics after storing in the disinfectant medium, while similar results were found in the studies done by Smitha *et al.*^[3] (42.7%) and Sethi *et al.*^[16] (54.8%), this could be due to the fact that dentin hardness decreases as both organic and inorganic constituents are affected by the various disinfection methods.

Most of the study participants had used hydrogen peroxide (56%) and only 9% of them used formalin as a storage medium for EHT, comparable to the studies done by Amith *et al.*^[14] (93.8%), Kumar^[11] (81.7%), Deogade *et al.*^[13] (57%), Smitha *et al.*^[3] (42.2%), and Omar OM *et al.*^[15] (18.8%). Despite the evidence demonstrated by numerous studies, students still use hydrogen peroxide because of its feasibility, availability, and cost-efficiency.

In the current study, 22% of them disclosed that they have experienced discomfort due to the usage of chemicals for storage/disinfection of EHT, whereas a higher percentage (61.1%) of them reported the same in the study done by Amith *et al.*^[14] Exposure to EHT stored in formalin may cause irritation of the eyes, nose, and throat since they were absorbed through the epithelium due to the fact that tooth preserved in formalin

releases toxic vapours that may cause local irritation, so when working with preserved EHT requires safety precautions such as wearing protective equipment and opening the container preserving EHT in a safe, well ventilated area.

Majority of the participants have disposed EHT in the red bin (66.1%) which was in contrast with the studies done by Omar OM *et al.*^[15] (27.2%), Riswana^[17] (10.9%), and Smitha *et al.*^[3] (27.2%). Only 16% answered correctly that EHT have to be disposed in yellow color-coded bags which depicts a lack of knowledge among the dental students on disposal of EHT.

Almost 88.5% of the participants proclaimed that they have not referred to any published scientific literature regarding the use of an appropriate medium for storage/disinfection of EHT and similar results are observed in the studies done by Smitha *et al.*^[3] (81.3%) and Omar OM *et al.*^[15] (53.8%). As a source of knowledge, self-reading of articles and research papers was not so common in this sample community. This was demonstrated in their response and practice of using a suitable medium or procedure to sterilize/disinfect EHT. With this background, students need to be educated with the published literature. This often highlights incomplete knowledge in the dental curriculum on this subject that should be addressed in depth.

CONCLUSION

Patient care is the very essence of what dentists do, and it is the way dental students truly learn to become dentists. The results of this investigation warranted that the awareness and knowledge of undergraduate dental students on the management of EHT

were found to be deficient. However, proper teaching, training, and education are necessary to handle, storage, and practice of EHT for their educational purpose. The other safety measures such as the use of gloves, masks, and safety eyeglasses should be stressed while practicing preclinical work on these teeth. Incorporation of guidelines on disinfection, storage, usage, and disposal of EHT should be included in their curriculum.

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Conflicts of interest

There are no conflicts of interest.

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Depression, suicidal ideation, and suicidal behaviors among dental students of Neo-state capital region in India

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Parveen Sultana Shaik, K. Krishna Priya³, Suresh Chand Yaddanapalli

Abstract:

BACKGROUND: The challenges of dental education place students at high risk of stress. Because dental education is a highly demanding and challenging course, placing heavy demands on the mental resources of the students, making them vulnerable to high levels of stress, this study aimed to determine the prevalence of depression, suicidal ideation, and suicidal behaviors in dental students.

MATERIALS AND METHODS: A descriptive cross-sectional study was done on a convenience sample of 388 dental students in a teaching dental institution. Data were collected using standard, prevalidated, self-administered questionnaires (Patient Health Questionnaire-9 and Suicidal Behaviours Questionnaire-Revised [SBQ-R]). Analysis was done using SPSS version 20. Descriptive statistics, Chi-square test, and logistic regression were performed.

RESULTS: At least half of the students were in major depression with different levels of severity. When students from different years were analyzed, majority from each year fell under II and III categories ($P < 0.001$). Most of the students reported that they do not have any suicidal ideation or behavior (SBQ-R). Nearly 16% of the students either presented the threat of suicidal attempt or likelihood of suicidal behavior in future. Almost 13% of the dental students thought of killing themselves in the past year for at least two times. Around 16% of them expressed to someone about their thought of committing suicide.

CONCLUSION: Even though depression and suicidal tendencies were not prominent in dental students, there is a need to shed light on those who responded positively and take necessary reforms to relieve academic stress.

Keywords:

Postgraduates, stress, suicidal tendency, undergraduates

Introduction

Suicide is the act of killing yourself, most often as a result of depression or other mental illness.^[1] Depression is a key risk factor for suicide; others include psychiatric disorders, substances used, chronic pain, a family history of suicide, and a prior suicidal attempt. Impulsiveness often plays a key role among adolescents who take their life.^[2] The prevalence

of suicide is often underestimated due to cultural, political, and economic circumstances. Recent reports inform that around a million people die by suicide annually, representing an annual global age-standardized suicide rate of 11.4/100,000 populations (15.0 for males and 8.0 for females),^[3] which is one person every 40 s and many more may attempt suicide. Suicide occurs throughout the lifespan and is the second leading cause of

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death among 15–29 year olds globally.^[2] According to the data by the accidental deaths and rate of suicides in India for the year 2019, the percentage distribution of suicide victims by the students was 10.4%, whereas that of for the year 2019 was 7.4%.^[4]

While the link between suicide and mental disorders in particularly, depression, and alcohol use disorders is well established in high-income countries, many suicides happen impulsively in moments of crisis with a breakdown in the ability to deal with life stresses.^[5] Nowadays humans are exposed to more stress than ever due to increasing competition and various environmental factors. The challenges of dental education place students at high risk of stress.^[6,7] Dental education is a highly demanding and challenging course, placing heavy demands on the mental resources of the students, making them vulnerable to high levels of stress.^[8] Suicidal ideation aids the thoughts of harming or killing oneself as it is a nonfatal, self-inflicted destructive act with explicit or inferred intent to die. Suicidality refers to all suicidal-related behaviors and thoughts including completing or attempting suicide, suicidal ideation, or communication.^[9]

Depression refers that common mental disorder, characterized by persistent sadness and a loss of interest in activities that you normally enjoy, accompanied by an inability to carry out daily activities, for at least 2 weeks.^[10] Hence, recognizing depression levels in vulnerable dental students plays a crucial role in making changes in the present dental curriculum. The aim of the study is to determine the prevalence of depression, suicidal ideation, and suicidal behaviors in dental students; to assess whether there is any association between depression and suicidal ideation; and to evaluate whether gender is a predictor of prevalence of these conditions.

Materials and Methods

Study design and setting

A descriptive cross-sectional study was done among dental students from a teaching dental institution of southern India in the month of February 2020.

Study participants and sampling

A total of 388 dental students were included in the study using convenience sampling method. Students from the clinical years (third and final years of undergraduation and postgraduation) were only included because they are the students who deal with the patients and are under high stress levels compared to the students from nonclinical years, and the study was not conducted during university exams to avoid exaggerated reporting.

Ethical considerations

Prior to the study, study details were explained and ethical clearance was obtained from the institutional ethical committee (239/IRB/SIBAR/2019).

Inclusion and exclusion criteria

students who were willing to participate and present on the day of data collection were included in the study, whereas students who were absent on the day and not willing to participate were excluded from the study.

Data collection tool and technique

The study pro forma has two part: the first part consisted of information regarding age, gender, and year of the study and the second part consisted of standard, self-administered, structured questionnaires (Patient Health Questionnaire^[11] and Suicidal Behaviours Questionnaire–Revised^[12]) to collect data regarding depression, suicidal ideation, and suicidal behavior [Annexure 1]. Patient Health Questionnaire (PHQ-9) is a self-reported depression assessment specifically developed for use in primary care. The questionnaire was taken from a previous study^[11] which was pretested and validated in 233 adolescent Indian students aged 14–18 years. It has good diagnostic accuracy with a sensitivity of 87.1%, a specificity of 79.7%, good content validity, good 1-month test–retest reliability ($r = 0.875$), high internal consistency (Cronbach's $\alpha = 0.835$), and high convergent validity with the Beck Depression Inventory ($r = 0.76$; $P = 0.001$).^[11] This depression questionnaire consists of 9 items to assess the level of depression and a 10th functional health assessment item to know the perceived difficulty of the problems faced by the individuals. Each of the 9 items was scored on a 4-point Likert scale ranging from score 0 (not at all) to 3 (nearly every day). After obtaining the total score by adding the scores of the individual 9 items, the severity of depression was graded as follows: 0–4 (minimal), 5–9 (mild), 10–14 (moderate), 15–19 (moderately severe), and ≥ 20 (severe).^[11] Suicidal Behaviours Questionnaire (SBQ) consists of 4 items, of which responses of item number 2 and 4 were measured on 5-point and 6-point Likert scales, respectively. For item no. 2, the responses ranged from “never” to “very often.” For item no. 4, the responses ranged from “never” to “very likely.” Item no. 1 and 3 had 6 and 5 responses, respectively. It was validated in psychiatric inpatient adolescents, high school students, psychiatric inpatient adults, and undergraduate students. SBQ has good diagnostic accuracy with 93% sensitivity and 95% specificity.

Statistical analysis

Data analysis was done using IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0 Armonk, NY: IBM Corp. Descriptive statistics, Chi-square test, and logistic regression tests were performed.

Results

Most of the study participants were female (74.7%) and are undergraduate (76%), while majority of them belonged to internship (28.6%).

Table 1 shows that 58.3% of the participants had minimal to mild depressive symptoms, while most of the students felt that any of their depressive symptoms made it “not difficult at all” (33%) or “somewhat difficult” (56.2%) to do their work, take care of things at home, or get along with other people.

Table 2 shows that 35.8% ($n = 139$) of students reported that they had a thought of killing themselves; of those, 12.6% of the individuals had a suicidal plan at least once, while 3.4% have tried to attempt, whereas 32.9% of the students have the thought of attempting suicide at least once in the past year. Out of the 139 participants with the suicidal intention, 63 members have discussed about their suicidal ideation at least once with someone. Nearly 7.5% ($n = 29$) of the students reported that they are likely to have future thoughts of attempting suicide.

Table 3 shows that the association between year of the study and PHQ scores was highly significant ($P < 0.001$). Most of the 3rd-year BDS and 1st-year MDS students (33.3% and 54.2%, respectively) were having minimal depressive symptoms. The number of 4th-year BDS students with both minimal and moderate depression with mild severity was equal. Interns and 2nd-year postgraduates were within normal range or full remission. Third-year postgraduates were having major depression with mild severity. The association of PHQ-9 scores and gender shows that 15.2% of the female participants were with moderate and severe major depressive symptoms that warrant treatment. Nearly 22.4% of the male participants

were with moderate and severe major depressive symptoms that warrant treatment and this association showed statistical significance ($P < 0.001$).

Table 4 shows the relation between PHQ and SBQ where with one unit increase in PHQ score, there were 1.283 units of increase in SBQ score. 19% of the variance in SBQ was explained by PHQ alone.

Discussion

The present study reports that majority of the study participants were perceiving depression of various levels while most of the participants were with low to high level of suicidality, which was higher in a study done by Deeb *et al.*^[6] In the present study, the participants perceived higher PHQ-9 scores when compared to that of the study done by Deeb *et al.*^[6] and less when compared to that of the study conducted by Galan *et al.*^[13]

PHQ instrument also includes a functional health assessment. This extracts the information regarding emotional difficulties or problems that impact work, things at home, or relationships with other people. Patient responses can be one of the following four: not difficult at all, somewhat difficult, very difficult, and extremely difficult. The last two responses suggest impaired functionality. Whereas in the present study, certain percentage of the students were with impaired functionality and needed compulsory treatment.^[11]

The participants had a plan of killing themselves at least once and even had attempted to kill themselves. This gives an alarming sign that reflects their failure to manage stress. While the study participants had the thought of attempting suicide in the past year at least once, similar results were observed in a survey done by Galan *et al.*^[13]

Table 1: Distribution of study participants according to their level of depression severity (Patient Health Questionnaire-9 scores) and perceived difficulty of the problems faced by the participants (functional health assessment)

	Frequency (%)
Level of depression severity (PHQ-9 scores)	
0-4 (minimal)	98 (25.3)
5-9 (mild)	129 (33.0)
10-14 (moderate)	96 (24.7)
15-19 (moderately severe)	43 (11.1)
≥20 (severe)	22 (5.9)
Total	388 (100)
Perceived difficulty of the problems faced by the participants (functional health assessment)	
No difficult at all	128 (33.0)
Somewhat difficult	218 (56.2)
Very difficult	34 (8.8)
Extremely difficult	8 (2.1)
Total	388 (100)

PHQ=Patient Health Questionnaire

Table 2: Frequency distribution for various questions in the Suicidal Behaviours Questionnaire

Questions	n (%)
SBQ-1. Have you ever thought or attempted to kill yourself?	
Never	249 (64.2)
It was just a brief passing thought	77 (19.8)
I have had a plan at least once to kill myself but did not try to do it	37 (9.5)
I have had a plan at least once to kill myself and really wanted to do it	12 (3.1)
I have attempted to kill myself, but did not want to die	3 (0.8)
I have attempted to kill myself, and really hoped to die	10 (2.6)
Total	388 (100)
SBQ-2. How often have you thought about killing yourself in the past year?	
Never	268 (69.1)
Rarely (1 time)	70 (18.0)
Sometimes (2 times)	26 (6.7)
Often (3-4 times)	13 (3.4)
Very often (5 or more times)	11 (2.8)
Total	388 (100)
SBQ-3. Have you ever told someone that you were going to commit suicide, or that you might do it?	
Never	76 (54.6)
Yes, at one time, but did not really wanted to die	29 (20.8)
Yes, at one time and really wanted to die	18 (12.9)
Yes, more than once, but did not want to do it	7 (5.03)
Yes, more than once, and really wanted to do it	9 (6.4)
Total (students at least with the thought of suicide)	139 (100)
SBQ-4. How likely is it that you will attempt suicide someday?	
Never	306 (78.9)
Unlikely	53 (13.7)
Likely	19 (4.9)
Very likely	10 (2.6)
Total	388 (100)

SBQ=Suicidal Behaviours Questionnaire

Table 3: The Patient Health Questionnaire-score and gender, year of the study

Variables	PHQ-score					P
	0-4 (minimal)	5-9 (mild)	10-14 (moderate)	15-20 (moderately severe)	≥20 (severe)	
Gender (%)						
Female	24.5	36.2	24.1	11.4	3.8	0.001*
Male	27.6	23.5	26.5	10.2	12.2	
Year of study, n (%)						
III BDS	18 (20.0)	30 (33.3)	21 (23.3)	15 (16.7)	6 (6.7)	0.001*
IV BDS	13 (13.8)	28 (29.8)	28 (29.8)	15 (16.0)	10 (10.6)	
Interns	43 (38.7)	37 (33.3)	19 (17.1)	10 (9.0)	2 (1.8)	
I MDS	4 (16.7)	13 (54.2)	6 (25.0)	1 (4.2)	0	
II MDS	14 (40.0)	11 (31.4)	8 (22.9)	1 (2.9)	1 (2.9)	
III MDS	6 (17.6)	9 (26.5)	14 (41.2)	1 (2.9)	4 (11.8)	

*Statistically significant; Chi-square test. PHQ=Patient Health Questionnaire

Table 4: Association between scores of Patient Health Questionnaire and Suicidal Behaviours Questionnaire

Independent variable	OR	P	R ²
PHQ	1.283	<0.001**	0.190

**Highly statistically significant, logistic regression test. PHQ=Patient Health Questionnaire, OR=Odds ratio

With suicidal thoughts, the participants had discussed about their suicidal ideation with others at least once, whereas half of them were not sharing with anyone, which

is not a good indicator for prevention. For a segment of the participants, it was likely to get a thought of suicide in future, which is an alarming sign. Final-year postgraduate students were having major depression with mild severity. The reason could be academic pressure as they have to clear university examinations. All these findings indicate failure of students in managing stress.

The link between suicide and depression is well established in high-income countries; many suicides

happen impulsively in moments of crisis with a breakdown in the ability to deal with life stresses. Stress describes external demands (physical and mental) on an individual's physical and psychological well-being. Although it has been hypothesized that some stress may actually be beneficial as a stimulus for learning, the negative consequences of stress are significant which progress sometimes to depression and tend to initiate suicidal ideation. Although various situations tend to elicit different patterns of stress responses, there are also individual differences in stress responses to the same situation. This tendency to exhibit a particular pattern of stress responses across a variety of stressors is referred to "response stereotypy."^[14] A study was done by Schmitter *et al.* to compare chronic stress between medical and dental students which concluded that chronic stress was more pronounced among dental students.^[15]

In this study, the PHQ scores were directly related to the SBQ scores, which suggests that students with depression were having high suicidal behavior. There was a statistically significant association between depression and suicidal behavior, which was similar to a study done by Galan *et al.*^[13] There was a statistically significant difference in PHQ scores between males and females, with females perceiving higher depression compared to males, which was in accordance with the study by Shetty *et al.*^[8] and in contrast to a study done by Galan *et al.*^[14] and Deep *et al.*^[6]

Case control studies can be done exploring the factors associated with suicidal behavior. Early identification; treatment; and care of people with mental disorders, substance use disorders, chronic pain and acute emotional distress can prevent suicides. While training of non specialized health workers in the assessment and management of suicidal behavior; follow up care for people who attempted suicide; and provision of community support are needed.

Limitation and recommendation

Limitations

Because it is a cross-sectional self-reported survey, no causal relationship can be determined and as the study was done in one institution, the results cannot be generalized. The cross-sectional nature of the study, the possibility of retrospective bias especially in the aftermath of the suicidal attempt, and lack of scales for assessing psychiatric morbidity (especially depression and stressors) are limiting our results.

Recommendation

Suicides are preventable, and there are a number of measures that can be taken at population, sub-population, and individual degrees to save an individual from committing suicide. Preventable measures such as by

reducing ingress to the means of suicide (e.g., pesticides, firearms, and certain medications); by reporting through media in a legitimate responsible way; and by introducing alcohol notions to reduce the harmful intake of alcohol are also important.

Suicide is a complex issue, and therefore suicidal prevention efforts require coordination and collaboration among multiple sectors of the society. Suicides come into limelight only when people with celebrity status commits it, therefore governmental efforts must be comprehensive and integrated as no single approach alone can make an impact on an issue as complex as suicide.

Many nongovernmental organizations are providing toll free numbers, hence it is essential for all of us as a society to frame a safe network for people going through arduous times and be prepared to listen and remind them of their ingenuity while it is high time that the government should concentrate on mental health issues and provide the toll free number that might help individuals with suicidal thoughts and at the same time bring a policy that every educational institution should have a team to monitor such acts.

Conclusion

As depression and suicidal tendencies are very prominent in students nowadays especially dental students, there is a need to shed light on these and take necessary reforms to relieve academic stress. The current investigation uncovered a wide scope of causative variables of self-destruction among dental understudies which is because of a progression of academical pressures, future settlement, and finance.

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Conflicts of interest

There are no conflicts of interest.

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Annexure

Annexure 1

QUESTIONNAIRE:

Age: Gender: M/F Year of the study:

PHQ-9 QUESTIONNAIRE

Over the last 2 weeks , how often have you been bothered by any of the following problems? (Use "✓" to indicate your answer)	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

SBQ-REVISED QUESTIONNAIRE

Instructions: Please check the number (only one) beside the statement or phrase the best applies to you.

•Have you ever thought about or attempted to kill yourself?

1. Never
2. It was just a brief passing thought.
3. a. I have had a plan atleast once to kill myself but did not try to do it.
3. b. I have had a plan atleast once to kill myself and really wanted to die.
4. a. I have attempted to kill myself, but did not wanted to die.
4. b. I have attempted to kill myself and really hoped to die.

•How often have you thought about killing yourself in the past year?

1. Never
2. Rarely (1 time)
3. Sometimes (2 times)
4. Often (3-4 times)
5. Very often (5 or more times)

•Have you ever told someone that you were going to commit suicide, or that you might do it?

1. Never
- 2a. Yes, at one time but did not really want to die
- 2b. Yes, at one time, and really want to die
- 3a. Yes, more than once but did not want to do it
- 3b. Yes, more than once, and really wanted to do it

•How likely is it that you will attempt suicide someday?

- Never
- Unlikely
- Likely
- Very likely


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Differential Manifestation of COVID-19 Anxiety and Adherence to Precautionary Measures as a Function of Socioeconomic Status: A Longitudinal Study in Coastal Andhra Pradesh

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Abstract

Introduction: There is a possibility that the coronavirus disease (COVID-19)-related anxiety may differ between people from varied socioeconomic status for a variety of reasons. Furthermore, the adherence to COVID-19 precautionary measures could be different between socioeconomic strata in light of the differences in opportunities to more effectively follow these measures for people from different socioeconomic status. **Aim and Objectives:** The aim of this study was to check the differences in COVID-19 anxiety and precautionary measure adherence between subjects from different socioeconomic strata and to conduct a stratified analysis of the association between anxiety and adherence based on socioeconomic status of the participants. **Materials and Methods:** This longitudinal study was conducted in the months of July and November 2020 among 648 subjects belonging to the coastal districts of Andhra Pradesh. The COVID-19 Anxiety Scale (CAS) was used to assess the disease-related anxiety among the study participants, and self-reported adherence (SRA) scores were collected to document the adherence of the study subjects to COVID-19 precautionary measures. Statistical analysis was performed using SPSS version 20 software. **Results:** Significant differences were found in the CAS scores between subjects from different socioeconomic strata in the month of July, while no such observations were made in November. For the outcome of SRA scores, there were significant differences between socioeconomic strata at both the study time points. Overall, the mean CAS and SRA scores decreased from July (20.35 ± 5.54 and 14.48 ± 3.45 , respectively) to November (15.96 ± 5.67 and 12.57 ± 4.17 , respectively). **Conclusion:** The study results highlight the need to reinforce the necessity and importance of adhering to COVID-19 precautionary measures in light of the potential danger of the second wave of COVID-19 in the country.

Keywords: Compliance, COVID-19 anxiety, health behaviors, socioeconomic status

INTRODUCTION

Coronavirus disease (COVID-19) has been one of the largest pandemics the world has witnessed in the recent years. Worldwide, 42.3 million people were affected by the disease by the middle of December 2020. There have been 1.66 million COVID-19 deaths globally, and new disease-specific deaths have been adding to the tally every day. The number of COVID-19 cases in India accounted for 13.3% of the global burden of the disease, and the disease-specific deaths in India were 8.67% of the global COVID-19 deaths. The recovery rate in India is substantially higher at 95.2% compared to the

global recovery rate of 56.4%.^[1] It has widely been established that COVID-19 has a significant impact on the mental health of people.^[2-4] Besides the fear of infection, there has been enormous economic pressure on populations.^[5] At this juncture, it is imperative to refer to the fact that there is no specific treatment available for COVID-19, and the medical support

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offered is predominantly symptom based. Many nations across the globe have been relying on practice of precautionary measures and imposing nonpharmaceutical interventions such as lockdown to combat the spread of the disease.^[6] There has been a stronger emphasis on promotion of the importance of practicing precautionary measures to prevent COVID-19 ever since the initial outbreak of COVID-19. In India, this campaign was endorsed by noted people from different professions such as cinema, sports, and politics. Aarogya Setu, a mobile app developed by the Government of India, utilizes contact tracing in identification of information relating to people, the users of the app might have contacted. In case any of these people tests positive for COVID-19 in future, the app instantaneously conveys the information to the users and arranges for medical care.^[7] However, it is obvious that not everyone gets an equal opportunity to practice the suggested precautionary measures. While most cases of nonadherence are circumstances driven, there is a possibility that the perceived severity of the pandemic and the associated disease-related anxiety at an individual level would influence the adherence of individuals to precautionary measures suggested. Moreover, it is understandable that socioeconomic status of a person can influence the adherence to precautionary measures and may also influence the anxiety levels of the subjects.^[8] Till date, there were no studies conducted in India which evaluated the differences in COVID-19 anxiety and adherence to COVID-19 precautionary measures based on socioeconomic status. Furthermore, the relationship between anxiety and adherence in a stratified analysis based on socioeconomic status has not been explored. With this background, the aim of this study was to check the differences in COVID-19 anxiety and precautionary measure adherence between subjects from different socioeconomic strata and to conduct a stratified analysis of the association between anxiety and adherence based on socioeconomic status of the participants.

MATERIALS AND METHODS

This longitudinal study was conducted from July 2020 to November 2020 among 648 subjects aged 18 years and older regardless of gender. Data were collected on two occasions from the same panel of participants in the months of July and November. This study was conducted in the coastal districts of Andhra Pradesh using a stratified systematic random sampling. All the participants were informed about the nature and purpose of the study, and written informed consent was obtained. The ethical approval of the study was obtained from the institutional review board (14/20/IRB/SBDC) on June 23, 2020. Sample size was calculated to be 449 using G*Power 3.1.9.2 software (Dusseldorf University, Germany). Based on the longitudinal nature of the study, a sample size of 650 was considered in the study.

A thorough review of literature was performed to identify the validated psychometric tools which measure the COVID-19 anxiety.^[9-15] Of all the scales assessed, the COVID-19 Anxiety Scale (CAS) developed among the Telugu-speaking population

of the state of Andhra Pradesh was deemed relevant. CAS is a seven-item scale on a four-point semantic differential scale with overall scale scores ranging from 7 to 28.^[12] To measure the adherence of the study participants to COVID-19 precautionary measures, an interviewer-administered structured questionnaire with three questions rated on a six-point Likert scale was used. The three questions included in the questionnaire were the adherence to precautionary measures suggested to prevent the spread of COVID-19: use of face mask, frequent handwashing, and maintaining social distance. The score obtained by a participant in the adherence questionnaire is identified as the self-reported adherence (SRA) score and ranges from 3 to 18, with higher scores indicative of better adherence. Demographic details of the study participants such as age, gender, and socioeconomic status were also documented. Socioeconomic status of the participants was recorded using the modified BG Prasad scale updated in 2020 based on the per-capita monthly income.^[16] Since there were a very less number of participants in the lower and upper socioeconomic strata, subjects belonging to these strata were combined with subjects from lower-middle and upper-middle socioeconomic strata, respectively. There was a loss of 77 subjects to follow-up, and only 571 subjects were assessed for COVID-19 anxiety and adherence to precautionary measures during the follow-up in November. Figure 1 presents the study flowchart. Statistical analysis was performed using SPSS version 20 software (IBM SPSS Statistics for Windows version 20, Armonk, NY, USA). Descriptive statistics, one-way analysis of variance with Tukey's *post hoc* tests, paired *t*-tests, and Spearman's correlation tests were used in data analysis.

RESULTS

The mean age of the study participants was 47.54 ± 8.9 . There were no significant age differences between subjects from different socioeconomic strata. The mean CAS score was 20.35 ± 5.54 in the month of July which decreased to 15.96 ± 5.67 in the month of November. Similarly, mean SRA scores dropped from 14.48 ± 3.45 to 12.57 ± 4.17 during this time. Significant differences were found in the CAS scores between subjects from different socioeconomic strata in the month of July, while no such observations were made in November [Table 1 and Figure 2]. For the outcome of SRA scores, there were significant differences between socioeconomic strata at both the study time points. *Post hoc* tests revealed similar CAS scores in the month of July between subjects from middle and upper-middle, upper socioeconomic strata [Table 2]. The middle and upper-middle, upper socioeconomic strata demonstrated similar SRA scores as well in the month of November. Table 3 shows that in each socioeconomic stratum, there was a significant decline in the mean CAS scores and mean SRA scores from July to November. There was a significant strong positive correlation between CAS and SRA scores in upper, upper-middle socioeconomic strata (Spearman's $\rho = 0.69$; $p = 0.001^*$). A significant weak positive correlation was observed between

these two parameters in the middle, lower-middle, and lower socioeconomic strata at any of the study time points

substantial decline in the COVID-19 anxiety levels of the population and also people's adherence to COVID-19 precautionary measures over the course of this study. While a decrease in anxiety levels is anticipated in light of the consistently reducing number of COVID-19 cases detected per day and the decreasing COVID-19 case fatality rates,

DISCUSSION

It is clearly evident from this study there has been a

Table 1: Differences in COVID-19 Anxiety Scale and self-reported adherence scores based on socioeconomic status

Time	Parameter	Socioeconomic status	n	Mean ± SD	95% CI for mean	P
July	CAS	Lower and lower middle	220	18.76±5.55	18.02–19.5	0.001*
		Middle	297	20.63±5.22	20.03–21.22	
		Upper middle and upper	131	22.37±5.5	21.42–23.32	
	SRA	Lower and lower middle	220	11.82±3.71	11.33–12.32	
		Middle	297	15.4±2.51	15.11–15.68	
		Upper middle and upper	131	16.89±1.47	16.63–17.14	
November	CAS	Lower and lower middle	183	16.14±5.46	15.34–16.94	0.816
		Middle	272	15.82±5.64	15.14–16.49	
		Upper middle and upper	116	16.08±6.04	14.97–17.19	
	SRA	Lower and lower middle	183	10.92±3.71	10.28–11.46	
		Middle	272	13.45±4.16	12.95–13.95	
		Upper middle and upper	116	13.12±4.14	12.36–13.88	

*Statistical significance. One-way analysis of variance, $P \leq 0.05$ considered statistically significant. CAS: COVID-19 Anxiety Scale, SRA: Self-reported adherence, SD: Standard deviation, CI: Confidence interval

Table 2: Multiple pairwise comparisons of COVID-19 Anxiety Scale and self-reported adherence scores based on socioeconomic stratification

Time	Parameter	SES reference category	SES comparison category	Mean difference	P
July	CAS	Lower and lower middle	Middle	-1.867	0.001*
			Upper middle and upper	-3.615	0.001*
		Middle	Upper middle and upper	-1.748	0.006
	SRA	Lower and lower middle	Middle	-3.57	0.001*
			Upper middle and upper	-5.06	0.001*
		Middle	Upper middle and upper	-1.48	0.001*
November	SRA	Lower and lower middle	Middle	-2.534	0.001*
			Upper middle and upper	-2.203	0.001*
		Middle	Upper middle and upper	0.332	0.73

*Statistical significance. Tukey's *post hoc* tests, $P \leq 0.05$ considered statistically significant. CAS: COVID-19 Anxiety Scale, SRA: Self-reported adherence, SES: Socioeconomic status

Table 3: Stratified analysis of differences in COVID-19 Anxiety Scale and self-reported adherence scores between the study time points based on socioeconomic status

Parameter	Socioeconomic status	Time	Mean difference	P
CAS	Lower and lower middle	July	2.28	0.001*
		November		
	Middle	July	4.67	
		November		
	Upper middle and upper	July	5.69	
		November		
SRA	Lower and lower middle	July	0.94	0.001*
		November		
	Middle	July	1.9	
		November		
	Upper middle and upper	July	3.69	
		November		

*Statistical significance. Paired *t*-test, $P \leq 0.05$ considered statistically significant. CAS: COVID-19 Anxiety Scale, SRA: Self-reported adherence

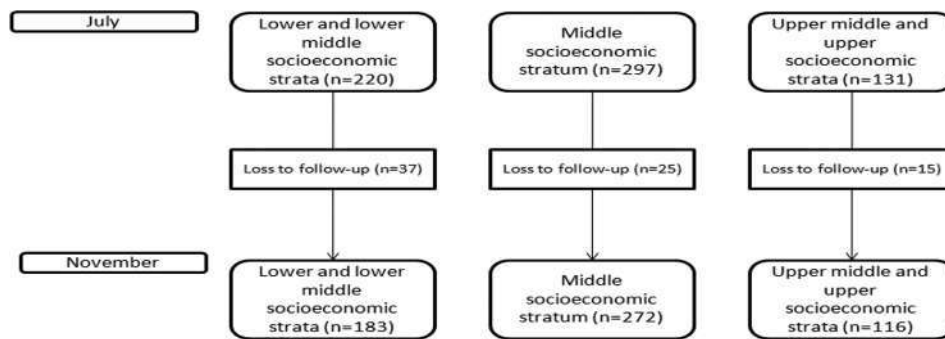


Figure 1: Study flowchart

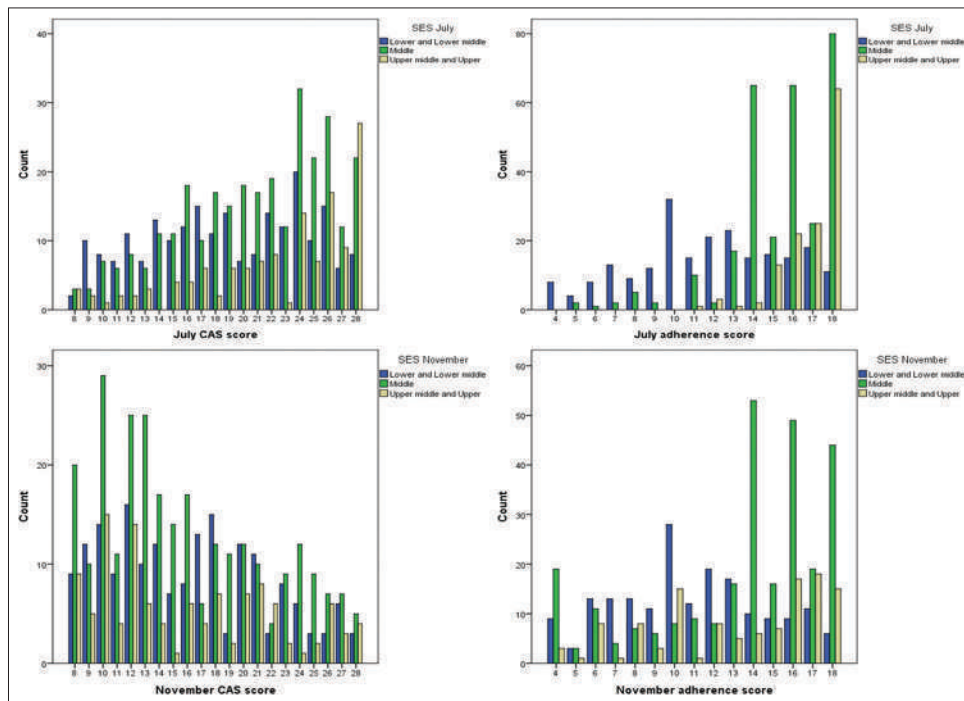


Figure 2: Clustered bar charts showing differences in COVID-19 Anxiety Scale and self-reported adherence scores between socioeconomic strata at the two study time points

poor adherence to precautionary measures is unwarranted. An area of significant concern is the difference in SRA scores between subjects from different socioeconomic strata. This observation persisted throughout the course of this study, the reasons for which could be found in the limited opportunities in adherence to precautionary measures for people from low socioeconomic strata possibly living in relatively overcrowded places and working in environments which are not conducive to the practice of social distancing. Furthermore, frequent handwashing and use of masks may not be financially feasible for this population resulting in poor SRA scores. Another significant area of concern is the magnitude of reduction in the adherence to COVID-19 precautionary measures among subjects from upper and upper-middle socioeconomic strata. A significant reduction in COVID-19 anxiety scores among these people could have contributed toward decreased adherence scores among

these people in light of the strong positive correlation between CAS and SRA scores in the upper and upper-middle socioeconomic strata.

The results from this study were observed to be consistent with the results reported by Bazaid *et al.* in Saudi Arabia where increased adherence to precautionary measures was observed among participants with high socioeconomic status.^[17] Paykani *et al.* reported that socioeconomic status was not found to be associated with compliance to stay-at-home advice among Iranian adults.^[18] Nevertheless, it was observed that subjects who rated their social class to be relatively poor compared to others demonstrated limited compliance. Higher levels of anxiety were reported among subjects from higher socioeconomic strata in a study conducted by Salameh *et al.* in May 2020 in Lebanon which is consistent with the observations made in this study.^[19] In another study conducted

by Agberotimi *et al.*^[20] in Nigeria, in the months of March and April 2020, results inconsistent with this study were demonstrated with similar anxiety levels between subjects from different socioeconomic strata; however, there were significant differences in depressive symptoms with higher prevalence among lower socioeconomic strata.

Clark *et al.* reported that the feeling of vulnerability to COVID-19 had little influence on the subjects' compliance to precautionary measures among an international sample of 8317 people.^[21] Similar findings were observed in this study with a weak correlation between COVID-19 anxiety scores and the SRA to precautionary measures among subjects from lower, lower-middle, and middle socioeconomic strata. However, there was a strong positive correlation between anxiety and adherence to precautionary measures among the subjects from upper-middle and upper socioeconomic strata. Chandu *et al.* reported limited adherence to COVID-19 precautionary measures among a student population in India.^[22] While there were no studies in India which directly assessed the influence of socioeconomic status on the adherence to COVID-19 precautionary measures, it was identified from an Indian study that educational level, an integral component of socioeconomic status, of the subject is an important predictor for adherence to precautionary measures.^[23] Wong *et al.* reported that increase in COVID-19 anxiety between two given time points was a significant predictor for increase in adherence to precautionary measures between those two time points.^[24] This observation was similar to that made in this study among subjects from higher socioeconomic strata. Outside the COVID-19 context, a relation between anxiety and adherence was thoroughly explored. People with any severity of anxiety demonstrated reduced adherence to antihypertensive medication in a study conducted by Bautista *et al.* in 2012.^[25] Similar results were reported by Sundbom and Bingeors in 2013 where nonadherence was more common among those prescription drug users who were identified to have symptoms of anxiety.^[26] Gaining insights into the differential manifestation of COVID-19 anxiety among people belonging to different socioeconomic strata is important in the identification of people who require mental health services. The results observed in this study contribute to the primary care provision by population-level identification of the differing COVID-19 anxiety levels based on socioeconomic status which helps not only in preparing the health-care delivery systems for the provision of required mental health services but also enables the administrative authorities to focus on the reasons for the emergence of COVID-19 anxiety as a function of socioeconomic status. The limitations of this study include a substantial loss to follow-up and lack of information on the reasons for the dropout. Moreover, the study did not collect information on the COVID-19 infection status of the participants and their family members which could have a direct influence on both the outcome parameters of anxiety and adherence. The study findings of decreased SRA to COVID-19 precautionary measures from July to November 2020 highlight

the need to reinforce the necessity and importance of adhering to COVID-19 precautionary measures in light of the potential danger of the second wave of COVID-19 in the country. Furthermore, people from lower socioeconomic strata must be provided with enough opportunities to more effectively practice the suggested precautionary measures. There is also a need to restructure the campaigns from delivering information to showing directions on how to effectively practice the precautionary measures.

CONCLUSION

Within the limitations of this study, the difference in COVID-19 anxiety scores was observed based on socioeconomic status at the beginning of the study, with people from lower socioeconomic status demonstrating the least anxiety. There had been a decline both in the COVID-19 anxiety scores and the SRA to COVID appropriate behavior with time.

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Conflicts of interest

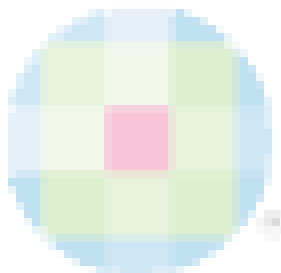
There are no conflicts of interest.

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Research Article

Knowledge, attitude in handling medical emergencies among clinical dental students: an institutional survey

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ABSTRACT

Aim: To assess the awareness of clinical dental students regarding medical emergencies encountered in dental practice and their perceived confidence in managing them.

Materials and Methods: A cross-sectional study was conducted among 217 dental students with a pretested validated questionnaire consisting of questions pertaining to knowledge, attitude and their confidence in handling medical emergencies. Data collected was statistically analyzed by using SPSS version.20.

Results: Out of 217 study subjects, 187 returned completed questionnaires with response rate of 86%. 99.5% study subjects felt that taking medical history is useful in designing treatment plan. 81.3% said that syncope was the most commonly seen dental emergency during dental practice.

Conclusion: Handling of medical emergencies should be taught early from 3rd year as that could help them to retain the knowledge of basic sciences from the preclinical years and apply them in their practice during their clinical years.

Keywords: Dental students, knowledge, medical emergency, preparedness

INTRODUCTION

There are too many causes in the dental office which may provoke medical emergencies. Common causes of emergency cases include the frequent administration of local anaesthetics and other medications, the use of dental materials with high sensitizing potential, the attention of medically compromised patients and adult patients, the anxiety of uncertain surgical procedures in many patients.¹³

Generally, by taking a detailed medical history, assessing the patient and formulating a structured treatment plan with necessary improvements to dental care if needed, a medical emergency may be prevented.^{8,12} Lack of training and inability to cope with medical emergencies can lead to tragic consequences and sometimes legal action. Therefore, dental professionals should be able to properly understand and communicate with relevant health conditions, as well as have sufficient knowledge of oral health treatment and possible interactions with medical conditions.⁸

In dental profession, unexpected medical emergencies might happen and can be life-threatening in certain instances. Negative reactions to local anaesthesia, syncope, epileptic

seizures, hypoglycemia and angina pectoris, both of which have the ability to be life-threatening, are the most frequent medical complications in the dental clinic.⁹ To prevent morbidity and death, the dentist should be able to initiate primary care, and this warrants the need for basic information and material preparedness to recognize, access and handle emergencies in one's practice.^{9,10} So, with this background the current study was done to assess the awareness of clinical dental students regarding medical emergencies encountered in dental practice and their perceived confidence in managing them.

MATERIALS AND METHODS

A cross-sectional study was conducted in a dental college among 217 dental students who are posted in clinical practice were given a pre-tested validated self-administered questionnaire consisting of questions about knowledge, attitude and confidence in handling medical emergencies. Students who are present during the study period and those who returned the completed questionnaires are included in the study. Ethical approval was taken from the institutional ethical committee (IEC) prior to the commencement of

the study. The questionnaire consisted mainly of objective questions, and informed consent was taken prior from all the study participants. Reliability of the questionnaire was determined by using Cronbach's alpha coefficient test, which gave a value of 0.76. Data thus obtained were entered into Excel sheet and analyzed by using Statistical Package for Social Sciences (SPSS) version.20 whilst Descriptives and Chi-square test were done where p value ≤ 0.05 is considered as statistically significant.

RESULTS

Out of 217 study subjects, 187 returned completed questionnaires with a response rate of 86%. While 99.5% of study subjects felt that taking a medical history is useful in designing the treatment plan. 155 students felt that exposure to previous dental treatment will reduce the incidence of a medical emergency. The majority of the participants said that anxiety is the common cause of the medical emergency.

81.3% said that syncope was the most commonly seen dental emergency during dental practice, of which 44(72.1%) third BDS, 81(83.5%) fourth BDS, and 27(93.1%) interns. 82.8% of interns, 80.4% of fourth BDS and 54.1% of third BDS students are aware of emergency drugs and the kit to be available in the dental office, which is statistically significant. 86.2% of interns underwent BLS training when compared with fourth and third BDS students, which is statistically significant.

The majority of the students from 93.8% final and 93.1% interns said BLS training is required, which is statistically significant. More than half of the study participants are not confident in handling a medical emergency situation.

DISCUSSION

Although, the life-threatening emergencies in the dental offices was infrequent, many factors can escalate the likelihood of such events which include an increasing number of older persons seeking dental care, therapeutic advances in dentistry, longer appointments and increased drug usage. A medical emergency may arise on the dental chair despite efforts to minimize them. Lack of training and inability to cope with these can lead to tragic consequences.

In the present study, 94% of the students told recording medical history is useful in designing a treatment plan, which is similar to a study conducted by Alsahrani.F (2017)³ 85% of the students thought recording medical history is useful in designing a treatment plan. 64% of students in the present study are aware of the BLS technique, while in the study done by Ibrahim Aliyu et al⁷ 73.3% are aware, which was in

contrast to the study done by Neha Baduni.N (2014)⁸ where none of the participants had complete knowledge about BLS. In this study, 72% of students were aware of the emergency kit which is; in contrast, to a study conducted by Alsahrani.F (2017)³ where 60% of students were aware of emergency kit which is to be available in the dental office.

49% of students in the present study were confident to handle the emergency situation, which is similar to the study done by Aadil Ahamed (2016)¹ and Sharma P (2019)¹¹ and which was seen different results from a study conducted by Fasoyiro.O (2019)⁶ where 72% of students were competent enough to handle an emergency situation.

On analyzing the results fourth BDS students and interns were more aware of emergency situations and the way to handle them when compared to third BDS students whilst fourth BDS students had almost equal levels of knowledge as interns. This could be because of guest lecture regarding medical emergencies, the fourth BDS students had before the day of the survey

In the present study, 81% observed syncope as most common medical emergency, which is similar to the study conducted by Sheikho.M.A (2018)¹², in his study most common medical emergency was vasovagal syncope and second most common medical emergency was hypoglycemia, which is, in contrast, to study done by Elanchezhiyan.S (2013)⁵, were 43% reported that syncope is the commonest medical emergency in dental office.

In the present study 69% of the participants said anxiety is the common cause for a medical emergency, which is similar to the study done by Elanchezhiyan.S (2013).⁵ In this study, 81% agreed to check vital signs before to dental treatment, which is less when compared to study by Sheikho.M.A (2018)¹² 97.3% of the respondents check the vital signs prior to dental extraction.

Nearly half of the participants experienced at least one emergency situation. In a study conducted by Sharma P (2019)¹¹ 29.4% of them obtained vital signs before commencing any treatment.

In this study, nearly 78% of participants said that BLS training is necessary to everyone, but only 64.2% of dental students are trained in BLS, which is in contrast to the study done by Elanchezhiyan.S (2013)⁵ even though 96% reported that the BLS course is mandatory for dentists, only 56% said they had trained in BLS, and which is similar to the study done by Joshi.S (2016).¹³ In present study only 69% responded



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that patient anxiety is the common cause for medical emergencies.

In the present study 83% of the students felt recording medical history plays a vital role in medical emergency management, which is similar to the study conducted by Elanchezhiyan.S (2013)⁵ 100% agreed that medical history plays a vital role in emergency management. Analysis of the study showed that awareness level of students was not satisfactory and special gridlines are required to enlighten the students regarding medical emergencies encountered in dental practice and handling them efficiently

As Goldberger said in the year 1990, that the emergency ceases to exist as you prepare for an emergency. So, for the worst case, we need to be prepared.

CONCLUSION

The observation of the survey showed that most of the participants were lacking confidence in handling medical emergencies. The study showed that there is finding a dearth of knowledge among dental students and interns in dealing with medical emergencies. Annual basic life support and emergency courses should be made mandatory in the dental teaching curriculum, and further hands-on training is required to update their knowledge to enhance their capability to recognize and manage a medical emergency and to become well-qualified practitioners.

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Table 1: Showing the responses by dental students regarding medical emergencies

Question	Third year	Fourth year	Internship	P-value
RECORDING MEDICAL HISTORY IS USEFUL				0.354
Yes	60(98.4%)	97(100%)	29(100%)	
No	1(1.6%)	0(0%)	0(0%)	
EXPOSURE TO PREVIOUS DENTAL TREATMENT WILL HELP IN REDUCING INCIDENCE OF A MEDICAL EMERGENCY				0.013*
Yes	44(72.1%)	87(89.7%)	24(82.8%)	
No	6(9.8%)	6(6.2%)	4(13.8%)	
No idea	11(18%)	4(4.1%)	1(3.4%)	
IS ANXIETY IS THE COMMON CAUSE OF MEDICAL EMERGENCY				0.708
Yes	40(65.6%)	70(72.2%)	19(65.5%)	
No	13(21.3%)	19(19.6%)	8(27.6%)	
No idea	8(13.1%)	8(8.2%)	2(6.9%)	
PREMEDICATION FOR ANXIETY REDUCE MEDICAL EMERGENCY				0.697
Yes	40(65.6%)	63(64.9%)	22(75.9%)	
No	11(18%)	14(14.4%)	4(13.8%)	
No idea	10(16.4%)	20(20.6%)	3(10.3%)	
MOST COMMON MEDICAL EMERGENCY				0.143
Syncope	44(72.1%)	81(83.5%)	27(93.1%)	
Seizures	9(14.8%)	5(5.2%)	2(6.9%)	
Bronchospasm	5(8.2%)	5(5.2%)	0(0%)	
Others	3(4.9%)	6(6.1%)	0(0%)	
AWARE OF EMERGENCY DRUGS AND KITS AVAILABLE IN DENTAL OFFICE				0.001*
Yes	33(54.1%)	78(80.4%)	24(82.8%)	
No	28(45.9%)	19(19.6%)	5(17.2%)	
RECORDING VITAL SIGNS IS REQUIRED BEFORE DENTAL PROCEDURE.				0.313
Yes	50(82%)	80(82.5%)	22(75.9%)	
Yes	7(11.5%)	14(14.4%)	7(24.1%)	
No	4(6.6%)	3(3.1%)	0(0%)	
No idea				
AWARE OF BLS TECHNIQUE				0.001*
Yes	26(42.6%)	69(71.1%)	25(86.2%)	
No	35(57.4%)	28(28.9%)	4(13.8%)	
BLS TRAINING IS REQUIRED.				0.001*
Yes	29(47.5%)	91(93.8%)	27(93.1%)	
No	8(13.1%)	1(1%)	1(3.4%)	
No idea	24(39.3%)	5(5.2%)	1(3.4%)	
CAPABLE OF HANDLING MEDICAL EMERGENCY.				0.166
Yes	24(39.3%)	50(51.5%)	17(58.6%)	
No	37(60.7%)	47(48.5%)	12(41.4%)	

*statistically significant


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Effectiveness of N95 Respirator versus Surgical Mask against Sars-Cov2 - Systemic Review and Meta Analysis

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Abstract

Introduction: The effect of N95 respirators for the protection against the infection is still undetermined. Hence in this study we evaluated the effectiveness of N95 respirators against surgical masks for inhibition of SARS- COV2 by gathering randomized controlled trials (RCTs).

Materials and Methods: Online data from PubMed, EMBASE, Cochrane Library was searched for period of one year from Jan-2020 to jan-2021. The studies between 2009 and 2020 were searched. The systematic reviews were considered. The RCTs incorporated in systematic reviews were recognized. We also searched for the RCTs done independently. Risk of the bias was evaluated by 2 reviewers independently after data extracted. Meta-analyses were done to calculate pooled estimates by RevMan 5.3 software.

Results: A total of six RCTs involving 9171 participants were included. There were no statistically significant differences in inhibiting laboratory-confirmed SARS- COV2 (RR = 1.09, 95% CI 0.92- 1.28, P > .05), laboratory-confirmed respiratory viral infections (RR = 0.89, 95% CI 0.70-1.11), laboratory-confirmed respiratory infection (RR = 0.74, 95% CI 0.42-1.29) and influenza like illness (RR = 0.01, 95% CI 0.33-1.14) using N 95 respirators

and surgical masks. Meta-analysis indicated a protective effect of N95 respirators against laboratory-confirmed bacterial colonization (RR - 0.58, 95% CI 0.43-0.78).

Conclusion: The use of N95 respirators matched with surgical masks is not associated with a lower risk of laboratory-confirmed SARS- COV2. It suggests that N95 respirators should not be recommended for general public and non high-risk medical staff those are not in close contact with SARS- COV2 patients or suspected patients.

Keywords: Surgical masks, N95 respirator, Respiratory tract infections.

Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have mortality rates about 10% and 37%, correspondingly.¹ Ever since the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), facemasks have been deliberated to be vitally important to reduce the risk of infection because vaccination or specific anti-infective treatments are unavailable.^{2,3} N95 respirators are used to protect users from inhaling small airborne particles and must fit tightly to the user's face. Surgical masks are intended to protect wearers from microorganism transmission and fit slackly to the user's face.^{5,6} Though surgical masks cannot stop inhalation of small airborne particles, both of them can defend users from large droplets and sprays.^{7,8} There are inconsistent recommendations for severe acute respiratory syndrome (SARS) and pandemic influenza: the World Health Organization (WHO) recommends using masks in low-risk situations and respirators in high-risk situations, but the Centers for Disease Control and Prevention (CDC) endorses using respirators in both low and high-risk situations.⁹ However, N95 respirators may play a incomplete role in low-resource settings, where there are a limited number of N95 respirators, or it may be too expensive.⁹ Also, previous meta-analyses determined there was insufficient evidence to determine the effect of N95 respirators due to a small number of studies that is prone to lack of statistical power.^{10,11} Furthermore, these meta-analyses were limited by the small number of included randomized control trials (RCTs). More, well planned RCTs of comparing N95 respirators with surgical masks against influenza published in recent years were not incorporated in previous meta-analyses.¹²⁻¹⁴ In light of the growing number of RCTs of masks use for protecting against, this systematic review and meta-analysis intended to evaluate the effectiveness of N95 respirators versus surgical masks for prevention of SARS- COV2.

Materials and method

This meta-analysis was piloted based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.¹⁵ We included only the studies that were

- (1) RCT (including cluster randomized trial) and nonrandomized controlled study;
- (2) COVID patients
- (3) N95 respirators and surgical masks
- (4) RTPCR- confirmed cases only was taken as primary outcome variable
- (5) Other lab confirmed respiratory viral, bacterial infections are taken as secondary outcome variables
- (6) Hospital or community.



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All the other human studies with improper design were excluded.

Search approach

Online data from PubMed, EMBASE, Cochrane Library was searched for period of one year from Jan-2020 to Jan-2021. The terms like N95 mask, surgical mask, SAR-COV2, systemic reviews, meta analysis. Later, primary RCTs included in the systematic reviews were identified. Furthermore, we piloted an additional search to identify RCTs published in the past five years from January 27, 2015, to January 27, 2020, using the databases and search strategies described above.

Data mining:

Two reviewers independently selected the articles based on the titles, abstracts and full texts. Then, two reviewers independently extracted the following data from included studies: first author, publication year, country, disease, details of study population and intervention, study design, sample size, settings, and results.

Risk of bias assessment

Two reviewers independently assessed the risk of bias of the selected RCTs using the Cochrane Risk of Bias tool,¹⁶ which includes domains on random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, and selective reporting. For each RCT, every domain was judged among 3 levels: high risk, unclear risk, and low risk. Disagreements were resolved by discussion.

Data analysis

All statistical analyses were performed using Review Manager (RevMan) version 5.3. Comparable data from studies with similar interventions and outcomes were pooled using forest plots. Relative risk (RR) with 95% confidence intervals (CIs) for dichotomous data was used as the effect measure. Between-study heterogeneity was assessed using the I^2 for each pooled estimate.¹⁷ We adopted a random-effects model for heterogeneity $P < 0.10$. We performed a subgroup analysis based on the settings (hospital, community) due to the possibility of clinical heterogeneity. A sensitivity analysis was conducted to evaluate the robustness of the results by excluding individual studies for each forest plot. Funnel plots were planned to assess publication bias. Because of the small number of studies available for each pooled estimate, we failed to assess publication bias.

Results

Search results and study characteristics

In total, we included six RCTs (12,18-22) and found no unpublished data of RCTs from ClinicalTrials.gov. The characteristics of these RCTs were presented in Table 1. The included studies published between 2009 and 2019. A total of 9171 participants in Canada, Australia, China, or America were included, and the number of participants in each RCT ranged from 435 to 5180 patients. The follow-up duration varied from 2 to 15 weeks. Five studies included participants in hospitals,^{12,18,20-22} and one in households.¹⁹ Because of different definitions of outcome in included studies, we redefined the laboratory confirmed respiratory infection as respiratory SARS- COV2, other viruses or bacteria infection. 3.2

Risk of bias

The results of the risk of bias assessment can be found in Figure 2. Five studies reported the computer-generated random sequences, while only one mentioned randomization. All studies did not mention allocation concealment. Participants and trial staff were not blinded in two studies, and the other two studies failed to mention the blinding of participants and personnel. Four studies did not report whether the outcome assessors were blinded. All studies had complete outcome data or described comparable numbers and reasons for withdrawal across groups and prespecified outcomes.

Effectiveness

Five RCTs involving 8444 participants reported laboratory-confirmed influenza &/ SARS-COV2.^{12,18-21} Meta-analysis with fixed-effects model revealed that there was no statistically significant differences in inhibiting influenza using N95 respirators and surgical masks (RR = 1.09, 95% CI 0.92-1.28, $P > .05$) (Figure 3). The results of subgroup analyses were consistent with this regardless of the hospital or the community. The results of the sensitivity analysis were not altered after excluding each trial. Four RCTs¹⁸⁻²¹ involving 3264 participants reported laboratory confirmed respiratory viral infections. Meta-analysis with fixed-effects model revealed that there were no statistically significant differences in inhibiting respiratory viral infections using N95 respirators and surgical masks (RR = 0.89, 95% CI 0.70-1.11, $P > .05$) (Figure 4). The results of subgroup analyses were consistent regardless of the hospital or the community. However, the sensitivity analysis after excluding the trial by Loeb et al¹⁸ showed a significant effect of N95 respirators on stopping respiratory viral infections (RR=0.61, 95% CI 0.39-0.98, $P < .05$). Two RCTs^{21,22} involving 2538 participants reported laboratory confirmed bacterial colonization. Meta-analysis with fixed-effects model revealed that compared with surgical masks, N95 respirators significantly decreased bacterial colonization in hospitals (RR = 0.58, 95% CI 0.43-0.78, $P < .05$) (Figure 5). The sensitivity analysis showed that the results did not change after excluding each trial. Two RCTs^{12,22} involving 6621 participants reported laboratory confirmed respiratory infection. Meta-analysis with random-effects model revealed that there were no statistically significant differences in inhibiting respiratory infection using N95 respirators and surgical masks in hospitals (RR = 0.74, 95% CI 0.42-1.29, $P > .05$) (Figure 6). However, the sensitivity analysis after excluding the trial by Radonovich et al¹² showed a significant effect of N95 respirators on inhibiting respiratory infection (RR = 0.53, 95% CI 0.35-0.82, $P < .05$). Five RCTs involving 8444 participants reported influenza like illness.^{12,18-21} Meta-analysis with random-effects model revealed that there were no statistically significant differences in inhibiting influenza like illness using N95 respirators and surgical masks (RR = 0.61, 95% CI 0.33-1.14, $P > .05$) (Figure 7). The results of subgroup analyses indicated that statistically significant superiority of N95 respirators over surgical masks against influenza like illness (RR = 0.37, 95% CI 0.20-0.71, $P < .05$) in the community (only one RCT). The sensitivity analysis showed results remained unchanged after excluding each trial.



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Figure 1: Flowchart of retrieved studies.

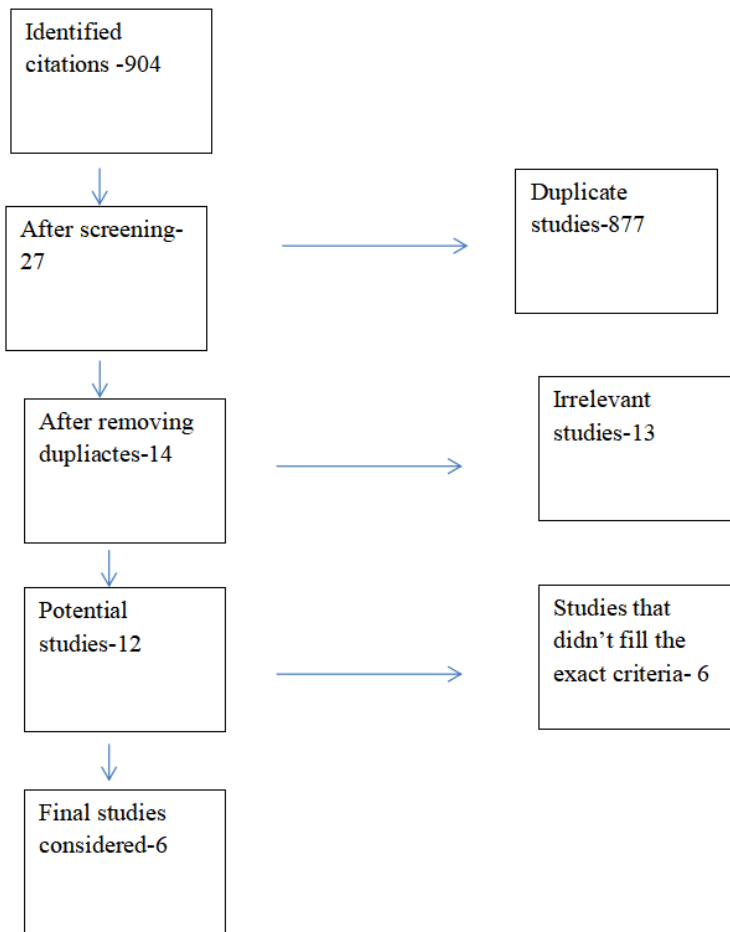


TABLE 1: Characteristics of studies included in the meta-analysis

Study	Setting	Participants	Intervention	Outcomes	Notes
Loeb et al 2009¹⁸	8 hospitals in Ontario, Canada: emergency departments, acute medical units and pediatric units	446 nurses; individual-level randomization	<ul style="list-style-type: none"> Intervention: targeted use, fit-tested N95 respirator Control: targeted use, surgical mask 	<ul style="list-style-type: none"> Laboratory-confirmed respiratory infection, influenza-like illness, workplace absenteeism 5-week follow-up 	<ul style="list-style-type: none"> Noninferiority trial Detection of influenza A and B, respiratory syncytial virus, metapneumovirus, parainfluenza virus, rhinovirus-enterovirus, coronavirus and adenovirus
MacIntyre	145	145 index	Intervention	•Laboratory-	Detection of

et al 2009 ¹⁹	households in Sydney, Australia	patients and 290 household contacts in 145 households; cluster randomization by household	1: continual use, surgical mask • Intervention 2: continual use, nonfit-tested N95 • respirator • Control: lifestyle measures	confirmed respiratory virus infection, influenza-like illness • 2-week follow-up	influenza A and B, respiratory syncytial virus, parainfluenza virus, rhinovirus-enterovirus, coronavirus, coronavirus, adenovirus
MacIntyre et al 2011 ²⁰ / 2014 ²	15 hospitals in Beijing, China: emergency departments and respiratory wards	1441 nurses, doctors and ward clerks; cluster randomization by hospital	• Intervention 1: continual use, fit-tested N95 respirator • Intervention 2: continual use, nonfit-tested N95 respirator • Control: continual use, surgical mask	Laboratory-confirmed respiratory infection, influenza-like illness • 5-week follow-up	Detection of influenza A and B, respiratory syncytial virus, metapneumo virus, parainfluenza virus, rhinovirus-enterovirus, coronavirus, adenovirus, streptococcus pneumoniae, bordetella pertussis, chlamydia pneumoniae, mycoplasma pneumoniae and haemophilus influenzae type B
MacIntyre et al 2013 ²¹	19 hospitals in Beijing, China: emergency departments and respiratory wards	1669 nurses, doctors and ward clerks; cluster randomization by ward	• Intervention 1: continual use, fit-tested N95 respirator • Intervention 2: targeted use, fit-tested N95 respirator • Control:	• Laboratory-confirmed respiratory infection, influenza-like illness • 5-week follow-up	Detection of influenza A and B, respiratory syncytial virus, metapneumo virus, parainfluenza virus, rhinovirus-

			continual use, surgical mask		enterovirus, coronavirus, adenovirus, S. pneumoniae, B. pertussis, C. pneumoniae, M. pneumoniae and H. influenzae type B
Radonovich et al 2019¹²	7 hospitals in US: primary care facilities, dental clinics, adult and pediatric clinics, dialysis units, urgent care facilities and emergency departments, and emergency transport services	5180 nurses/nursing trainees, clinical care support staff, administrative/clerical staff, physicians/advanced practitioners/physician trainees, registrations/clerical receptions, social workers/pastoral cares and environmental service workers/housekeepers; cluster randomization by outpatient clinic or outpatient setting	<ul style="list-style-type: none"> Intervention: targeted use, fit-tested N95 respirator Control: targeted use, medical mask 	<ul style="list-style-type: none"> Laboratory-confirmed respiratory infection, laboratory-confirmed influenza, laboratory-detected respiratory illness, influenza-like illness, acute respiratory illness 12-week follow-up 	<p>Effectiveness study</p> <ul style="list-style-type: none"> Detection of influenza A and B, respiratory syncytial virus, metapneumo virus, parainfluenza virus, rhinovirus-enterovirus, coronavirus, coxsackie/echovirus



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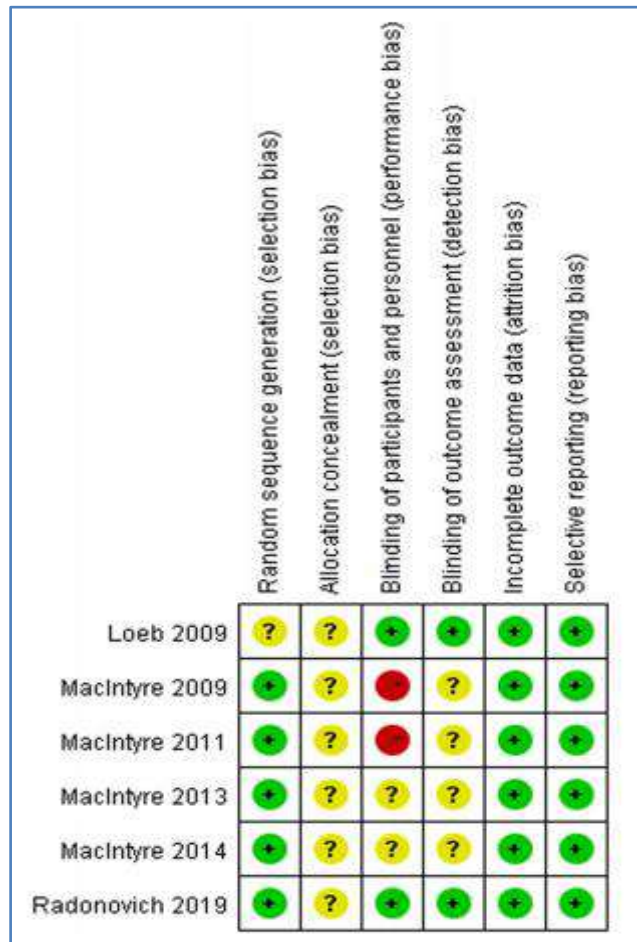


FIGURE 2: Risk of bias summary

FIGURE 3: N95 respirators vs surgical masks against laboratory-confirmed “SARS-COV2”

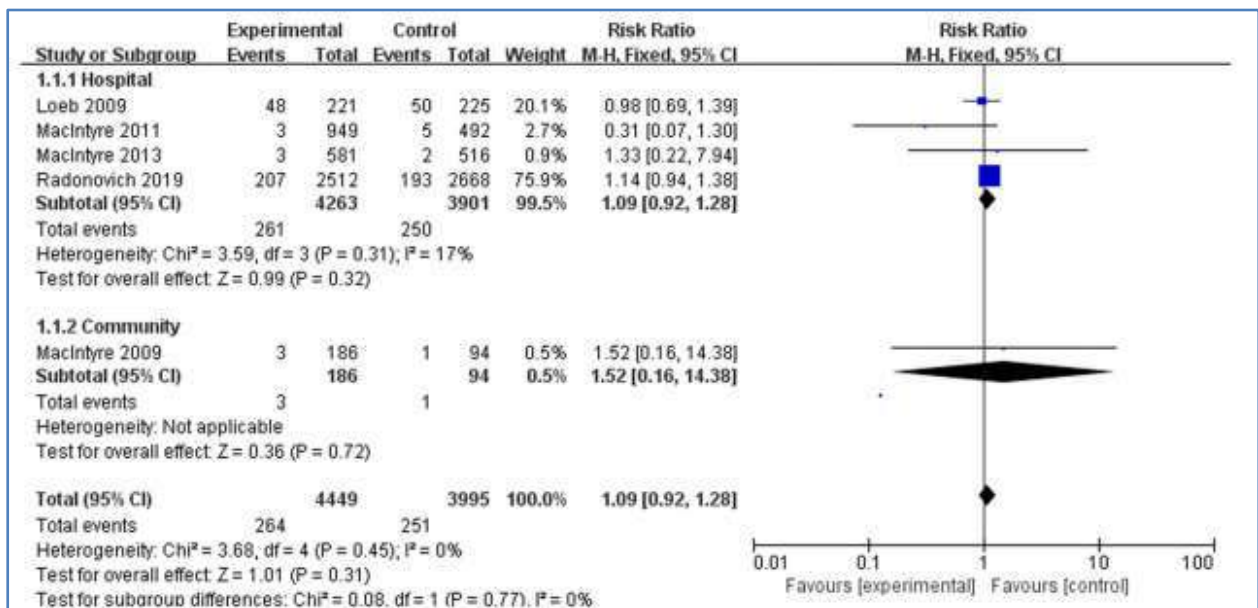


FIGURE 4: N95 respirators vs surgical masks against laboratory-confirmed respiratory “viral infections”

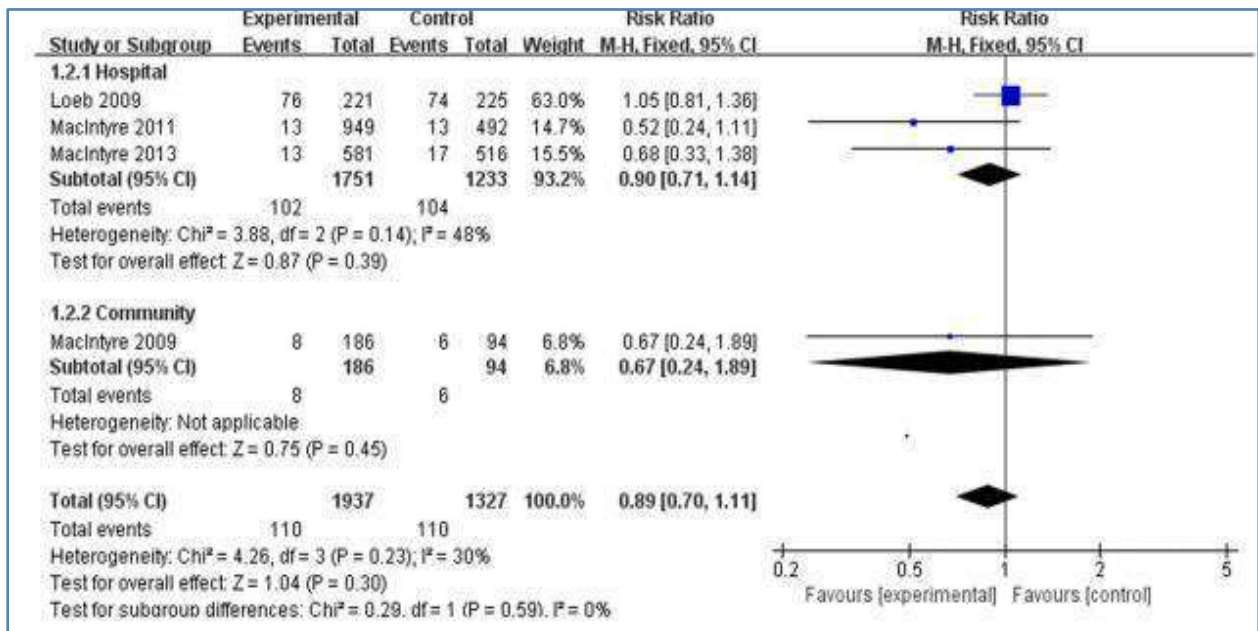


FIGURE 5: N95 respirators VS surgical masks against laboratory-confirmed bacterial colonization

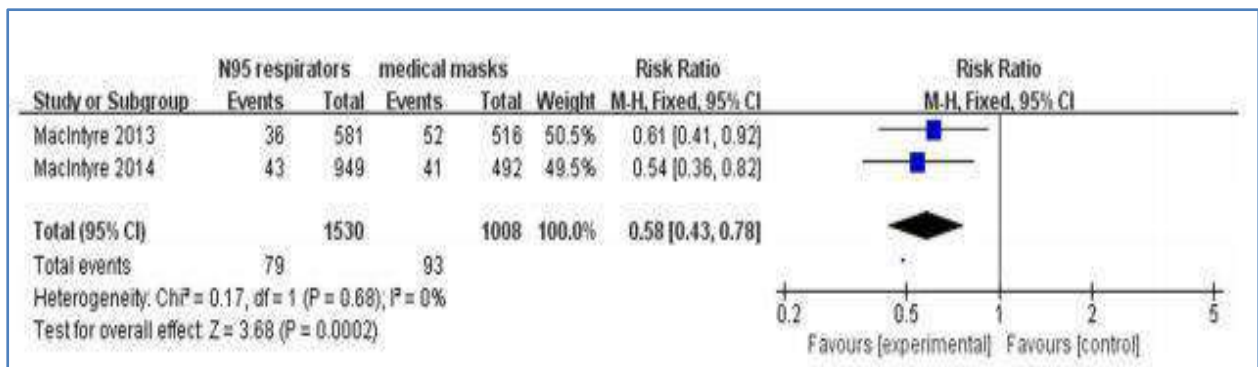


FIGURE 6: N95 respirators VS surgical masks against laboratory-confirmed respiratory infection

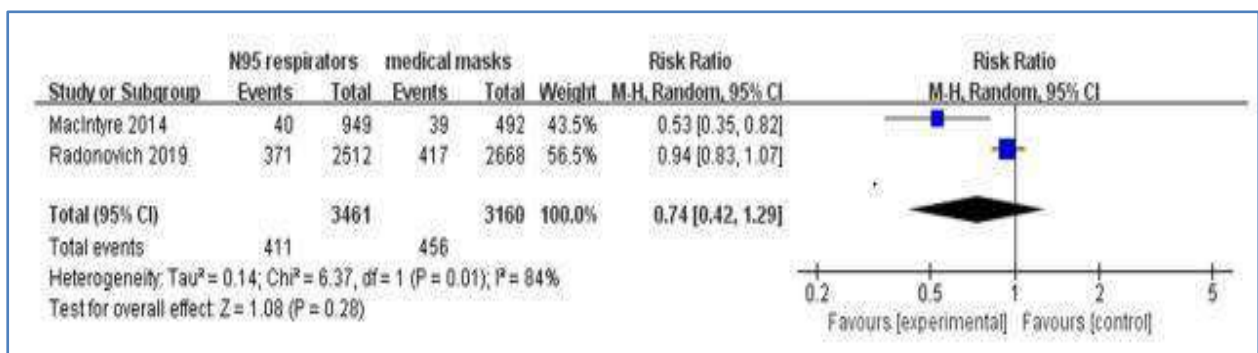
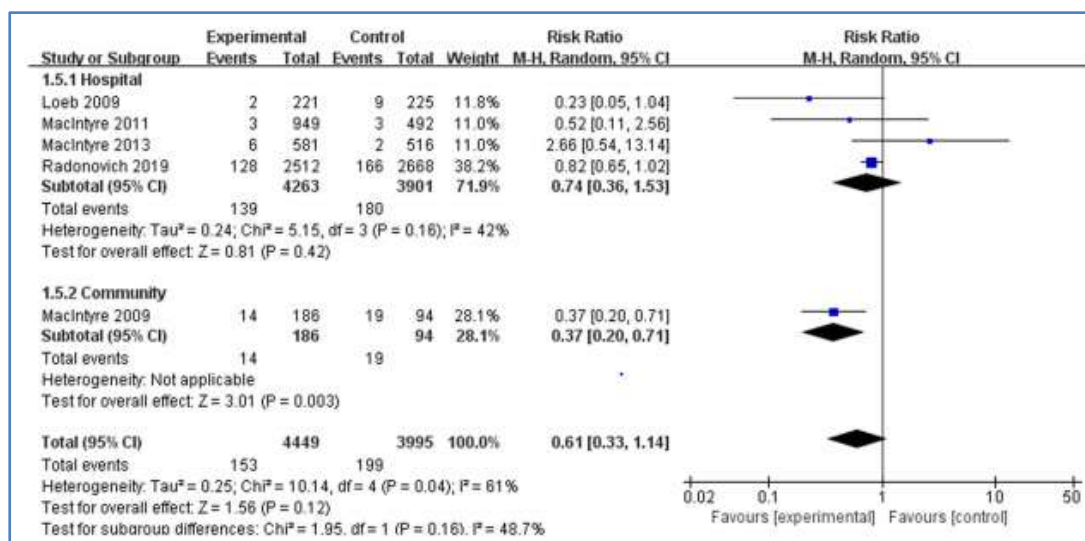


FIGURE 7: N95 respirators VS surgical masks against influenza like illness

Discussion

This meta-analysis showed that there were no statistically significant differences in inhibiting laboratory-confirmed SARS-COV2, laboratory confirmed respiratory viral infections, laboratory-confirmed respiratory infection and influenza-like illness using N95 respirators and surgical masks. N95 respirators delivered a protective effect against laboratory-confirmed bacterial colonization. In subgroup analysis, similar results could be found in the hospital and community for laboratory-confirmed influenza/ SARS- COV2 and laboratory-confirmed respiratory viral infections. Nonetheless, sensitivity analysis showed unstable results for the prevention of laboratory-confirmed respiratory viral infections and laboratory-confirmed respiratory infection. Over the course of influenza/ SARS- COV2 pandemics, large numbers of facemasks may be required to use in long periods to guard people from infections.²³ Using N95 respirators is probable to result in discomfort, for instance, headaches.²³ A earlier study³ reported that there was an converse relationship between the level of defiance with wearing an N95 respirator and the risk of clinical respiratory illness. It is problematic to ensure high compliance due to this discomfort of N95 respirators in all studies. The reason for the similar effects on inhibiting influenza/ SARS- COV2 for the use of N95 respirators versus surgical masks may be connected to low compliance to N95 respirators wear,²³ which may lead to more common doffing compared with surgical masks.¹³ Though N95 respirators may confer more protection in laboratory studies designing to achieve 100% intervention adherence,²⁴ the routine use of N95 respirators seems to be less acceptable due to more substantial discomfort in real-world practice.¹¹ Consequently, the benefit of N95 respirators of fitting tightly to faces is offset or subjugated.¹³ Nevertheless, it should be noted that the surgical masks are primarily designed to protect the environment from the wearer, whereas the respirators are supposed to protect the wearer from the environment.²⁵ There are some limitations to this study. First, some RCTs had a great risk of bias due to lack of allocation concealment and blinding; though it is impractical to blind participants who would know the type of masks they are wearing. Second, the number of comprised studies focusing on the community was small. Thus, the results of the subgroup analysis might be unreliable. Third, we identified RCTs from published systematic reviews, which may end in the omission of relative RCTs. Finally, there might be publication bias, and we cannot measure it due to an insufficient number of included RCTs.

Conclusion

In conclusion, the present meta-analysis shows the use of N95 respirators equated with surgical masks is not related with a lower risk of laboratory-confirmed influenza/ SARS-COV2. It proposes that N95 respirators should not be endorsed for the general public and non-high risk medical staffs those are not in close interaction with influenza/ SARS- COV2 patients or suspected patients.

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Is the Profession of Dentistry Losing its Yesteryear's Glory? An Exploratory Study from Dental Students' Perspective

Abstract

Introduction: The regard for dentistry as a profession has been declining over the recent years in light of the increasing number of dental students graduating per year with negligible improvement in the utilization of oral health care services. In this context, it is important to document the perceptions and apprehensions of the current dental students as these feelings would have an influence on the roles they assume as dentists in future. **Aim:** With this background, this study attempts to document the willingness of dental students from three South Indian states to re-choose dentistry given an opportunity. **Materials and Methods:** This cross-sectional survey was conducted among house surgeons from 12 dental institutions, 4 each from the southern Indian states of Andhra Pradesh, Telangana, and Tamilnadu. The semi-structured questionnaire used in this study consisted of five primary questions along with details on the participants' gender, type of admission. It aimed at documenting the willingness to re-choose dentistry given an opportunity, and the reasons for their willingness or otherwise. A total of 822 students constituted the final sample. SPSS version 20 software was used to analyse the data. **Results:** Majority of the study participants were from Tamil Nadu, not reported dentistry as their primary career choice, and demonstrated reluctance in re-choosing dentistry given an opportunity. A significantly higher percentage of dental students from Tamil Nadu reported dentistry as their first professional choice. This observation persisted in the response of students for the question on their willingness to re-choose dentistry. **Conclusion:** The study results demonstrate the declining interest and regard for dentistry among the current dental students with nominal variations between students from the three South Indian states.

Keywords: Dental, dentistry, education, qualitative research

Introduction

Though dentistry is regarded as one of the oldest medical disciplines dating back to 7000 B.C., it is only over the last century that this discipline in health care got streamlined into a definite profession.^[1] In the Indian context, refinement of dentistry as a profession became possible with the inception of formal dental education. The first dental college in India was established in 1920 by Dr. Rafiuddin Ahmed in Kolkata.^[2] Since then the profession has evolved by leaps and bounds becoming one of the most sought after professions in the country. There has been a consistent increase in the number of dental colleges in India over the years with a pronounced increase seen during 1998 – 2014.^[3] At present, there are 315 dental educational institutions in India producing nearly 31,000 dental graduates every year, making India the largest producer of dentists across the world.^[4] However, this increase in the number of dental institutions in the country was not

demand-driven and neither was this choice methodically informed. Once the supply component in dental care delivery began to outstrip the demand component, the delivery systems started suffering from a reduced number of care seekers which consequently had given rise to a notion that dentistry as a profession is not fruitful anymore, at least not as yielding as it once was. The success of any profession is a complex interplay of a multitude of factors among which financial returns in the profession and societal regard for the profession could be considered the most determining. It is this reduction in societal regard for the profession and the reduced financial returns that prompted the aforementioned notion on the profession of dentistry.

Indian medical education system is an intricate organization with academic merit being not the only factor that warrants admission. While the distinction of this system is largely debated, from the educational institutions' perspective this mechanism ensures full

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enrollment. On the other side, the mechanism also offers solutions for parents of those students who demonstrated insufficient standards in the entrance examination, to choose a profession of their choice. It is under these circumstances that dentistry enjoyed almost full enrollment throughout the country despite an enormous increase in the number of dental institutions. However, the scenario has apparently been changing. There has been a consistent and considerable decline in the number of undergraduate admissions in dentistry over the last few years.^[5,6] While there could be a number of reasons for this phenomenon, the experiences of current dental students with dentistry and their understanding of the future in dentistry cannot be ignored. In this context, this study attempts to gain insights into the perceptions of current house surgeons in various dental colleges in Southern India to inform policymakers on the prospective ways to streamline the profession, should there be a need.

Materials and Methods

This cross-sectional questionnaire study was conducted among house surgeons from 12 dental institutions, 4 each from the southern Indian states of Andhra Pradesh, Telangana and Tamilnadu. Two-stage cluster random sampling was done, where three states were selected in the first stage and four dental institutions from each of these states were randomly selected in the second stage. All the house surgeons from the selected dental institutions were invited to participate in the study. The primary investigator made a correspondence with the coordinators of this study at each of the 12 dental institutions to discuss the study protocol. The participation in this study is voluntary and consent was obtained from all the willing participants before the questionnaire was administered. The questionnaire was administered to the consented house surgeons among the twelve dental institutions considered in this study in April 2019. The semi-structured questionnaire used in this study was divided into four sections: whether dentistry was participant's first choice; the participant's reasons for choosing dentistry as a career; whether the participant is willing at the present time to choose dentistry if he/she was given an opportunity to re-choose; participant's reasons for their willingness or otherwise in re-choosing dentistry. Details regarding participants' gender and type of admission were collected. The questionnaire was assessed for content validity by four experts in the field of dental education and a pilot test was done among 25 students to evaluate the face validity of the questionnaire. Students who participated in the pilot study were not included in the final sample. Of the 967 students approached, 98 students opted not to participate in the study, while 47 students returned incomplete or partially filled questionnaires. The final sample consisted of 825 house surgeons from the twelve selected dental institutions in Southern India. Approval obtained on 16th February, 2018. SPSS version 20 software (IBM SPSS statistics for Windows version 20, Armonk, USA) was used to analyze the study data. Descriptive statistics, Chi-square

test were performed to check the association of participants' regard for dentistry with background characteristics and the geographical location of the institution, and to extract themes that determined participants' responses on the willingness to re-choose dentistry given an opportunity.

Results

Of the 825 study participants, 193 (23.39%) were males. Majority of the participants (36.37%) were dental students from Tamil Nadu, followed by 273 (33.09%) from the state of Andhra Pradesh, and 252 (30.54%) from Telangana. Only 18.06% of the students reported dentistry as their first professional choice. The most common reason for choosing dentistry was reported to be the inability to secure an admission in medicine. There was a statistically significant difference based on geographical location with regard to students' response to dentistry as their first professional choice [Table 1] and the reasons given for choosing dentistry [Table 2]. A significantly higher percentage of dental students from Tamil Nadu reported dentistry as their first professional choice. This observation persisted in the response of students for the question on their willingness to re-choose dentistry if they were given an opportunity [Table 1]. A total of 35% of participants from Tamil Nadu demonstrated willingness to re-choose dentistry, while only 12.3% of students from Telangana and 23.07% of students from Andhra Pradesh showed willingness to re-choose, given an opportunity. However, no significant differences were noted in the interest of students to advise dentistry as a professional choice for their friends based on the geographical location of the students [Table 1]. Not more than a quarter of the students from all the three states included in the study expressed interest in recommending dentistry as a career choice for their friends. The scope that the profession offers to make people smile was the major reason for students' willingness to re-choose dentistry [Figure 1], while mental stress was the most common reason for the unwillingness to re-choose dentistry. The other reasons for students' reluctance in re-choosing dentistry were financially non-profitable nature of the profession and physically demanding nature of the profession [Figure 2].

Discussion

It is beyond doubt that the profession of dentistry has made great strides over the years. There has been tremendous expansion of the dental education in India since 1998. During the period of 16 years from 1998 to 2014, 206 new dental institutions were established in the country which accounts for an increase by 215.9%.^[3] The reports on the quality of dental education and the students' viewpoints on the learning environment in dental institutions are not very encouraging.^[7] In these circumstances, the results of this study that the majority of students were reluctant in re-choosing dentistry and would not suggest dentistry as a professional choice for their friends are not surprising. One of the

Table 1: Geographical variations in the responses given by students regarding their career choice

Question	Category	Andhra Pradesh	Telangana	Tamil Nadu	P value
Is dentistry your first professional choice	Yes	27	36	84	0.001*
	No	246	216	216	
Given an opportunity, would you re-choose dentistry	Yes	63	31	105	0.001*
	No	210	221	195	
Would you recommend dentistry as a professional choice to your friends	Yes	56	63	57	0.212
	No	217	189	243	

Chi-square test; $P \leq 0.05$ considered statistically significant; *denotes statistical significance

Table 2: Geographical variations in the responses given by students regarding the reasons for choosing dentistry

Reason	Category	Andhra Pradesh	Telangana	Tamil Nadu	P-value
Could not get an admission into medicine	Yes	207	189	196	0.008*
	No	66	63	104	
Societal recognition	Yes	42	35	56	0.29
	No	231	217	244	
Economically fruitful	Yes	16	36	29	0.005*
	No	257	216	271	
Good scope abroad	Yes	36	49	37	0.04*
	No	237	203	263	
To get a well-settled partner	Yes	59	39	19	0.001*
	No	214	213	281	

Chi-square test; $P \leq 0.05$ considered statistically significant; *denotes statistical significance

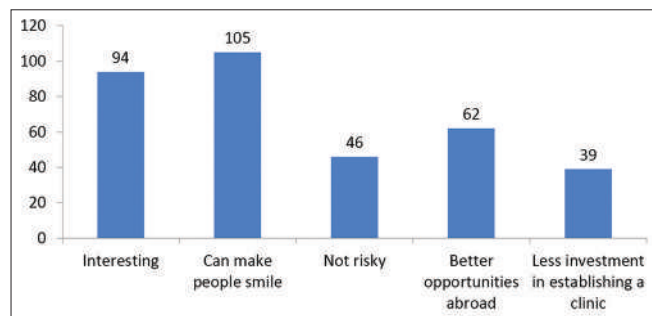


Figure 1: The reasons reported by the students for their willingness to re-choose dentistry

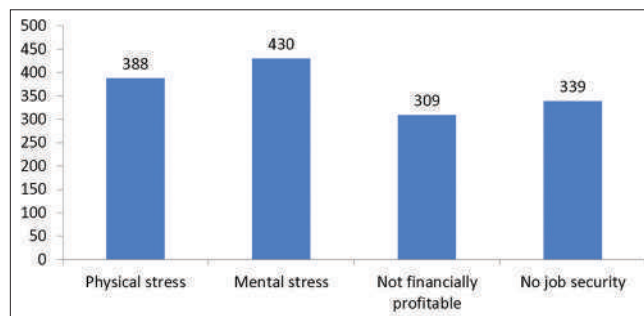


Figure 2: The reasons reported by the students for their reluctance to re-choose dentistry

important basis for the observation of geographical variation in students' preferences relating to re-choosing dentistry could be found in the cultural and attitudinal differences between the students from different states in general. Another obvious reason was dentistry being the first career choice for considerably more students from Tamil Nadu compared to the states of Telangana and Andhra Pradesh, which highlights the inherent interest among students from Tamil Nadu towards the profession that could have prompted them to respond positively about re-choosing dentistry given an opportunity. The fact that less than a quarter of the students participated in this study were willing to recommend dentistry as a career choice for their friends is reflective of the skeptical nature of students regarding their future as dental health professionals and another reason could be to avoid further competition in an already competitive environment.

This study highlights the apprehension of dental students about their future roles as oral health care professionals.

The analogy of supply-demand consideration in marketing applies in this context. In the financial market, when the supply component exceeds the demand component, the prices of the products drop. Similarly, when the available dental manpower is more than what is required to meet the demand for oral care, the regard for the profession diminishes among prospective students as the financial returns from the existing demand for oral health care have to be shared among an ever-increasing supply component.^[8] A report on the available dental manpower in Tamil Nadu by Prabu *et al.* revealed that the dentist to population ratio in Tamil Nadu was 1: 3667 while the recommended ratio by the world health organization was 1:7500.^[9] These numbers in the states of Andhra Pradesh and Telangana would not be too different. Barring the discussion on the reasons for geographical disparities in the available dental manpower between rural and urban areas, the available manpower is clearly more than what is demanded from the public.

When the students were probed about the reasons for their reluctance to re-choose dentistry, financially non-profitable nature of the profession, its physically and mentally demanding nature emerged as the major reasons. These observations highlight the expectations students have about their future career roles. It was reported in the literature that the image of oral health care professionals in some parts of the world is associated with wealth and prestige.^[10] However, the current Indian dental students cannot associate their future careers with wealth and prestige, given the circumstances. Few students who demonstrated willingness to re-choose dentistry given an opportunity opined that the relatively inexpensive nature of dental education compared to medical education suits them, and some students felt that the profession has more scope abroad. In a study conducted in British University of Egypt in 2016, 73.3% of students expressed satisfaction about their career choice as a dental professional which is in contrast with the results of the present study.^[11] Societal reputation, financially profitable nature of the profession were reported to be the main reasons for choosing dentistry by the dental students of Damascus University, Syrian Arab Republic.^[12] These findings are in accordance with the thought process of the students in the current study who demonstrated reluctance in re-choosing dentistry because of these reasons. In a study conducted by Aguiar CM *et al.* among Pernambuco dental students in Brazil, dentistry was not the first career choice for only less than 15% of the students, which is in contrast with the observations made in the present study where dentistry was the first career choice for only 18.06% of the students.^[13] Halawany SH reported dentistry as the primary career choice among Saudi dental students in 2014.^[14] Similar to this study, in a study conducted among dental students in Bhopal, 22.6% of final-year students reported choosing dentistry out of their own interest.^[15] This is a reflection for the fact that the dental students in the present study lack extensive knowledge and realistic expectations about the careers they are assuming, but follow the path of least resistance in making their career choices.

Conclusion

The study results demonstrate the declining interest and regard for dentistry among the current dental students. Though slight geographic variations were observed between the students belonging to different states in their responses regarding dentistry being the first career choice, majority of students regardless of their geographical location, were reluctant to re-choose dentistry given an opportunity. These findings raise concerns about the future of the profession in the country that produces the largest number of dental graduates per annum in the world, and must receive careful attention from all the stakeholders.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have

given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Development and psychometric evaluation of COVID-19 Psychological Burden Scale for Indian Health Care Workers

ABSTRACT

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Background: It is beyond doubt that the health care systems across the globe have been experiencing burdens of unprecedented magnitude in the coronavirus disease (COVID-19) era. However, no psychometric tools were validated in India to assess the impact of COVID-19 on the psychological well-being exclusively among health care workers, given their obvious risk for exposure. **Objectives:** This study was conducted to develop the first of its kind psychometric tool that measures the psychological burden posed by COVID-19 on Indian health care workers. **Materials and Methods:** One hundred and sixty-nine health care workers attending COVID-19 duties in four different states of India took part in the study. The initial scale designed was tested for face and content validity. Exploratory factor analysis using direct oblimin rotation with Kaiser normalization was employed to determine the factor structure. Differential item functioning (DIF) analysis with ordinal regression based on the type of COVID-19 facility at which the participant is serving, educational background was done for identification of item bias. **Results:** COVID-19 Psychological Burden Scale for Indian Health Care Workers (CPBS-IHCW), with 17 items loaded on four components, demonstrated good internal consistency reliability (Cronbach's Alpha 0.873). DIF revealed no item bias based on type of facility and educational background. Significant differences in CPBS-IHCW scores were noted between health care workers serving at different COVID-19 facilities and belonging to different educational backgrounds. **Conclusion:** CPBS-IHCW is a 17-item, rapidly administrable scale, demonstrating good internal consistency reliability, and temporal stability, which can be used in the assessment of psychological burden among health care professionals catering to the needs of the COVID-19 affected.

Keywords: Health personnel, psychological burnout, psychological stressors, psychometrics, social stigma

The World Health Organization (WHO) declared coronavirus disease (COVID-19) as a pandemic on March 11, 2020.^[1] According to the WHO situation report on June 8, 2021, more than 172 million people across the

globe tested COVID-19 positive.^[2] A huge burden has been placed by COVID-19 on the health care systems across

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the globe. In this context, health care professionals as front-line workers embraced a multitude of roles at various levels such as community awareness building, laboratory sample collection, monitoring and supervision of suspect cases, and provision of requisite care for the confirmed COVID-19-positive cases. Literature on previous outbreaks underscores the negative impacts of assuming front-line duties on the mental health and psychological well-being of health care workers.^[3-5] It is estimated that the magnitude of anxiety and stress, depression levels, and suicidal inclinations would rise as a consequence of the psychological burden posed by COVID-19.^[6] The vulnerability of health care workers in developing psychological symptoms such as insomnia, depression, and anxiety has been discussed in the context of COVID-19.^[7,8] In this fight against the unanticipated COVID-19 pandemic, there are many factors contributing to the appearance of burn-out among health care workers such as working in high-risk areas for infection, availability of suitable personal protective equipment, long work hours to meet the escalating demand, staying away from families, increasing reports on health care professionals communicating the disease, and uncertainty about the future.^[7]

Safeguarding the health of health care workers is one of the fundamental responsibilities of any health care system, which includes psychological well-being. The WHO Department of Mental Health and Substance Use developed a series of messages aimed at diverse audiences to reinforce mental health and psychological well-being in these challenging times.^[9] In order to provide requisite mental health and psychological support for health care workers in need, it is important to assess the psychological burden among the individuals. Although there are a number of available psychometric scales that measure the impact of COVID-19 on mental health,^[10-15] to the best of our knowledge, there are no validated psychometric measures intended for health care professionals to assess the psychological burden posed by COVID-19 in the Indian context. With this background, the objective of this study was to develop a scale to measure COVID-19 imposed psychological burden among Indian health care workers (IHCW), henceforth referred to as COVID-19 Psychological Burden Scale for IHCW (CPBS-IHCW), and evaluate its psychometric properties.

MATERIALS AND METHODS

This study was conducted during July and September 2020 among health care professionals attending COVID-19 duties in the Indian states of Andhra Pradesh, Telangana, Madhya Pradesh, and Odisha. Ethical approval for this study was obtained from the Institutional Review

Board of Sibar Institute of Dental Sciences (244/Sibar/IEC/2020;17/5/2020). The Government of India has categorized COVID-19 dedicated facilities into three types: COVID Care Center (CCC); Dedicated COVID Health Center (DCHC); Dedicated COVID Hospital (DCH).^[16] In addition to these COVID-19 dedicated facilities, field surveillance, field supervision, laboratory sample collection teams were instituted by the health authorities. CCC offers services only for COVID-19 suspect cases having mild and very mild symptoms (fever/upper respiratory tract infection). At DCHC, services for all moderate COVID-19 suspect cases (pneumonia with no signs of severe disease; respiratory rate 15–30 cycles/min; SpO₂ 90%–94%) shall be offered, whereas DCH is primarily for the provision of comprehensive care to severe COVID-19 suspect cases (respiratory rate \geq 30 cycles/min; SpO₂ <90% in room air).^[16] This classification of patients based on the severity of symptoms can be found in the ‘algorithm for isolation of suspect/confirmed cases of COVID-19’ proposed by the Ministry of Health and Family Welfare, Government of India.^[16] The study sample constituted of a convenience sample of 169 health care professionals, from the disciplines of modern medicine, dentistry, and nursing, who were involved in COVID-19 duties at CCCs, DCHCs, and DCHs during July and September 2020. The administrative heads at the district level were approached to gain access to the eligible participants. The scale was administered to these participants online via their registered contact numbers. All the participants provided informed consent. The background characteristics of age, gender, educational background, and qualification were documented from the study participants. For convenience, the methodology adopted in this study was discussed under the following subheadings: (i) generation of item pool; (ii) assessment of content validity and face validity; (iii) evaluation of corrected item-total correlations (ITC) and internal consistency reliability; (iv) identification of factor structure and scale purification; (v) differential item functioning (DIF) analysis for estimating item bias; (vi) Establishment of the temporal stability of the scale.

1. Generation of item pool: Thorough review of literature, regarding factors linked with health care professionals’ fear of acquiring the disease and the sources of anxiety, preceded the generation of item pool.^[7,17] Independent reviews by the investigators resulted in the drafting of initial questionnaire with 18 items in English. All the items were designed to assess the psychological burden, associated with COVID-19, among health care workers on a four-point (1–4) semantic differential scale, where the estimating facet was clearly mentioned in the choices, with higher scores representing increased psychological burden

2. The assessment of content validity and face validity: The initial scale with 18 items in English was assessed by a six-member expert panel for content validity that comprised of three psychiatrists and three members from the departments of community medicine. The authors who articulated the initial scale were not a part of the expert panel. The expert panel rated the 18 items on a four-point scale: “quite relevant;” “highly relevant;” “moderately relevant;” “poorly/not relevant.” Seventeen items in the scale were rated to be either “quite relevant” or “highly relevant” by all the panel members, and the item that received “moderately relevant” or “poorly/not relevant” response from few of the panel members was removed from the scale after consensus building. The inter-rater agreement was measured with Intraclass Correlation Coefficient (ICC) (Two-way mixed model, mean of six raters, absolute agreement).^[18] The scale level ICC was 0.89 for the 17 item scale, which was pilot tested on 10 health care workers attending COVID-19 laboratory sample collection duties to assess the face validity of the questionnaire. Two items were rephrased after conduct of the debriefing interviews as semantic discrepancy was noted with 3 members, i.e., discrepancy between what the item intends to assess and what the respondents understood from the item
3. Evaluation of corrected ITC and internal consistency reliability: Corrected ITC to assess the correlation of each of the scale items with the total scale score were used for initial scale purification.^[19] Items demonstrating a corrected ITC of <0.3 were intended to be eliminated from the scale, as values below the cut-off indicate limited association of the corresponding item to the overall construct measured by the scale.^[20] Internal consistency reliability of the scale was measured using Cronbach’s alpha
4. Identification of factor structure and scale purification: Exploratory factor analysis (EFA) was employed to determine the factor structure. Direct oblimin rotation with Kaiser normalization was done and items with significant cross loadings across components, should there be any, were intended to be removed if the difference between loadings is <0.2^[21]
5. DIF analysis for estimating item bias: DIF was done to check item equivalence.^[22] A difference in item responses across different categories of a grouping variable (e.g. COVID-19 health care facility), while controlling for the underlying construct measure is suggestive of DIF. The total score obtained on the scale was considered the conditioning variable and the “interaction term” between the grouping variable and the conditioning variable was also used in DIF analysis to assess the presence of nonuniform DIF.

A significant increase in R^2 value (>0.02) from model 1, where the conditioning variable was the only covariate, to model 3, where the grouping variable and the “interaction term” were added to model, is suggestive of DIF^[22]

6. Establishment of temporal stability of the scale: Temporal stability of the instrument was evaluated by re-administering the scale to 25 health care workers 1 week after the initial administration. ICC (Two-way mixed effects model, single rater, absolute agreement) was used as a measure for the temporal stability of the scale.

Statistical analysis

SPSS version 20 software (IBM SPSS statistics for windows version 20, Armonk, NY, USA) was used to analyze the data. The following were employed in data analysis: descriptive statistics; EFA using direct oblimin rotation with Kaiser Normalization for determination of factor structure; DIF analysis with ordinal regression based on the type of COVID-19 health care facility the participant serves in and educational background of the participant for identification of item bias; Mann–Whitney test for determining gender variation in CPBS-IHCW scores; Kruskal–Wallis ANOVA, with *post hoc* Bonferroni-adjusted Mann–Whitney tests for multiple pairwise comparisons, for identifying differences in CPBS-IHCW scores based on the type of COVID-19 health care facility, educational background, and qualification. For DIF analysis, the difference in Nagelkerke R^2 values from the three models employed in DIF analysis was used to assess the magnitude of DIF.

RESULTS

Majority of the study participants were females with master’s degree in modern medicine. The mean age

Table 1: Background characteristics of the study population (n=169)

Variable	Category	n (%)
Gender	Male	54 (31.95)
	Female	115 (68.05)
Health care facility	CCC	81 (47.9)
	DCHC	52 (30.8)
	DCH	36 (21.3)
Educational background	Modern medicine	82 (48.5)
	Dentistry	61 (36.1)
	Nursing	26 (15.4)
Educational qualification	Bachelor’s degree	57 (33.7)
	Master’s student	50 (29.6)
	Master’s degree	62 (36.7)
Age (years)	Mean (95% CI)-28.86 (27.89-29.83)	

CCC=COVID care center, DCHC=Dedicated COVID health center, DCH=Dedicated COVID hospital, CI=Confidence interval

Table 2: Item level descriptive statistics, item-total correlation, and internal consistency reliability estimates

Item	Mean (SD)	Median (IQR)	Corrected item-total correlations	Cronbach's alpha*
Item 1: How afraid are you of acquiring COVID-19 at work?	2.56 (0.83)	3 (1)	0.51	0.865
Item 2: How afraid are you that your family members may acquire COVID-19 because of you?	2.28 (0.91)	2 (1)	0.6	0.861
Item 3: How frequently are you feeling that you have acquired COVID-19?	1.81 (0.94)	2 (1)	0.55	0.863
Item 4: How concerned you are about the possibility of you propagating COVID-19 to your colleagues at workplace?	2.25 (0.87)	2 (1)	0.63	0.86
Item 5: How afraid are you that you and your family may experience discrimination from society as you are involved in COVID-19 duties?	3.2 (0.91)	3 (1)	0.49	0.866
Item 6: How anxious are you about the perceptions of the community during the isolation/self-quarantine period you will have to undergo after getting relieved from COVID-19 duties?	2.52 (0.98)	2 (1)	0.42	0.87
Item 7: How concerned are you that your involvement in COVID-19 related work may negatively affect your future professional duties?	2.22 (1.03)	2 (2)	0.49	0.866
How frequently is your sleep getting affected because of thoughts relating to COVID-19? (Item 8)	2.2 (1.01)	2 (2)	0.51	0.865
Item 9: How afraid are you of dying from COVID-19?	1.69 (0.81)	1 (1)	0.6	0.862
Item 10: How satisfied are you with the training received in preparation for COVID-19 duties?	2.14 (0.8)	2 (0)	0.51	0.865
Item 11: How confident are you in fulfilling the COVID-19 related responsibilities delegated to you?	1.69 (0.71)	2 (1)	0.52	0.865
Item 12: How assured are you about the quality and comprehensiveness of COVID-19 related evidence that you have received?	2.08 (0.74)	2 (1)	0.32	0.872
Item 13: How frequently have you been thinking of not attending COVID-19 duties delegated to you because of pressure from family members or close acquaintances?	3.02 (0.89)	3 (2)	0.35	0.872
Item 14: How assured are you about receiving necessary support from the community in case you develop COVID-19 related symptoms?	2.88 (0.8)	3 (1)	0.31	0.873
Item 15: How worried are you about the wellbeing of your family when you are fulfilling the delegated COVID-19 responsibilities?	2.27 (0.9)	2 (1)	0.61	0.861
Item 16: How apprehensive are you that the family members of an infected person may resort to violence against you or the facility in which you serve?	2.18 (0.8)	2 (1)	0.52	0.865
Item 17: How satisfied are you with the current access you have to personal protective equipment?	2.18 (0.79)	2 (1)	0.51	0.865

*Depicts internal consistency estimates of remaining items if the corresponding item is removed from the total score. SD=Standard deviation, IQR=Interquartile range

of the study sample was 28.86 ± 6.38 years. Table 1 shows the background characteristics of the health care workers who participated in this study. All the 17 items of CPBS-IHCW demonstrated corrected ITC >0.3 (0.31–0.63). Table 2 presents the corrected ITC and the Cronbach's alpha (if item deleted) values for the 17 item scale. The scale demonstrated good internal consistency reliability (Cronbach's alpha 0.873). Underlying factor structure of the scale was evident from Kaiser Meyer Olkin measure of 0.789. EFA using direct oblimin rotation with Kaiser normalization resulted in a four-component solution (eigenvalue >1). This four-component solution suggested by the "total variance explained" was confirmed by the scree plot [Figure 1]. The four components of CPBS-IHCW were identified as follows, after a series of consensus-building based on the content of items loaded on these components: "Personal vulnerability" (items 1, 3, 7, 8, 9); "workplace preparedness and safety" (items 10, 11, 12, 16, 17); "fear of societal prejudice and discrimination"

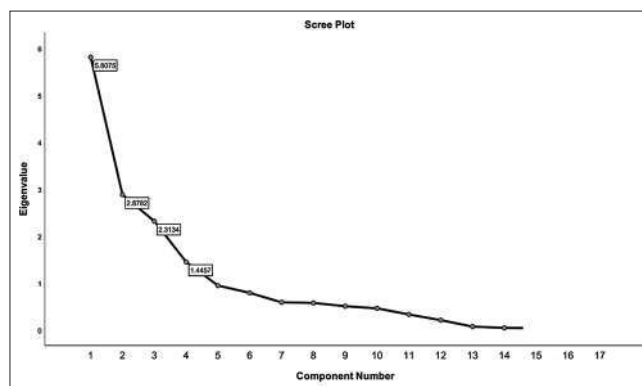


Figure 1: Scree plot

(items 5, 6, 13, 14); "family well-being" (items 2, 4, 15). The maximum possible score for CPBS-IHCW is 68, while the minimum score is 17. The component loadings, communalities, and the total variance explained by the factors in EFA are presented in Table 3. The four sub-scales of CPBS-IHCW accounted for slightly more than 73% of total variance. Item level DIF analysis,

Table 3: Pattern matrix from exploratory factor analysis showing the component loadings and communalities for the seventeen items of the CPBS-IHCW

Item	Factor 1	Factor 2	Factor 3	Factor 4	Communality
Item 1: How afraid are you of acquiring COVID-19 at work?	0.52				0.481
Item 2: How afraid are you that your family members may acquire COVID-19 because of you?				0.978	0.968
Item 3: How frequently are you feeling that you have acquired COVID-19?	0.615				0.55
Item 4: How concerned you are about the possibility of you propagating COVID-19 to your colleagues at workplace?				0.975	0.98
Item 5: How afraid are you that you and your family may experience discrimination from society as you are involved in COVID-19 duties?			0.72		0.686
Item 6: How anxious are you about the perceptions of the community during the isolation/self-quarantine period you will have to undergo after getting relieved from COVID-19 duties?			0.704		0.56
Item 7: How concerned are you that your involvement in COVID-19 related work may negatively affect your future professional duties?	0.95				0.845
Item 8: How frequently is your sleep getting affected because of thoughts relating to COVID-19?	0.94				0.86
Item 9: How afraid are you of dying from COVID-19?	0.504				0.54
Item 10: How satisfied are you with the training received in preparation for COVID-19 duties?		-0.95			0.908
Item 11: How confident are you in fulfilling the COVID-19 related responsibilities delegated to you?		-0.57			0.548
Item 12: How assured are you about the quality and comprehensiveness of COVID-19 related evidence that you have received?		-0.667			0.427
Item 13: How frequently have you been thinking of not attending COVID-19 duties delegated to you because of pressure from family members or close acquaintances?			0.87		0.715
Item 14: How assured are you about receiving necessary support from the community in case you develop COVID-19 related symptoms?			0.72		0.552
Item 15: How worried are you about the wellbeing of your family when you are fulfilling the delegated COVID-19 responsibilities?				0.974	0.971
Item 16: How apprehensive are you that the family members of an infected person may resort to violence against you or the facility in which you serve?		-0.97			0.931
Item 17: How satisfied are you with the current access you have to personal protective equipment?		-0.96			0.915
Eigen value	5.8	2.87	2.31	1.44	
Percentage of total variance explained	34.16	16.93	13.6	8.5	

KMO measure of sampling adequacy (0.789), Bartlett's test of sphericity - χ^2 (df)=3104.33 (136), $P < 0.001$, Extraction method=Principal components, Rotation=Direct oblimin rotation with Kaiser normalization, Factor 1=Perceived vulnerability, Factor 2=Work place preparedness and safety, Factor 3=Fear of societal prejudice and discrimination, Factor 4=Family well-being. df=Degree of freedom

done with ordinal regression, showed item bias for Item 11 with COVID-19 health care facility as the grouping variable [Table 4]. However, the magnitude of DIF was small (0.052). There were significant differences in CPBS-IHCW scores between participants serving at different COVID-19 health care facilities, with those participants from DCH demonstrating higher psychological burden scores compared to those serving in other types of facilities. This difference was also noted based on educational background, as the CPBS-IHCW scores of nurses were significantly higher than the participants from modern medicine and dentistry [Table 5]. An ICC (Two-way mixed effects model, single rater, absolute agreement) of 0.93 between test and re-test among 25 health care workers confirmed temporal stability of the instrument.

DISCUSSION

It is undeniable that the world would not be able to finish this COVID-19 marathon without the services of health care workers on the front line. In this process, the health care workers are exposed to numerous uncertainties, fears, and stressors. The aftermath of any epidemic would include psychological distress and social stigma.^[7,8] The world has experienced these psychological consequences previously during the time of influenza,^[23] SARS outbreak,^[24,25] and with the gradually evolved AIDS epidemic.^[26] Shanafelt *et al.* summarized the possible sources of fear and anxiety among health care workers during the COVID-19 pandemic; they have also discussed the conventional self-reliant nature of health fraternity as an important contributor to anxiety while dealing with a previously un-encountered

Table 4: Differential item functioning analysis with ordinal regression

Grouping variable	Factor	Item	Model 1 (df=1)		Model 2 (df=3)		Model 3 (df=4)		DIF R ² difference (model 3-1)
			χ ²	R ²	χ ² (df)	R ²	χ ² (df)	R ²	
COVID-19 health care facility	Factor 1	Item 1	101.4	0.49	103.61	0.502	105.15	0.507	0.017
		Item 3	140.24	0.62	147.03	0.64	147.05	0.641	0.021
		Item 7	202.86	0.75	212.18	0.768	212.82	0.769	0.019
		Item 8	215.97	0.776	230.61	0.8	234.42	0.807	0.031
	Factor 2	Item 9	111.44	0.547	115.08	0.56	115.54	0.561	0.014
		Item 10	309.61	0.937	312.31	0.94	314.39	0.942	0.005
		Item 11	113.97	0.565	120.63	0.587	129.67	0.617	0.052*
		Item 12	101.53	0.507	104.4	0.518	105.44	0.521	0.014
	Factor 3	Item 16	365.46	0.985	380.64	0.996	380.64	0.996	0.011
		Item 17	322.91	0.947	329.61	0.953	330.02	0.954	0.007
		Item 5	176.53	0.717	176.54	0.717	176.84	0.718	0.001
		Item 6	149.29	0.63	158.02	0.652	158.03	0.652	0.022
	Factor 4	Item 13	209.24	0.77	210.48	0.779	210.56	0.729	0.009
		Item 14	126.82	0.584	133.64	0.604	133.8	0.605	0.021
		Item 2	425.05	0.99	428.73	0.996	428.73	0.996	0.006
	Educational background	Factor 1	Item 4	421.91	0.918	421.91	0.918	421.91	0.918
Item 15			401.17	0.98	403.6	0.986	406.37	0.987	0.007
Item 1			101.4	0.49	105.45	0.508	105.64	0.509	0.019
Factor 2		Item 3	140.24	0.62	140.66	0.623	140.78	0.624	0.004
		Item 7	202.86	0.75	203.7	0.752	204.19	0.753	0.003
		Item 8	215.97	0.776	218.36	0.78	219.97	0.783	0.007
		Item 9	111.44	0.547	111.74	0.548	116.3	0.563	0.016
Factor 3		Item 10	309.61	0.937	312.9	0.94	312.93	0.941	0.004
		Item 11	113.97	0.56	124.91	0.6	133.97	0.63	0.07
		Item 12	101.53	0.507	101.83	0.508	102.09	0.509	0.002
		Item 16	365.46	0.985	373.4	0.991	373.4	0.991	0.006
Factor 4		Item 17	322.91	0.947	327.29	0.951	327.88	0.952	0.005
		Item 5	176.53	0.717	177.4	0.719	177.45	0.719	0.002
		Item 6	149.29	0.63	151.45	0.635	153.52	0.641	0.011
		Item 13	209.24	0.77	218.98	0.794	220.38	0.797	0.027
Factor 4	Item 14	126.82	0.584	126.9	0.584	128.53	0.589	0.005	
	Item 2	425.05	0.99	427.61	0.996	427.61	0.996	0.006	
	Item 4	421.91	0.918	421.94	0.918	421.99	0.918	0.008	
		Item 15	401.17	0.98	403.48	0.986	406.51	0.987	0.007

*Presence of DIF, total score obtained for the items within a factor is considered the conditioning variable in all the models, Model 1 - Model with the item score as dependent variable and conditioning variable as the covariate; Model 2 - Model with item score as dependent variable, conditioning variable as the covariate, and grouping variable as the uniform term, Model 3 - Model with item score as dependent variable, conditioning variable as the covariate, grouping variable as the uniform term, and interaction term (Grouping variable × conditioning variable) as the nonuniform term. DIF=Differential item functioning, χ²=Chi-squared value, df=Degrees of freedom, R²=Nagelkerke's R-squared (coefficient of determination) value

Table 5: Differences in CPBS-IHCW scores based on the background characteristics

Variable	Category	n	Median (IQR)	Mean rank	χ ² (df, n)	P	P (post hoc tests) ¹		
							CCC-DCHC	CCC-DCH	DCHC-DCH
COVID-19 health care facility	CCC	81	39 (12)	79.54	13.18 (2, 169)	0.002*	0.564	0.001*	0.002*
	DCHC	52	36 (15)	75.49					
	DCH	36	45 (9)	111.01					
Educational background	Modern medicine	82	40 (13)	87.08	6.99 (2, 169)	0.03*	0.11	0.113	0.01*
	Dentistry	61	37 (11)	74.18					
	Nursing	26	46 (10)	103.8					
Educational qualification	Bachelor's degree/Diploma	57	37 (14)	84.12	0.63 (2, 169)	0.72	0.79	0.62	0.43
	Master's student	50	39 (14)	81.43					
	Master's degree	62	39 (11)	88.69					

Kruskal-Wallis ANOVA test, P ≤ 0.05 considered statistically significant, ¹Post hoc tests were done using Mann-Whitney U-tests with Bonferroni adjustment (P ≤ 0.016 considered statistically significant) for multiple pairwise comparisons. IQR=Interquartile range, CCC=COVID care center, DCHC=Dedicated COVID health center; DCH=Dedicated COVID hospital



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disease which is outside the professionals' area of clinical expertise.^[8] A validated scale to assess the psychological burden among health care professionals offering COVID-19 services goes a long way in supporting these professionals from the mental health and psychological end. In light of lack of psychometric tools to assess COVID-19 imposed psychological burden among health care workers, CPBS-IHCW offers a rapidly administrable and valid solution. To the best of our knowledge, this is the first COVID-19 psychometric instrument developed to assess the psychological burden among Indian health care professionals.

CPBS-IHCW showed good internal consistency reliability with Cronbach's alpha of 0.87, well above the cut-off suggested in literature.^[27] It is a rapidly administrable 17-item tool, using a semantic differential scale, which was considered over Likert scale to eliminate the potential acquiescence bias.^[28] The evaluative dimension is explicitly stated on a semantic differential scale rather than asking the subject to respond in affirmative or negative to a directive statement. EFA produced a four-factor solution reflecting the multidimensional structure of CPBS-IHCW. The correlation matrix between CPBS-IHCW and its four subscales confirmed that all the subscales are specific with regard to the measurement of the underlying construct of psychological burden; with moderate inter-sub scale correlation coefficients. The negative component loadings for items in "work place preparedness and safety" component were because the items were phrased in a reverse manner, that strong disagreement to these items increases the CPBS-IHCW scores, while it is the opposite for items loaded on the other three components. DIF analysis confirmed that there is no item bias in CPBS-IHCW, with no differences in item responses based on the type of COVID-19 health care facility and educational background, except for Item 11, the magnitude of DIF for which, however, is very small. As DIF assumes unidimensionality, DIF analysis was done separately for items loaded on the individual factors.^[29] It was identified that the health care workers serving in DCH had higher CPBS-IHCW scores which could be due to the more comprehensive and demanding nature of medical care provision for severe COVID-19 cases. This finding could be understood as provisional evidence for the construct validity of CPBS-IHCW. Nurses demonstrated higher CPBS-IHCW scores compared to health professionals from modern medicine and dentistry, the rationale for which, in the Indian context, could be the discrepancy between the level of information nurses have with regard to COVID-19 and the nature of duties they assume which demand closely working with infected persons. A study by Tan *et al.* among health care workers in Singapore in relation to the psychological impact posed by COVID-19 reported

anxiety among 14.5% and depression among 8.9% of the study sample.^[30] In the present study, no attempt was made to categorize participants based on CPBS-IHCW. Though there is limited consensus available on the recommended sample size for EFA, larger samples are conventionally advised. The sample size considered in this study is in accordance with the recommended subjects to item ratio of 30:1 in EFA.^[31] Smaller subject to item ratios of 6:1 were recommended and used in psychometric research.^[32,33] It is also noteworthy that psychological aspects are hugely culture-specific and no single instrument can be universally valid. Culture-specific, contextual instruments tested for psychometric properties are the need of the hour owing to immense variations in the work environments, cultural differences, health-related attitudes, and beliefs between health care workers from different countries across the globe. The results of this study need to be assimilated in light of the convenience sampling technique adopted in this study and the unequal gender distribution in the study sample.

CONCLUSION

Based on the results of this study, we conclude that CPBS-IHCW is a valid and reliable measure to evaluate psychological burden among health care workers operating at various levels, which is of great value in the assessment of psychological burden among health care professionals catering to the needs of the COVID-19 affected.

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Conflicts of interest

There are no conflicts of interest.

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COVID-19 Psychological Burden Scale for Indian Health Care Workers (CPBS-IHCW)

1. How afraid are you of acquiring COVID-19 at work?
Extremely afraid ○ ○ ○ ○ Not at all afraid
2. How afraid are you that your family members may acquire COVID-19 because of you?
Extremely afraid ○ ○ ○ ○ Not at all afraid
3. How frequently are you feeling that you have acquired COVID-19?
Almost always ○ ○ ○ ○ Never
4. How concerned you are about the possibility of you propagating COVID-19 to your colleagues at workplace?
Extremely concerned ○ ○ ○ ○ Not at all concerned
5. How afraid are you that you and your family may experience discrimination from society as you are involved in COVID-19 duties?
Extremely afraid ○ ○ ○ ○ Not at all afraid
6. How anxious are you about the perceptions of the community during the isolation/self-quarantine period you will have to undergo after getting relieved from COVID-19 duties?
Extremely anxious ○ ○ ○ ○ Not at all anxious
7. How concerned are you that your involvement in COVID-19 related work may negatively affect your future professional duties?
Extremely concerned ○ ○ ○ ○ Not at all concerned
8. How frequently is your sleep getting affected because of thoughts relating to COVID-19?
Almost always ○ ○ ○ ○ Never
9. How afraid are you of dying from COVID-19?
Extremely afraid ○ ○ ○ ○ Not at all afraid
10. How satisfied are you with the training received in preparation for COVID-19 duties?
Extremely satisfied ○ ○ ○ ○ Not at all satisfied
11. How confident are you in fulfilling the COVID-19 related responsibilities delegated to you?
Extremely confident ○ ○ ○ ○ Not at all confident
12. How assured are you about the quality and comprehensiveness of COVID-19 related evidence that you have received?
Very much assured ○ ○ ○ ○ Not at all assured
13. How frequently have you been thinking of not attending COVID-19 duties delegated to you because of pressure from family members or close acquaintances?
Almost always ○ ○ ○ ○ Never
14. How assured are you about receiving necessary support from the community in case you develop COVID-19 related symptoms?
Very much assured ○ ○ ○ ○ Not at all assured
15. How worried are you about the wellbeing of your family when you are fulfilling the delegated COVID-19 responsibilities?
Extremely worried ○ ○ ○ ○ Not at all worried
16. How apprehensive are you that the family members of an infected person may resort to violence against you or the facility in which you serve?
Extremely apprehensive ○ ○ ○ ○ Not at all apprehensive
17. How satisfied are you with the current access you have to personal protective equipment?
Extremely satisfied ○ ○ ○ ○ Not at all satisfied

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Tobacco Related Oral Lesions in South Indian Industrial Workers

Abstract

Introduction: Tobacco is the leading causative factor for both oral potentially malignant disorders and oral cancer. Tobacco use is higher among lower income population. Low-income population of India are majorly employed as industrial workers. The aim of this study is to estimate the prevalence of oral lesions associated with tobacco related habits among industrial workers. **Materials and Methods:** Cross-sectional epidemiological investigation was conducted among 1000 industrial workers using simple random sampling technique. Information on patient demographics, tobacco related (smoke and smokeless) and other deleterious habits, and clinical examination details were recorded in a structured format. The data were analyzed using Statistical Package for Social Sciences version 20.0. Results were tabulated using frequency distribution and mean with a standard deviation. Multiple logistical regression was used to analyze oral lesions by different variables. **Results:** Among the 1000 industrial workers screened, smoking habit was observed in 13.20%, while 86.8% were using smokeless tobacco. The prevalence of tobacco related oral lesions among individuals with smoke/smokeless tobacco habit was 13.8%. The study documented tobacco related oral lesions such as leukoplakia (6.5%), oral submucous fibrosis (2%), smoker's palate (2.7%), tobacco related pigmentation (1.9%), erythroplakia (0.3%), and oral squamous cell carcinoma (0.2%). **Conclusion:** The study documented potentially malignant disorders and oral cancer among users with tobacco related habits. The results also revealed that higher prevalence of potentially malignant disorders over oral cancer. Thus, preventive programs for early detection of oral precancer and oral cancer such as tobacco cessation, tobacco counselling programs are emphasized for industrial workers.

Keywords: *Epidemiology, occupation, oral lesions, tobacco, smoke, smokeless*

Introduction

Tobacco is considered a common risk factor for major noncommunicable diseases (NCDs). WHO stated that tobacco use is a major risk factor for NCDs, such as cardiovascular disease, cancer, chronic respiratory disease, and diabetes. Majority of tobacco consumers (particularly smokers) begin their habits during their early teen age. WHO predicted that tobacco users among younger individuals may lead to deaths of 250 million children and young people alive today. Majority of such populations are identified from the developing countries.^[1] WHO estimated in 2004 that 194 million males and 45 million females from India are consuming tobacco products to chew or smoke.^[2] In addition, WHO mentioned that India will observe the fastest death rate due to tobacco related conditions; however, this notation to death

reflects to NCDs and not necessarily to cancer related death. India has the highest number of oral cancer cases in the world due to the use of smokeless tobacco. Smokeless tobacco increases the risk of oral cancer development by sixfold.^[3] Oral cancer contributes to 30% of all cancer cases in India. Evidence-based reports mentioned that India has the highest prevalence of tobacco chewing (40%) and smoking (20%) population. Beedi smoking is a common type of smoking practice in India. Higher prevalence of smokeless tobacco users may be due to low cost, and often it is harder to recognize the habit of an individual by a member of their family, thus the habit of smokeless tobacco consumption remains unknown to family members.^[4]

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Another justification for higher tobacco consumption in India may be attributed to Indian tradition which permits an individual to consume tobacco and areca nuts in different forms.^[5] A National Sample Survey from India stated that the prevalence of alcohol use is 4.5%, smoking tobacco is 16.2%, and chewing tobacco (smokeless tobacco) is 14%. The report also stated that tobacco related practices are common among males than females and rural than urban population.^[6]

Tobacco consumption is a harmful habit with the increased risk of oral potentially malignant disorders (OPMDs) and oral cancer. The changes in the oral mucosa may just begin as change in surface color or texture. Thus, lesions receive lesser attention by the tobacco consumer due to innocent appearing clinical presentation and are often neglected for early detection and care. Tobacco smoking is also a risk factor for periodontal disease and due to lack of awareness of routine dental examination among Indians, the cases with periodontal diseases are undiagnosed at an early stage. Various types of tobacco related products popular across Indian states are listed in Table 1. Although anti-smoking campaigns and mass media communication on patient education in newspapers, television, and radios are available, awareness on tobacco related practices remains an ongoing challenge in making an effective change. The challenges may be due to economic status, illiteracy, religious practices, psycho-social, peer and celebrity member influence, emotional, and family burden.^[5,7] Peer and celebrity member influence begins as a fashion smoking practice that may eventually turn into serious habit.

OPMD is a recent term used for referring to precancer, precursor, or premalignant lesion of oral cavity.^[8] The term OPMDs was coined with the notation that all lesions and conditions listed under this category are associated with increased potential for malignant transformation.^[9]

Table 1: Various types of tobacco related products identified across India^[7]

Region in India	Tobacco related products
Madhya Pradesh	Gutka
Gujrat	Gutka, Mawa, Bajjar
Arunachal Pradesh	Gutka, Gul
Bihar and Jharkhand	Khaini
Eastern India	Pan masala
Goa	Mishri, creamy stuff
Uttar Pradesh and Uttarakhand	Gul, Gudakhu
Manipur	Pan with tobacco, Hidakphu
Maharashtra	Mishri
Mizoram	Tuibur
Almost all regions of India	Smokeless tobacco or chewing tobacco, lal dant manjan

Existing literature on the frequency of tobacco related habits and oral lesions associated with tobacco related habits majorly focuses on youth/students, medical/dental students, loom workers, and tobacco workers. As mentioned earlier, two top reasons for the communication gap in attaining effective change from the anti-smoking campaigns are economic status and illiteracy.^[10] The authors of this study believe that the target population on low economic status and illiteracy may be identified in industrial workers. In addition, the number of industrial sectors in India is continuously rising in the recent decade.^[11] Hence, the data on tobacco related habits and oral lesions associated with tobacco habits will be useful for policy making decisions. Considering the importance of morbidity, quality of life, and influence on mortality, the study preferred to investigate the oral lesions related to tobacco habits linked to higher morbidity, negative impact on quality of life, and mortality [Table 2].^[12] Thus, the present study attempted to identify the prevalence of tobacco related habits and oral lesions associated with tobacco related habits among industrial workers in Andhra Pradesh, South India.

Materials and Methods

The current study is a cross-sectional epidemiological investigation conducted in industrial workers in Andhra Pradesh, South India from March 2018 to August 2018. The study was approved (protocol no. 128/IEC/ SIBAR/ 2018) by Institutional Ethics committee SIBAR Institute of Dental Sciences, Guntur on 6th Jan. 2018. Based on the industrial workers population in the study region, the sample size was estimated as 900 individuals with a 95% accuracy rate, a 95% confidence interval, and marginal error of 2%. Ten percentage oversampling with an additional approximation of 10 individuals was planned; hence, a total of 1000 individuals were recruited for the study.

Table 2: Tobacco related oral lesions categorized based on the level of morbidity impacting quality of life and mortality

Highly like to be associated with higher morbidity, negatively impact quality of life, and mortality	Highly likely to be associated with lesser morbidity, impact quality of life, and mortality
Oral squamous cell carcinoma/oral cancer	Discoloration of teeth, restorations, or denture
Oral leukoplakia	Periodontal disease
Erythroplakia	Reduced ability to taste or smell
Oral submucous fibrosis	Coated or hairy tongue
Smoker’s palate	Oral candidiasis
	Dental caries
	Increased failure rates for dental implants
	Smoker’s melanosis/pigmentation

The main aim of the study was to focus on tobacco related OPMDs; however, nontobacco related OPMDs may be encountered during data collection. Hence, for the convenience of the study, OPMDs were classified into the following sections:

- (1) Tobacco related OPMDs – Oral leukoplakia, erythroplakia, palatal changes with reverse smoking, oral submucous fibrosis
- (2) Nontobacco related OPMDs – Oral lichen planus, oral lichenoid lesions, graft versus host disease, discoid lupus erythematosus, epidermolysis bullosa, dyskeratosis congenita, actinic Cheilitis.

Study criteria

Sectional criteria of the study:

Inclusion criteria

- Individuals of 28 years and above
- Have signed informed consent form
- Individuals available on the day of screening

Exclusion criteria

Individuals who had acute and painful dental problems

Questionnaire focused on demographic details, tobacco related habit, and alcohol history was collected. The clinical examination was performed by a calibrated examiner with oral disease background. The lymph nodes were examined for all the participating individuals during extraoral examination. Basic dental examination instruments were used to conduct oral cavity examination. The individuals with clinically evident oral mucosal changes were subjected for toluidine blue and acetic acid staining for detection of OPMDs. The clinical information was computerized using Microsoft Excel documents and later subjected for statistical analysis using Statistical Package for Social Sciences version 20.0, Armonk, NY, IBM Corp.

Statistical analysis

The continuous variables in the data were mentioned as number, standard deviation, mean values, and proportions as percentages. The chi-square test was employed to assess the difference between the groups. Student *t* test was used to identify the mean exposure of tobacco habits. Multiple logistic regressions were used to analyze the oral lesions by different variables.

Results

The present study was conducted using a cross-sectional design with a WHO proforma for recording oral mucosal lesions and tobacco related habits among industrial workers in Guntur city. A total of 1000 workers from various industries who satisfied the exclusion and inclusion criteria of the study were individuals of 28 years and above and

individuals available on the day of screening. The study consisted of 53.9% of males and 46.1% of females. Majority of study participants were identified from 28 to 38 years of age intervals. The minimum number of participants was in the age group from 59 to 68 years. The mean age of the study population was 34.95 ± 11.97 years. In this study, 86.8% of individuals were chewing various forms of tobacco, whereas 13.2% were tobacco smokers. Gender distribution of tobacco smokers and smokeless tobacco users is given in Table 3. Age distribution of study participants of various forms of tobacco use showed statistical significance with $P \leq 0.001$. Age distribution of study participants based on various forms of smokeless tobacco practice showed higher levels of consumption of *khaini* than other forms of smokeless tobacco habits. Comparison of age distribution of study participants based on smokeless tobacco users and tobacco smoker showed both habits were prevalent from 28 to 38 years age group. Age- and gender-based distribution of study participants with and without tobacco related oral lesions is presented in Table 4. Both smokeless tobacco and tobacco users are highly prevalent in the age group from 28 to 38 years. Distribution of tobacco related oral lesions in various anatomic locations of oral cavity on both genders showed prevalence in the following order: buccal mucosa, labial mucosa, vestibule, corner of mouth, and palate. Buccal mucosa is the most frequently involved site for tobacco related oral mucosal lesions in both males and females. Distribution of tobacco related oral lesions (OPMDs) based on the age is presented in Figure 1(a).

Table 3: The frequency of smoke and smokeless tobacco users by gender

Gender	Smokeless tobacco	Smoking tobacco	<i>P</i> value
Male	408 (75.70%)	131 (24.30%)	0.00001*
Female	460 (99.78%)	1 (0.22%)	
Total	868 (86.80%)	132 (13.21%)	

* $P < 0.05$ is statistically significant.

Table 4: Age and gender distribution of tobacco related oral lesions among study participants

Age (years)	With tobacco related oral lesions	Without tobacco related oral lesions	Gender	With tobacco related oral lesions	Without tobacco related oral lesions
28–38	102 (10.2%)	712 (71.2%)	Males	138 (13.8%)	401 (40.1%)
39–48	14 (1.4%)	103 (10.3%)	Females	12 (1.2%)	449 (44.9%)
49–58	7 (0.7%)	87 (8.7%)	Total	150 (15.0%)	850 (85.0%)
59–69	4 (0.4%)	21(2.1%)			

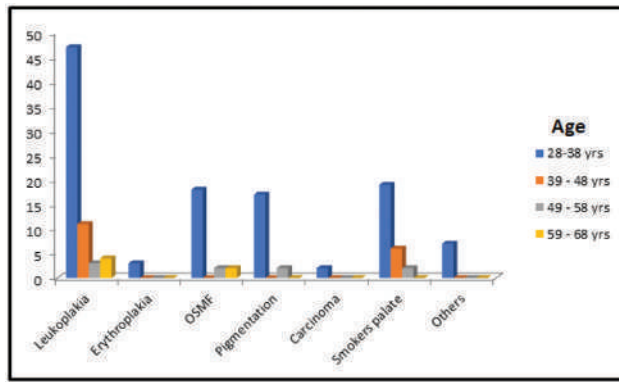


Figure 1: (a) Distribution of tobacco related oral lesions in relation to age

Oral leukoplakia, erythroplakia, and oral submucous fibrosis are more frequently seen in the age group from 28 to 38 years. Gender distribution of tobacco related oral lesions showed predilection for males [Figure 1b].

Discussion

The study observed 53.90% males and 46.10% females. Our interpretation was similar to findings of Gupta and Ray,^[12] who found the maximum number of cases to be males (61.20%) than females (39.80%). The mean age of the present study was 27.95 ± 11.97 years, which ranges from 15 to 69 years. Most of the study population were from 20 to 29 years. Similarly, an Indian study reported in 2008 observed higher predilection in the age group from 20 to 30 years.^[13]

The current study documented 868 (86.80%) individuals with smokeless tobacco habits and tobacco smoking habit in 132 (13.21%) individuals. The study observed a similar prevalence rate of tobacco habit with a report from Goa, India.^[14] The current study reported tobacco smoking as 13.21%, and Goa study stated 15%. Another study from Andhra Pradesh reported smoking prevalence as 67%.^[15] The variation in the prevalence within the same geographic area should be attributed to industrial workers who probably may prefer the type of tobacco habit based on affordability. The cost range for smokeless tobacco may be way cheaper than smoking form of tobacco.^[15] Interestingly, consumption of smokeless tobacco was predominantly observed in females (99.78%) than males (75.70%).^[16] Similar findings were reported by Sankaranarayan *et al.*^[17] in 1989. Higher prevalence of smokeless tobacco among females may be related to social barriers for smoking form of tobacco among the women. Our qualitative interpretation on this item is that smokeless tobacco allows an individual to be pouched or snuffed in the oral vestibule and may go unnoticed by fellow work partners or family members. A study stated that the prevalence of OPMDs was higher among individuals with higher lifetime consumption of smokeless tobacco such as Pan Parag, Gutka, and Mawa.^[17] Our study reported that cigarettes are the most common form of smoking tobacco product, followed by cigarettes without

filter. Similar observation was made in the study conducted by Chandra and Govindraj^[18] in 2012.

The study observed oral leukoplakia, oral submucous fibrosis, and oral cancer to be more prevalent among the age group from 20 to 29 years (36.22%), which can be attributed as early consequences of tobacco related habits. Similar observation was observed in the study conducted by Mehrotra *et al.*^[19] in 2010. This study reinforces the concepts on association of tobacco products with OPMDs through the available data. A literature mentioned that any form of tobacco products may be associated with the occurrence of potentially malignant lesions.^[20] Leukoplakia is more commonly seen among males followed by oral submucous fibrosis. A less number of oral mucosal lesions were observed in females. This could be due to the fact that tobacco smoking is more commonly associated with oral mucosal lesions than smokeless tobacco. Smokeless tobacco is usually associated with oral submucous fibrosis and no other forms of oral mucosal lesions. In addition, traditionally males are more known to be affected by workplace related stress, financial burden, and psychological trauma due to lack of life partner or family issues. This study also supports the view that smokeless tobacco (Pan Parag, Mawa, and pan masala) is the risk factor for causing potentially malignant disorders and cancer.^[21,22]

Recommendations on social accountability from the findings of the study

Based on the interpretation of results, the authors of the study recommend the following for the policy making individuals: (1) Educational videos on self-screening for oral precancer and cancer, (2) workplace restriction on tobacco related products, (3) facilitation of outreach services of oral diseases by the management of industries, (4) collaboration of dental schools with industries for periodic oral cancer screening, (5) tobacco cessation and counselling programs among industry workers, (6) educating family members on the impact of tobacco related habits and oral health, overall health, and quality of life, (7) knowledge on the hospital costs involved in the late or terminal stages of oral cancer, (8) oral precancer or cancer month programs in industries, and (9) targeting the schools where children of industrial workers usually obtain their education for early education on tobacco related health and social hazards.

Conclusion

Leukoplakia, oral submucous fibrosis, and oral cancer are early consequences of tobacco related habits, whereas pigmentation and smoker's melanosis are late consequences. The study documented higher prevalence of oral lesions related to tobacco habits among industrial workers. The study emphasizes the importance of special preventive programs targeting industrial workers including self-screening for oral precancer and cancer, tobacco cessation, counselling programs,

and early detection of oral cancer. Workplace restriction for smoking or smokeless tobacco, educating family members on tobacco related oral lesions and its impact on health, and overall health and lifestyle may also play a role in bringing forward a societal change.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Prosthetic Rehabilitation of A Post-Covid Mucormycosis Maxillectomy Defect Using A Fused Two-Piece Hollow Obturator: A Fabrication Technique

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Abstract

Purpose: Intraoral defects in the maxilla cause communication with the nasopharyngeal complex. The surgically removed palate can have a devastating effect on the appearance and speech of the patient. Obturators allow patients to eat and drink without any suspicion of food entering the oroantral cavities/ pharynx during mastication. This clinical report describes fabrication of a closed hollow bulb obturator using the two-piece double flask technique.

Method: After the final teeth arrangement was completed, teeth over the defect were removed and separately processed using heat cure acrylic resin. Both the segments are combined by autopolymerizing acrylic resin to form a single hollow-bodied obturator.

Conclusion: This technique uses a double flasking method of the two segments to control the thickness of the bulb in the defect area, thus decreasing the weight of the obturator.

Keywords: Maxillary defects, Obturators, Hollow bulb, Post-Covid Mucormycosis

1. Introduction

Resection of maxilla due to tumors or any infection leads to facial disfigurement, impaired functions like speech due to hypernasality, rumination, and a significant impact on patients quality of life. Post COVID-19 sepsis is that which occurs after SARS- CoV-2 had a rampage in the human body. It leads to ciliary dysfunction, cytokine storm, thrombo-inflammation, microvascular coagulation, and eventual immune exhaustion. This cascade of events facilitates secondary bacterial and fungal infections, especially in critically ill patients subjected to invasive emergency procedures, mechanical ventilation, prolonged hospital stays, and breaches in asepsis. Further, corticosteroid treatment in these highly susceptible hosts and high fungal spore counts in the environment creates the perfect setting for mold infections. ⁽¹⁾

The infection begins in the nose and paranasal sinuses due to the inhalation of fungal spores. ^(2, 3) This infection can spread to the orbital and intracranial structures by direct invasion or through the blood vessels. ^(4, 5) The fungus invades the arteries leading to thrombosis that subsequently causes necrosis of hard and soft tissues. Early diagnosis and treatment can reduce the mortality and morbidity of this lethal fungal infection. Treatment principles may include antifungal agents along with surgical debridement or resection.

This article discusses an innovative fabrication technique of obturator to treat an acquired maxillary defect due to post Covid-19 mucormycosis. Management of such maxillary defects when surgical resection was performed requires a multidisciplinary approach. Prosthodontic rehabilitation is the most practical, convenient, and cost-effective treatment mode and the added advantage of removing prosthesis and evaluating the infected site efficiently.

2. Case Report

A 65-year-old male patient reported to the Department of Prosthodontics with a chief complaint of difficulty in consumption of food and speech discrepancies. Intraoral examination revealed ~~large maxillary defect~~ on the left side with oroantral communication and partially edentulous arch. (Fig 1)



Fig. 1) Pre-operative intra oral view

On past medical history, it was found that the patient underwent maxillectomy for post Covid-19 necrosis of maxilla due to mucormycosis six months back. The treatment plan was to fabricate a hollow bulb obturator using a two-piece double flask technique.

3. Treatment

A linear design for a Class-IV defect was selected for this case according to design principles described by Aramany in 1978 in which remaining palatal tissues provided the support and retention was achieved from the ball end clasps and C clasps made on the remaining intact dentition. ⁽⁶⁾

Fabrication of the Obturator

A primary impression was made with irreversible hydrocolloid impression material (Fig 2 a) using a stock tray. After obtaining the primary cast, (Fig 2 b) a special tray was fabricated with self-cure acrylic resin.



Fig. 2 a) Primary impression with irreversible hydrocolloid



Fig. 2 b) Primary cast

Border moulding was done (Fig 3a) with greenstick material and the full extent of the defect was recorded using an impression compound. 1mm impression compound was scraped, and a light body secondary impression was made to record the undercuts which aid in anatomic retention. A final pick-up impression was made with alginate to record existing dentate segment (Fig 3 b). The master cast was obtained with type IV gypsum (Fig 3 c).



Fig. 3 a) Border moulded impression & the extended depth of defect recorded

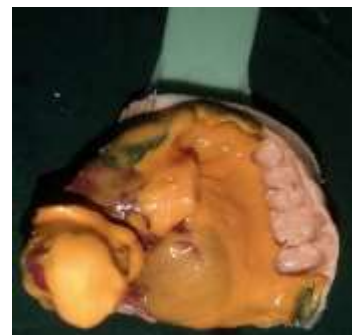


Fig. 3 b) Secondary pick up



Fig. 3 c) Secondary cast of maxilla

A temporary denture base with occlusal rim was fabricated on the master cast. Bite registration was done. Maxillary and mandibular casts were mounted on the semi-adjustable articulator (Hanau wide vue). Teeth arrangement and try-in were done. Occlusion, aesthetics, and phonetics were evaluated and found to be acceptable by the patient. A C-clasp on canine and molar as well as ball-ended clasps in the embrasures between canine and premolars were given (Fig 4). The final wax-up was done.



Fig 4) Final wax up & clasps placed for retention

Procedure for Two-Piece Hollow Obturator

The teeth overlying the defect area were cut (Fig 5), and the defect area was hollowed out. The hollow space was filled with putty material (Fig 6a), over which a segment with the posterior teeth was fabricated (Fig 6b).



Fig 5) Teeth portion over the defect area removed



Fig 6 a) Putty packed into the defect Fig. 6 b) Second segment fabricated

Orientation grooves were placed to prevent the misfit of the two pieces after fabrication and were re-checked for occlusion on the articulator. Both the segments were flaked and processed separately. (Fig 7, 8)



Fig.7) Flasking of the two segments separately



Fig 8) Mould spaces created

After retrieval, the segments were approximated with sticky wax before the obturator was removed from the cast (Fig 9a). Occlusion was checked by remounting the cast and the approximated segment onto the articulator (Fig 9b).



Fig 9 a) Approximated segments & stabilized using sticky wax



Fig. 9 b) Occlusion is checked after remounting

The necessary corrections were made, and finally, the two segments were adhered using auto polymerizing clear acrylic resin. After final finishing and polishing, the prosthesis was weighed 29 grams (Fig 10 a, b). Insertion of the prosthesis was done (Fig 11 a, b) and post-insertion instructions were given.



Fig. 10 a)



Fig. 10 b)

Final Prosthesis



Fig. 11 a) Intra Oral View



Fig. 11 b) Frontal View

Post-Operative View

4. Discussion

The primary goal of a prosthodontist is to rehabilitate a maxillary defect by closing the oronasal communication with a retentive and a stable seal using an obturator. Extension of the bulb into the maxillary defect is advantageous to provide resonance during the speech. The fabrication of a hollow bulb obturator in patients with severe maxillary defects can provide retention and stability by decreasing the weight. There are many techniques for fabricating hollow bulb obturators by using sugar, salt, or alum in the defect area while packing. Matalon and LaFuente⁽⁷⁾ used sugar during the processing of the obturator, which was removed by drilling a hole. Iramaneerat *et al.*⁽⁸⁾ used the technique of injecting argon gas into the obturator bulb. Buzayan *et al.*⁽⁹⁾ used a rigid thermoforming splint to fabricate a hollow bulb obturator. Asher *et al.*⁽¹⁰⁾ used plaster index as a matrix to fabricate a hollow obturator.

El Mahdy *et al.*⁽¹¹⁾ described the two-flask technique to process the obturator and the tooth portion separately. Mc Andrew *et al.*⁽¹²⁾ fabricated the prosthesis in two halves and sealed them using autopolymerizing resin. Few authors suggested the use of acrylic resin shim and polyurethane foam while packing.⁽¹³⁾ Use of attachment for hollow bulb obturators, though advantageous, the cost of treatment would be high.⁽¹⁴⁾

5. Conclusion

Fabrication of a two-piece obturator using the double flasking technique is an easy and conventional method. Hollowing of the obturators not only reduce the weight but also adds resonance to speech. The defect was closed, and a satisfactory esthetics, phonation and function was achieved. Prosthetic rehabilitation will improve the patient's quality of life and confidence.

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New Classification System for Cleft Alveolus: A Computed Tomography-based Appraisal

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ABSTRACT

Aim: The present study proposed a new classification system based on computed tomography (CT) scan appraisal; this enables the surgeon to identify the extent of the defect and helps to execute the proper treatment plan.

Background: Various terminologies and classifications were proposed to understand developmental defects. But none of the existing classifications/nomenclatures used the preoperative radiographic evaluation (i.e., computed tomography scan—CT scan) in the management and prognosis. Various treatments were advocated and practiced successfully for the surgical correction of lip and palate.

Materials and methods: The available CT scans from archives of the Department of Radiology and Oral and Dental Surgery were evaluated (retrospectively) for cleft alveolus and its morphology as per the proposal. The English language literature was searched in the MEDLINE database without date restriction to revise existing literature on numerous classification systems/nomenclatures using MeSH keywords related to cleft lip, palate, alveolus, developmental disturbance, facial clefts, and classification. Existing classification systems were revisited with a note on the drawbacks. After careful examination of morphological patterns of all clefts, the new CT scan-based alveolar cleft classification is proposed depending on the extent of cleft.

Results: The literature revealed a total of twenty-nine classifications of cleft lip and palate starting from the year 1922 to the year 2015, but none exclusively classified the cleft alveolus based on CT scan observations. The observation of three thousand CT scans showed five types of cleft alveolus, depending on the extent of involvement.

Conclusion: The CT scan-based classification is essential to the surgeon for successful surgical planning of cleft alveolus. The proposed classification is clinically relevant in this digital era for relating surgical outcomes. The three-dimensional viewing of a defect is essential for the surgeon for virtual planning. This paper provides a CT scan-based classification for universal acceptance in this era of digital technology, and CT scan aids in achieving these goals.

Clinical significance: The new proposal is based on preoperative evaluation of cleft using a CT scan. CT scan imaging provides a clear picture of the cleft in three dimensions for the operating surgeon. Advanced technology-enhanced surgical management modalities like CAD/CAM guided templates to support graft for successful management. The classification system will help the medical and surgical fraternity in various aspects. The three-dimensional modeling of defect and printing of a defect model using additive manufacturing technology helps the surgeon for presurgical visualization and virtual planning in a better way. This strategy of defect classification using a CT scan will help obtain better clinical outcomes and patient satisfaction.

Keywords: Alveolus, Anomaly, Cleft, Embryology, Nomenclature, Repair.

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INTRODUCTION

Various classification systems were proposed from the beginning of the era.¹ Many surgeons and clinicians came out with different classification systems. Time immemorial, the classification/nomenclature started with notations so that it can be stored and retrieved digitally.² The wide diversity of cleft lip and palate in terms of clinical presentation makes the universal acceptance of existing classifications difficult,³ and specific cleft alveolus classification does not exist.

The cleft alveolus is an entirely separate entity than a cleft of the palate. It should be viewed in the spectrum of cleft lip.⁴ The existing classifications are either combined with lip, palate, or both, because of the nature of cleft development (Table 1). The isolated occurrence of the cleft alveolus is rare, which might be one of the reasons for not having separate classification. The difference of opinion and need for a universally accepted classification made our team think of a computed tomography (CT) scan-based classification system and factors affecting the prognosis before surgical management. The existing literature proposed many treatment modalities, pre- and postoperative expansion of the maxilla, and schedules for

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Table 1: Existing classification chronologically with comments

S. no.	Proposed by	Year	Comments
1	Davis and Ritchie ⁵	1922	Based on the alveolus, patients with a unilateral or bilateral cleft of the lip and posterior palate with intact cleft alveolus fail to fit into a single group.
2	Victor Veau ⁶	1931	Used incisive foramen as an anatomic landmark for the division of types, but overlooked the cleft patients born with cleft lip, alveolus.
3	Fogh-Andersen ⁷	1946	Based on anatomy and embryology, and the concept of primary and secondary palates involved for grouping.
4	Kernahan and Stark ⁸	1958	Used embryological concepts and primary and secondary palates for description purposes.
5	Vilar-Sancho ⁹	1962	Used Greek nomenclature for classification and coded congenital cleft lip and cleft palate. It could not classify many of the cleft subtypes, even though included alveolus as "G" (gnato) with the right, left, complete, and incomplete. But not adopted worldwide probably because it was in Greek.
6	Harkins et al. ¹⁰	1962	Based on anatomic segmentation into the pre-palate and the palate with subclasses. The subclasses used severity, i.e., quantitative measurement of width and extent of cleft as 1/3, 2/3, or 3/3, but no specific mention of alveolar subclass was made in the classification.
7	Santiago ¹¹	1969	Based on digital and alphabetical notification involved all types for the use of machine coding but not specifically concerning the extent of alveolus as they mentioned either complete or incomplete, unilateral or bilateral.
8	Dahl ¹²	1970	A combination of clefts classification is not possible.
9	Fogh-Andersen ¹³	1971	Modified the original types by adding a new group to feature the median clefts.
10	Kernahan ¹⁴	1971	Described using a Y-shaped diagram representing lip, palate, and alveolus. This notification is merely visual and failed in the application aspect.
11	Elsahy ¹⁵	1973	This is a modified striped Y classification.
12	Spina ¹⁶	1973	Described a modification of terminology in international classification by adding Latin term "foraminal."
13	Balakrishnan ¹⁷	1975	Based on anatomical and embryological representation and was based on Dahl 1970 classification.
14	Sandham ¹⁸	1985	This is modified from Kernahan and Stark.
15	Kriens ¹⁹	1989	Proposed LAHSHAL classification, with acronym lip, alveolus, hard palate, soft palate. The last three words will be for the left side, and the first three words will be for the right side. Because of its verbal communication in different languages its use is limited.
16	Silva Filho et al. ²⁰	1992	Modified Spina classification with the inclusion of group I/III for stratification, but it did not assess cleft severity (cleft size) and the capacity for prognosis estimation.
17	Schwartz et al. ²¹	1993	Proposed an RPL system with numerical coding for easy presentation.
18	Koch et al. ²²	1995	Proposed LAHSN (lip, alveolus, hard and soft palates, and nose) based on the extent in all three directions.
19	Smith et al. ²³	1998	Modified classification of Kernahan striped to compensate for the shortcomings and added additional types. But it did not describe the submucous cleft palate levels nor the alveolus.
20	Ortiz-Posadas et al. ²⁴	2001	Developed mathematical expression for characterization to reflect overall complexity.
21	Castilla and Orioli ²⁵	2004	Presented ECLAMC numerical coding system. This classification presented numerical code for all possible varieties and extent, but did not mention the prognostic factors. It is similar to our presentation, but little variation exists in the use of terminology.
22	Liu et al. ²⁶	2007	Developed (LAPAL system) a five-digit numerical recording system based on Kernahan "Y" classification, Smith-modified Kernahan "Y" classification, and the RPL system. The extent of defects was represented by Arabic numerals (0-4).
23	Koul ³	2007	Used anatomical nomenclature (text) and symbols for proposal. It looks complicated because of the symbols involved in the presentation.
24	Yuzuriha and Muliken ²⁷	2008	Described less form labial clefts with measurements of 3 mm more or less as micro, mini-micro. This presentation includes the operative guide for optimal operative technique depending on the severity of the cleft.
25	Rossell-Perry ²⁸	2009	Proposed classification of severity and diagram for cleft description. Also discussed the selection of proper technique depending on the class of cleft. The severity of the cleft was assessed by the width of cleft and sum of width of the two palatal segments at the junction of soft and hard palates to indicate the tissue deficiency. Based on measurements, the index classifies three degrees of severity for the cleft palate but does not consider alveolus for the same.
26	Khan et al. ²⁹	2013	Revised Smith-modified Kernahan "Y" classification with submucous cleft varieties, but these subclassifications did not consider alveolus.

Contd...



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S. no	Proposed by	Year	Comments
27	Agarwal ³⁰	2014	Modified Indian classification described by Balakrishnan into groups with a notation of '+' for unilateral and bilateral combinations and abbreviations of the involved part.
28	Luijsterburg et al. ³¹	2014	Based on patho-embryological events like fusion, differentiation, and fusion and differentiation defects of the primary and secondary palates.
29	Allori et al. ¹	2015	Proposed a universal structured form in the form of CLAP notation.

Table 2: Proposed classification for alveolar cleft based on preoperative 3D computed tomography

Class	Description	Nature	Comments	Prognosis
I	One-third alveolus involvement	Submucosal, isolated, unilateral, or bilateral	Tooth involved may be erupted/partially erupted/impacted	Good
II	Two-thirds alveolus involvement	Submucosal, isolated, unilateral, or bilateral	Tooth involved may be erupted/partially erupted/impacted	Good, requires prosthetic or orthodontic treatment
III	Complete alveolus involvement	Isolated, unilateral, or bilateral	Tooth involved may be erupted/partially erupted/impacted	Good, requires prosthetic or orthodontic treatment
IV	Communication with nasal floor	unilateral, or bilateral complete	Tooth involved may be partially erupted/impacted	Depends on the width of cleft and requires prosthetic or orthodontic treatment
V-A	Concomitant with lip and palate	Associated with Class I	Any lip or palate cleft as described previously, might be associated with Class I alveolar cleft	Good with alveolus, but the overall result depends on the associated nature of clefts
V-B	Concomitant with lip and palate	Associated with Class II	Any lip or palate cleft as described previously, might be associated with Class II alveolar cleft	Good with alveolus, but the overall result depends on the associated nature of clefts
V-C	Concomitant with lip and palate	Associated with Class III	Any lip or palate cleft as described previously, might be associated with Class III alveolar cleft	Depends on the width of cleft and the associated nature of clefts and may require a second surgery

alveolar cleft grafting. Grafting of cleft alveolus uses autologous and synthetic bone substitute materials. With our knowledge, none of the published studies have given specific graft material nor advised a standard surgical technique in a particular defect class.

This study proposed a CT scan-based new classification appraisal for cleft alveolus exclusively, after morphological examination of cleft patterns using CT scans (archives of more than 3000 alveolar clefts—both unilateral and bilateral cases) in our center. This study delineates our center management strategy to understand the prognostic factors depending on the new CT-based cleft classification.

MATERIALS AND METHODS

The CT scans of the cleft patients with an age range of 9–20 years from the archives of the Department of Radiology and Oral and Dental Surgery were included for evaluation. The data were evaluated for cleft morphology, and other factors were noted retrospectively to understand the outcomes of surgery. Various cleft defects were appraised and noted as per the new classification. The grafting, defect correction, and its outcomes were noted for surgical emphasis as per our center's protocol based on CT scan.

The English language literature was searched in the MEDLINE database without date restriction to revise existing literature on numerous classification systems using MeSH keywords related to cleft lip, palate, alveolus, developmental disturbance, facial clefts, classification, and nomenclature. Cross-reference search was performed in various plastic and reconstructive journals, head, and neck, oral and maxillofacial journals for comprehensiveness.

RESULTS

The CT scan examination revealed five types of defects. These defects were grouped into five classes, annexed with subclasses (Table 2). The literature revealed a total of twenty-nine classifications/nomenclature of cleft lip and palate since the year 1922 till 2015 (Table 1). Still, none exclusively described cleft alveolus based on CT scan observations.

DISCUSSION

Historically, cleft classification or nomenclature descriptions were given by Davis and Ritchie⁵ way back in the year 1922, followed by many other studies^{1,3,6–31}, as noted in Table 1 with comments. But none of these existing classifications/nomenclatures mentioned cleft alveolus appraisal based on preoperative CT scan examination. The recent explanation of cleft alveolus has noted the historical discrimination of distinguishing it from the palate has not been made clear, which needs to be corrected for proper attention and treatment.⁴ The inadequacies of each classification are mentioned in Table 1 with comments. The advances in radiographic investigations from plain to three-dimensional imaging made the preoperative evaluation and virtual planning more easy and accurate. In the absence of a patient, the surgeon will be able to plan it accurately and discuss with peers before surgery for successful execution as planned using a CT scan.

Proposed Cleft Alveolus Classification

The new classification proposal is based on the preoperative CT scan views of defect patterns and morphology. In the proposed classification, cleft alveolus is divided into five major classes as Class I

to Class V with three subclasses in Class V (Table 2), either unilateral/bilateral depending on the extent of alveolar cleft measuring from the crestal bone of adjacent teeth. The schematic diagram and corresponding representational CT scan images delineate the proposed system of classification for better understanding (Figs 1A to 4A and Figs 1B to 4B). This system considers the depth and width of cleft and thereby helps the operating surgeon for proper planning of the treatment. In concomitant clefts of lip and palate (proposed Class V and its three subdivisions), the surgeon or clinician can adopt any one of the classifications depending on their geography and school of thought for the treatment. Many of our clefts fall in Class V with subclasses; this might require a second surgery for an esthetic correction after the growth of the patient ceases.

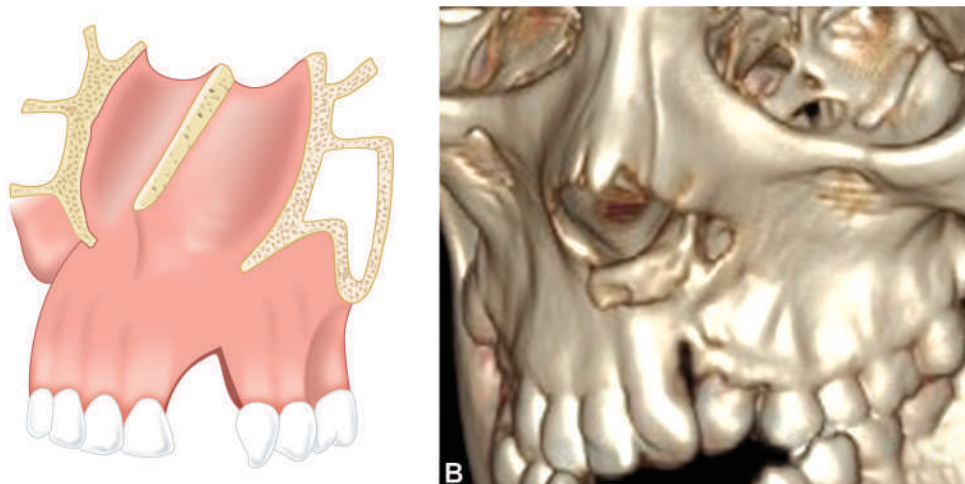
The timing for repair of cleft lip and palate were well-established facts in the published literature.³²⁻³⁵ The grafting for alveolus was also proposed and practiced widely.³⁶⁻³⁹ But none of the published literature classified cleft alveolus based on preoperative CT scan findings in three dimensions. The advances in diagnostic technology, both prenatal and postnatal, to assess developmental and growth disturbances for predicting anomalies are much better than earlier. Anomaly scanning of parents has

reduced the newer incidence of clefts reasonably. These reducing trends in the defects might be one of the reasons for not having enough number of cases to standardize the existing classification or providing a newer appraisal.⁴⁰ The historical presentations were based on clinical and intraoperative findings. Liu et al.,²⁶ Yuzuriha and Mulliken,²⁷ Rossell-Perry,²⁸ Khan et al.,²⁹ and Agarwal³⁰ classifications have similarities with the proposed classification, but none have a specific description of cleft alveolus using CT appraisal. The present appraisal is distinct and provides better prognostic features. The advances in technology help to create a virtual model for the reconstruction of these defects using CAD/CAM.

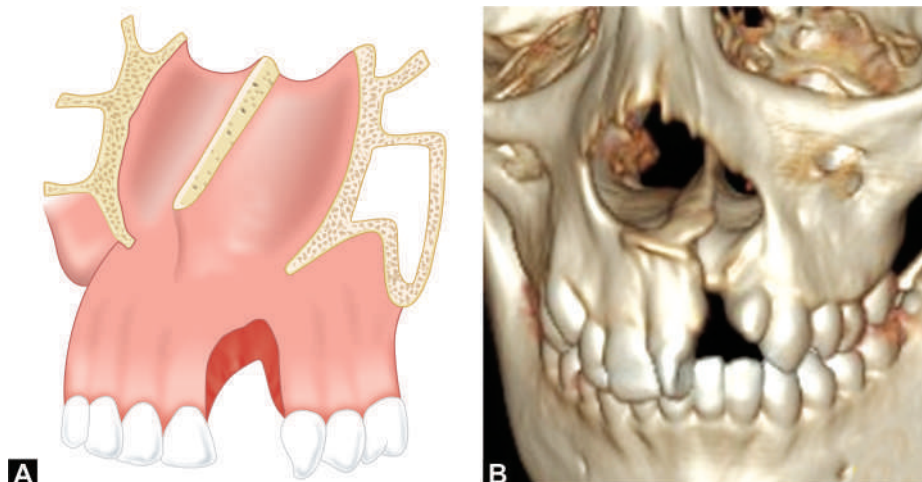
Our Center’s Protocol for the Management

Description of treatment options as per CT-based classification is as follows:

Classes I and II—use of cancellous alveolar bone grafting, platelet rich fibrin (PRF) membrane placed along the oral surface of the graft. Class III—use of corticocancellous alveolar bone grafting to provide mechanical strength to the graft. PRF membrane was placed along the oral surface of the graft. According to the subdivisions of the proposed classes, the involvement of the nasal floor and depression of the alar base on the affected side(s) should be considered. If the

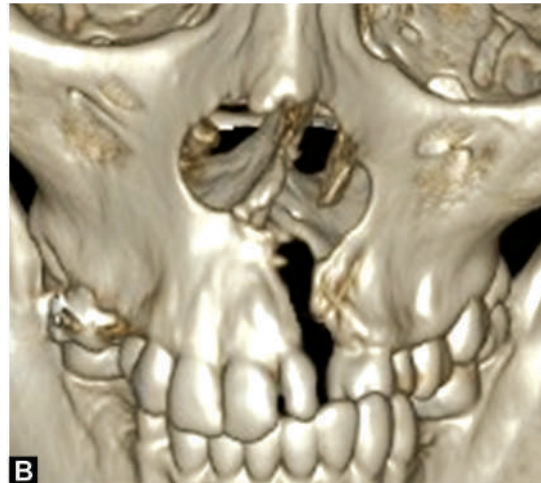
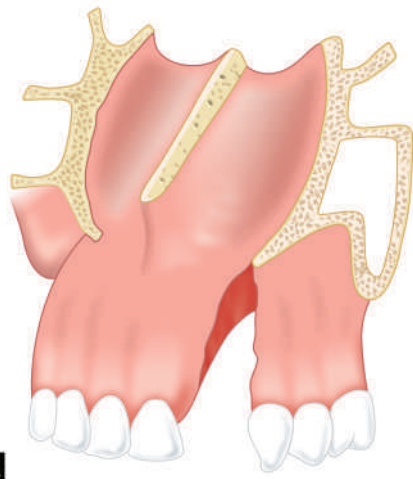


Figs 1A and B: (A) Schematic diagram and (B) representative 3D CT scan image showing Class I alveolar defect

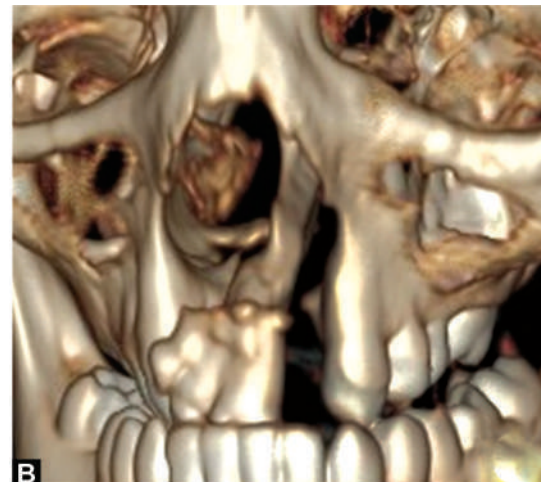
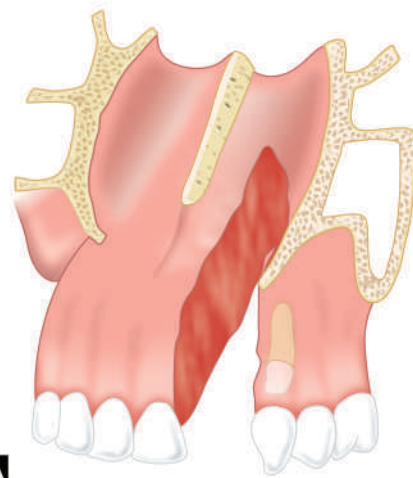


Figs 2A and B: (A): Schematic diagram and (B) representative 3D CT scan image showing Class II alveolar defect


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Figs 3A and B: (A) Schematic diagram and (B) representative 3D CT scan image showing Class III alveolar defect



Figs 4A and B: (A) Schematic diagram and (B) representative 3D CT scan image showing Class IV alveolar defect

nasal floor is involved, an additional PRF membrane has to be placed along the nasal floor mucosa and a thin piece of cortical bone is used to separate the nasal mucosa up to the level of nasal floor, for example, to the same height of the opposite unaffected side in the unilateral cleft. This cortical bone piece acts as resistance while packing the defective area with cancellous bone. The pyriform rim on the affected side[s] in the region of the depressed alar base also needs to be grafted with corticocancellous bone.

Factors Affecting the Prognosis of Cleft Alveolus

The prognosis of cleft alveolus surgery is multifactorial. It can be broadly divided into operator and patient factors. Patient factors include nature and type of cleft. The width of the cleft is considered as one of the critical factors for the success of the graft. Various surgical grafting techniques and adjunct presurgical treatment modalities like naso-alveolar molding for aligning the cleft segments are practiced by clinicians.¹² Grafting strategies of the cleft are well published in the literature.⁴⁰

Limitations of the Study

The study data were collected retrospectively, so the correlations of various factors for the outcome analysis were not considered.

The study also not considered scans of older age-group patients (above 20 years). The surgical modalities are not compared for the esthetic outcome, nor the data available were considered in the surgical and follow-up notes. The prospective study is ongoing to delineate the various factors affecting the prognosis of cleft grafting, taking into consideration new CT scan-based classification. The photographic outcome and patient satisfaction index studies are also ongoing in our center. This is preliminary work on the archival data of CT scan-based morphological examination of the cleft alveolus.

CONCLUSION

The proposed classification is useful for research and clinical purposes. Meticulous planning is required for the successful execution of surgery. By knowing the posterior extent of the cleft in the hard palate using a CT scan, one should be cautious to avoid overpacking of the defect. The new proposal is unique and provides insights into cleft alveolus may be isolated or concomitant. The prognosis of surgery invariably depends on the nature of clefts. It can be used for future reference in systematic reviews. The standardization of defects will provide proper evidence for treatment modalities in this evidence-based digital era.

AUTHOR CONTRIBUTIONS

Philip Mathew and Vivekanand S Kattimani drafted the protocol and developed a search strategy; Team 1, Philip Mathew, Vivekanand S Kattimani, Rahul Vinay Chandra Tiwari, and Team 2, Mohammad Shahid Iqbal, Aisha Tabassum, Khalid Gufran Syed, searched for literature. Vivekanand S Kattimani and Philip Mathew selected articles to include in this analysis. Philip Mathew and Rahul Vinay Chandra Tiwari performed archival data extraction for analysis. Mohammad Shahid Iqbal, Aisha Tabassum, and Khalid Gufran Syed obtained copies of publications of all included studies. Vivekanand S Kattimani and Rahul Vinay Chandra Tiwari extracted data from publications. Mohammad Shahid Iqbal and Aisha Tabassum verified the data entered for review. Vivekanand S Kattimani, Rahul Vinay Chandra Tiwari, and Philip Mathew interpreted the CT scan data; Philip Mathew and Vivekanand S Kattimani drafted the final evaluation and interpretations. All the authors read and approved the final version of the manuscript.

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Quality Assessment of Systematic Reviews of Temporomandibular Joint Ankylosis Surgical Treatment Outcomes

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ABSTRACT

Aim: Temporomandibular joint ankylosis (TMJA) management involves many surgical treatment modalities depending on the experience of the operator. A lot of literature has been published on various treatment modalities. Many systematic reviews (SRs) were published without any published prior protocol. So, the study aimed to evaluate the quality of SRs with meta-analysis of TMJA management.

Materials and methods: Systematic reviews with meta-analysis were included for the quality assessment using AMSTAR (assessment of multiple SRs) and Glenny et al. checklist by two independent teams. The search was limited to the Medline database archival (from January 1980 to December 2018).

Results: The primary search identified 1,507 related articles. After activation of different filters, abstracts screening, and cross-referencing, finally, a total of six studies were assessed to make the overview up-to-date.

Conclusion: The articles scored 8 to 11 with AMSTAR and 7 to 13 with the Glenny et al. checklist. None of the published reviews received maximum scores. The methodology and heterogeneity are essential factors to assess the quality of the published literature.

Clinical significance: None of the included meta-analysis was registered or published protocol with Prospero or Cochrane before publication for better validity of the studies. The authors are advised to follow reporting criteria so that in the future it is possible to provide the standards of care for TMJA with the highest quality of evidence.

Keywords: Condyle, Cranium, Diarthrodial joint, Evidenced-based dentistry, Systematic review.

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INTRODUCTION

Clinical decision making for the treatment approach in dentistry or medicine depends on evidence, published in peer-reviewed journals.¹⁻³ The decision for the standard of care for any treatment and recommendation depends on remarks of systematic reviews (SRs), randomized-controlled trials (RCTs), cohort studies, and least with retrospective studies.⁴ For few entities like temporomandibular joint ankylosis (TMJA), it is difficult to find RCTs because of less number of patients with similar clinical presentation, availability of many surgical modalities, and varied clinical scenario of ankylosis. So, it is difficult to ascertain and recommend a single modality of treatment or standardization of methodology. Because of these prevailing factors, the clinician will be in a dilemma during the selection of treatment modality.⁵ In recent years different protocols were developed, modified for the management of ankylosis.^{6,7} Thousands of articles were published regarding the management of TMJA. But this evidence is questionable because of varying methodologies and difficulty in reproducibility as ankylosis management and prognosis are multifactorial. So, biomedical journals started following standard publishing guidelines to maintain uniformity while reporting.⁸

In recent past, publishers adopted various reporting guidelines like CONSORT (Consolidated Standards of Reporting Trials), STROBE (STrengthening the Reporting of OBservational studies in Epidemiology), SPIRIT (standard protocol items for clinical trials), SQQR (standards for reporting qualitative research), etc., for various types of article publications.⁸ These guidelines help clinicians to prevent publication bias. Multiple tools were also developed to

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critically analyze the methodological quality of SRs and provide the recommendations based on the highest evidence.⁹⁻¹²

The available SRs showed that only 40.5% of the studies assessed the risk of bias/quality.³ Till date, quality assessment of the published reviews on TMJA had not been performed.^{2,13} In the wake of this diversity, the overview of SR articles with meta-analysis (MA) regarding comparative surgical treatment outcomes of TMJA was planned to assess and compare the quality using two types of tools: AMSTAR⁹ (assessment of multiple SRs) and the Glenny¹⁰ checklist.

MATERIALS AND METHODS

An electronic search was performed with the date and no language restriction. The search included articles published from January 1980 to December 2018 using various Boolean operators with multiple combinations of search strings in the Medline database. Depending on the title, abstract and full-text articles published in the English language were selected for reading with the following selection criteria to ascertain the objective. Only published articles of TMJA surgical treatment outcomes SR with MA were included for assessment. After initial screening, full-text articles were selected for critical reading and analysis. The authors were contacted for further clarification if the ambiguity in the published data exists. Two independent teams assessed the quality of included studies using AMSTAR⁹ and the Glenny et al.¹⁰ checklist.

The scoring was performed according to the characteristics of the study for a quality check as per the checklist. These criteria were based on the questions framed by AMSTAR⁹ and Glenny et al.¹⁰ for the assessment of quality check of published SR and MA. These questions assess how well the SR and MA were performed to reach the consensus on the treatment outcomes depending on the objectives of the study. The AMSTAR⁹ checklist consists of 11 questions, whereas the Glenny et al.¹⁰ list consists of 14 questions to assess quality. These questions/evaluative factors assess search criteria, methodology, the prior publication of the protocol, the validity of statistics of included studies, consideration of bias factors, any missing data, method of data collection, scientific quality, heterogeneity, and conclusive remarks based on the rationale supported by outcomes of the included primary studies. Differences in scoring among the two teams sorted with discussion. The methodological quality and statistics was assessed by a team of review members involving a public health dentist. The following search criteria with MeSH words were applied to generate the required data from the Medline archival.

Search Criteria and Data Screening

A literature search was performed using four search categories, which included various Boolean operators and MeSH keywords related to treatment outcomes of surgical management of TMJ ankylosis (Table S1 supplementary material).

Statistical Analysis

The data were collected using both quality assessment tools and were analyzed using descriptive statistics. The Spearman correlation test was performed for determining correlation between the two scoring criteria.

RESULTS

After going through the abstract of all final results of four search categories (Table S1) and narrowing down the search to address the objective of quality assessment, authors found 10 publications (Flowchart 1). After going through full-length articles, out of ten, one

Flowchart 1: Screening and selection process

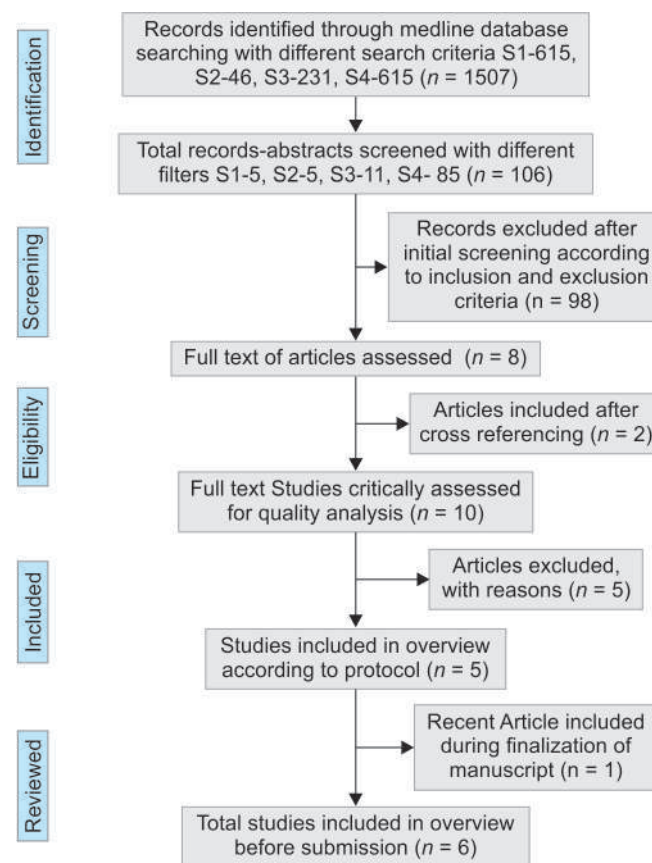


Table 1: List of excluded studies and the reason for their exclusion

Author and year	Reason for exclusion
Sporniak-Tutak et al. ¹⁴ (2011)	Descriptive review
Movahed and Mercuri ¹⁵ (2015)	Descriptive review
Sharma et al. ¹⁶ (2017)	Descriptive review
Bénateau et al. ¹⁷ (2016)	Other than the English language (in French)
AAOMS ParCare ¹⁸ (2012)	Not focused exclusively on comparative TMJ surgeries

was in the French language, another was American Association of Oral and Maxillofacial Surgeons (AAOMS) guidelines not focused on comparisons, and three were descriptive reviews focused mainly on the effects of various surgical treatment modalities in ankylosis without MA (Table 1).¹⁴⁻¹⁸ Five studies were found eligible for the review, but our team found one more recently published study,⁵ which has been added for final assessment to make an effort more complete and up-to-date and which rose the total number of included studies to six.

Study Characteristics

The scoring for included studies is presented in Table 2 (other characteristics continued in Tables S2 and S3 as supplementary material). Katsnelson¹⁹ included four studies²⁰⁻²³ searched between 1966 and May 2010. Al-Moraissi et al.²⁴ included 16 publications^{20-23,25-36} (nine retrospective studies and seven controlled clinical trials) searched in December 2013 without date restrictions. Ma et al.³⁷ included eight retrospective cohort studies

Table 2: Main characteristics and quality assessment scores obtained using AMSTAR and Glenny et al. checklist

Authors	Reference number	No. of studies included	Outcome measures	AMSTAR score (lowest 0, highest 11)	Checklist of Glenny et al. (lowest 0, highest 14)
Katsnelson	19	4	Maximal inter-incisal opening	8	7
Al-Moraissi	24	16	Maximal inter-incisal opening	11	10
Ma et al.	37	8	Maximal inter-incisal opening and incidence of reankylosis	11	12
Ma et al.	38	8	Maximal inter-incisal opening and incidence of reankylosis	11	13
De Roo et al.	41	38	Maximal inter-incisal opening	11	11
Mittal et al.	5	26	Incidence of reankylosis and maximal inter-incisal opening	11	11

Table 3: AMSTAR checklist and the number of studies that satisfied each of the criteria

S. no.	AMSTAR questions	Katsnelson ¹⁹	Al-Moraissi et al. ²⁴	Ma et al. ³⁷	Ma et al. ³⁸	De Roo et al. ⁴¹	Mittal et al. ⁵
1	Was an <i>a priori</i> design provided?	Yes	Yes	Yes	Yes	Yes	Yes
2	Is there a duplicate study selection and data extraction?	Yes	Yes	Yes	Yes	Yes	Yes
3	Was a comprehensive literature search performed?	Yes	Yes	Yes	Yes	Yes	Yes
4	Was the status of the publication (i.e., grey literature) used as an inclusion criterion?	Yes, no mention of grey literature	Yes, no mention of grey literature	Yes, no mention of grey literature	Yes, no mention of grey literature	Yes, no mention of grey literature	Yes, no mention of grey literature
5	Was a list of studies (included and excluded) provided?	No	Yes	Yes	Yes, but no list of excluded studies	Yes, but no record of excluded studies	Yes
6	Were the characteristics of the included studies provided?	Yes	Yes	Yes	Yes	Yes	Yes
7	Was the scientific quality of the included studies assessed and documented?	No	Yes	Yes	Yes	Yes	Yes
8	Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	Yes	Yes	Yes	Yes, using a customized framework	Yes
9	Were the methods used to combine the findings of studies appropriate?	Yes	Yes	Yes	Yes	Yes	Yes
10	Was the likelihood of publication bias assessed?	Yes	Yes	Yes	Yes	Yes	Yes
11	Was the conflict of interest stated?	No	Yes	Yes	Yes	Yes	Yes

and were searched up to October 11, 2014. Ma et al.³⁸ published one more study that included eight studies^{23,26,28,30,31,36,39,40} searched between 1946 and July 28, 2014. De Roo et al.⁴¹ publication included 38 studies^{21-23,28,31,42-73} with four prospective and one RCT, and other study types were not mentioned. Mittal et al.⁵ included 26 studies^{20-23,26-28,30-32,34,39,40,48,53,66,72,74-82} for MA.

Quality Assessment

None of the published reviews included in this overview met all the AMSTAR criteria (Table 3). The scores ranged from 8 points to

11 points. Point 4 of the AMSTAR guideline was partly explained in all publications. One study¹⁹ received the lowest score indicating poorly performed study involving four articles for review and MA, whereas the other publications^{5,24,37,38,41} received a score of 11. Two of the MA were published in the same year by the same author with different objectives involving eight studies each for MA.^{37,38}

The scores for the Glenny et al.¹⁰ checklist varied between 7 points and 13 points (Table 4). Spearman’s correlation was positive between the scores of two quality assessment tools, with a coefficient of 0.66 ($p = 0.15$) (Fig. 1). The mean and SD score for

Table 4: Checklist of Glenny et al. and the number of studies that satisfied each of the criteria

S. no.	Glenny et al. questions	Katsnelson ¹⁹	Al-Moraissi et al. ²⁴	Ma et al. ³⁷	Ma et al. ³⁸	De Roo et al. ⁴¹	Mittal et al. ⁵
1	Did the reviewer address a focused question?	Yes	Yes	Yes	Yes	Yes	Yes
2	Did the authors look for appropriate papers?	Yes	Yes	Yes	Yes	Yes	Yes
3	Did the authors attempt to identify all relevant studies?	Yes	Yes	Yes	Yes	Yes	Yes
4	Did the authors search for published and unpublished literature?	Unpublished not mentioned	Unpublished not mentioned	Unpublished not mentioned	Unpublished not mentioned	Unpublished not mentioned	Unpublished not mentioned
5	Were all languages considered?	Not mentioned	Yes	Not mentioned	Yes	Yes	Not mentioned
6	Was any hand-searching carried out?	Yes	Yes	Yes	Yes	Yes	Yes
7	Was it stated that at least two reviewers applied the inclusion criteria?	No, one author performed	Not mentioned	Yes	Yes	Yes	Yes
8	Did reviewers attempt to assess the quality of the included studies?	Partly assessed using publication bias	Assessed	Yes	Yes	Yes	Yes
9	If so, did they include this quality assessment in the analysis?	No, only publication bias assessed	Yes	Yes	Yes	No	Yes
10	Was it stated that the quality assessment was carried out by at least two reviewers?	No	No	Yes	Yes	No	Not mentioned
11	If the results have been combined, was it reasonable to do so?	Yes	Yes	Yes	Yes	Yes	Yes
12	Are the results clearly displayed?	Yes	Yes	Yes	Yes	Yes	Yes
13	Was an assessment of heterogeneity made, and were reasons for variation discussed?	No	Yes	Yes	Yes	Yes	Yes
14	Were the results of the review interpreted appropriately?	Yes	Yes	Yes	Yes	Yes	Yes

AMSTAR was 10.50 ± 1.22 and for Glenny et al. was 10.67 ± 2.06 (Table S4 supplementary material).

DISCUSSION

This overview is limited to SRs with a MA that evaluated the various surgical techniques used in the management of TMJA and its outcomes in humans. The TMJA prognosis is multifactorial; to date, no consensus on the standards of care has been advised. It might be attributed to patient and clinician factors broadly. Many attempts have been made in the past to assess the published literature.^{5,19,24,37,38,41} Few SRs are published with and without MA.^{5,14,15,19,24,37,38,41} Surprisingly, it was noted that variations in the number of included studies despite almost the same outcomes are being evaluated and published in the same year and same journal.^{37,41} This variation might be attributed to the inclusion and

exclusion criteria. Despite the increased number of publications, the quality of the publications has not reached the highest scoring.

The recent study⁵ is published after a gap of 4 years from the last published literature,⁴¹ but it includes a lesser number of studies compared to the previous research for interpretation even though the scope has been broadened by adding the distraction osteogenesis.⁵ The lack of inclusiveness of the previous MA (Ma et al., 2015^{37,38}) in discussion indicates methodological flaws in the search criteria.⁴¹ So, a more rigorous researching and reviewing approach is necessary for better evidence and conclusive remarks. The authors have not found the Prospero/Cochrane protocol for the published studies included in this overview. The SRs with or without MA should register its protocol in Prospero or Cochrane systemic protocol reviews for validity, which in turn prevents duplication of studies and methodological flaws. Leaving apart the Kabans protocol, the existing literature is unable to draw any further

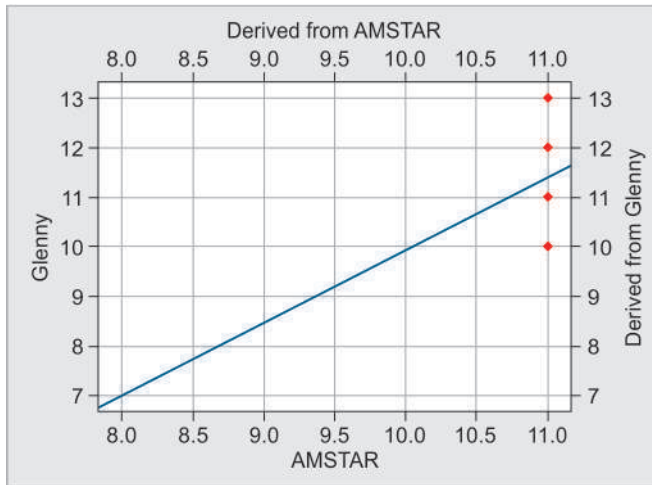


Fig. 1: Correlation between AMSTAR and Glennly et al. scores

conclusive remarks.^{6,7} So, the readers should exercise caution while adopting the interpretation and conclusive remarks.

The increase in scoring indicates an improvement in the reporting pattern from the first MA¹⁹ (the year 2012) to the latest published⁵ (the year 2019). The number of published articles were increased because of the shift of the thought process from gap arthroplasty to interposition arthroplasty and reconstruction arthroplasty using various techniques that have widened the scope of the study.^{5,19,24,37,38,41} This paradigm shift in surgical management made the prognosis better with reduced postoperative complications and improved patient compliance, function, and aesthetics. The reporting quality of RCT, controlled clinical trial (CCT), or case series needs to be improved for better evidence.⁸³ None of the published studies reported TMJA classification before intervention, so it might have given a better edge for correlation of the surgical method and prognosis. Although the publications reviewed had similar objectives in this overview, but they had high methodological heterogeneity. However, these SRs did not meet all of the criteria of the checklists used, indicating potential publication bias.

The risk of bias assessment is essential for individual studies.⁸³ Adopting the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach for the synthesis of evidence would be a significant step for improving the quality of clinical evidence.⁸³ The International Committee of Medical Journal Editors (ICMJE)⁸⁴ set many reporting guidelines for human clinical series or CCT or RCT publications. If authors adhere strictly to these reporting criteria, then better evidence can be provided, which can be universally adopted for developing the TMJA treatment protocol. High-quality reviews may help the clinicians and patients to have the best possible results.⁸³ The publication bias assessment and homogeneity are fundamental for the studies considered in MA.^{2,3} These factors clarify the reader the methodological quality opted for the same. Quality assessments, such as the Cochrane Collaboration's tool for assessing the risk of bias for RCTs and Newcastle–Ottawa Quality Assessment Scale for cohort studies, were used in the included studies.

The TMJA surgical management outcome analysis consists of several confounding factors. Some of them should be included in inclusion or exclusion criteria so that the effect can be minimized.

The time duration of ankylosis, type, age, treatment provided, postsurgical monitoring, etc., are important factors along with the surgical expertise of the clinicians for the better outcome. Even though the study included extensive quality checks using checklists but these lists lack of quantitative assessment. In our study, the lowest score obtained was 7. Despite the lack of standard reference scale for discrimination as poor or good study, the score below 3 was considered as poorly designed.^{4,85} But in our overview, we found moderate scored reviews.

The checklists used in this overview are more comprehensive and were used extensively with validation for the quality checks. Many other tools are available but are not so comprehensive and not provide scoring.^{9,11,12,86–88} Because of the scoring, it is possible to correlate using Spearman's correlation, which measures the relationship between two variables. So, we have used these two checklists. In this study, the scores were positively correlated, indicating a lower possibility of errors and a lower risk of bias in the scoring system implemented.

The study has limited the critical assessment to the English language literature archived in the Medline database for the accuracy, reproducibility, and quality of publications for evaluation. The increase in predatory journal publications is worrying some for the evidence published, and the involvement of such studies as reference might mislead the outcome or recommendation.^{13,84}

CONCLUSION

The clinicians and researchers, as a result of this, advised to look after the reporting guidelines and adhere to the protocol of reviews for better evidence. Authors cautioned to refer the valid, researchable, and indexed journals for better validity as few of the MA referenced predatory journals in their research, which may undermine the objective of SR and MA. The word of caution is always better for better evidence creation for the future generation and the standard of care.

AUTHOR CONTRIBUTIONS

Vivekanand S Kattimani and Abhishek Jairaj drafted the protocol; Vivekanand S Kattimani, Abhishek Jairaj, and Shaik Parveen Sultana developed a search strategy. Team 1, Vivekanand S Kattimani, Abhishek Jairaj, Shaik Parveen Sultana, and Team 2, Nikhil O Govindan, Paul Mathai, Swati Sahu, Abhishek Patley, searched for literature. Vivekanand S Kattimani, Abhishek Jairaj, and Swati Sahu selected articles to include in this analysis. Paul Mathai and Abhishek Patley obtained copies of publications of all included studies. Vivekanand S Kattimani, Nikhil O Govindan and Swati Sahu extracted data from publications. Abhishek Jairaj and Shaik Parveen Sultana verified the data entered for analysis. Shaik Parveen Sultana carried out the analysis part. Vivekanand S Kattimani, Abhishek Jairaj, and Shaik Parveen Sultana interpreted the analysis; Vivekanand S Kattimani and Abhishek Jairaj drafted the final review. All the authors read and approved the final version.

ETHICAL APPROVAL

Not required, as it does not involve humans or animals in the study.

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All authors have viewed and agreed to the submission.

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Table S1: Search methods performed in PubMed to retrieve the suitable data for assessment with search results obtained for evaluation

Search	The methodology of search and use of various Boolean operators and MeSH terms	Description of search criteria and results obtained
First search	("ankylosis"[MeSH Terms] OR "ankylosis"[All Fields]) AND (("surgical procedures, operative"[MeSH Terms] OR "surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "surgical"[All Fields]) AND ("organization and administration"[MeSH Terms] OR ("organization"[All Fields] AND "administration"[All Fields]) OR "organization and administration"[All Fields] OR "management"[All Fields] OR "disease management"[MeSH Terms] OR ("disease"[All Fields] AND "management"[All Fields]) OR "disease management"[All Fields])) AND (Review[ptyp] AND ("1980/01/01"[PDAT]: "2018/12/31"[PDAT])) AND "humans"[MeSH Terms])	The results obtained with the following filters activated: Review, Publication date from 1980/01/01 to 2018/12/31, Humans; the search team found total of 5 publications out of 615 items
Second search	("Temporomandibular ankylosis"[Supplementary Concept] OR "Temporomandibular ankylosis"[All Fields] OR "temporomandibular ankylosis"[All Fields]) AND (("surgical procedures, operative"[MeSH Terms] OR "surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "surgical"[All Fields]) AND ("organization and administration"[MeSH Terms] OR ("organization"[All Fields] AND "administration"[All Fields]) OR "organization and administration"[All Fields] OR "management"[All Fields] OR "disease management"[MeSH Terms] OR ("disease"[All Fields] AND "management"[All Fields]) OR "disease management"[All Fields])) AND (Review[ptyp] AND ("1980/01/01"[PDAT]: "2018/12/31"[PDAT])) AND "humans"[MeSH Terms])	The results obtained with following filters activated: Review, Publication date from 1980/01/01 to 2018/12/31, Humans; the search team found 5 publications out of 46 items
Third search	("Temporomandibular ankylosis"[Supplementary Concept] OR "Temporomandibular ankylosis"[All Fields] OR "temporomandibular ankylosis"[All Fields]) AND ("organization and administration"[MeSH Terms] OR ("organization"[All Fields] AND "administration"[All Fields]) OR "organization and administration"[All Fields] OR "management"[All Fields] OR "disease management"[MeSH Terms] OR ("disease"[All Fields] AND "management"[All Fields]) OR "disease management"[All Fields])) AND (Review[ptyp] AND ("1980/01/01"[PDAT]: "2018/12/31"[PDAT])) AND "humans"[MeSH Terms])	The results obtained with the following filters activated: Publication date from 1980/01/01 to 2018/12/31, Humans; the search team found 164 out of 231 items. Further activation of Filter: Review, resulted in 11 publications
Fourth search	("Temporomandibular ankylosis"[Supplementary Concept] OR "Temporomandibular ankylosis"[All Fields] OR "temporomandibular ankylosis"[All Fields]) AND ("1980/01/01"[PDAT]: "2018/12/31"[PDAT]) AND "humans"[MeSH Terms])	The results obtained with the following filters activated: Review, Publication date from 1980/01/01 to 2018/12/31, Humans; the search team found 85 publications out of 615 items



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Table S2: Study characteristics of involved reviews (continued) (supplemental material)

Study characteristics	Katsnelson ¹⁹	Al-Moraissi et al. ²⁴	Ma et al. ³⁷	Ma et al. ³⁸	De Roo et al. ⁴¹	Mittal et al. ⁵
Electronic databases search sites included	PubMed, Cochrane Central Register of Controlled Trials	PubMed, Cochrane database of systematic reviews, the Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, MEDLINE, CINAH, SPORTDiscus, and Electronic Journals Center	PubMed, EMBASE, OVID EBM reviews, and Web of Science	PubMed, EMBASE, Cochrane Library, Web of Science and China National knowledge, infrastructure	PubMed and the Web of Science and Cochrane Library	PubMed, Ovid, Embase, Web of Science, Scopus, and Cochrane central register of controlled trials (CENTRAL)
Protocol opted for data extraction	Standardized data extraction form	Customized data extraction form	Customized data extraction form	Customized data extraction form	Customized data extraction form with following Altman, STROBE (strengthening the reporting of observational studies in epidemiology) and Cochrane Library framework	Customized data extraction form
Method opted for scientific quality assessment	Not assessed	As per the checklist proposed by Rangel et al. and Versteegh et al.	Newcastle-Ottawa scale (NOS)	Newcastle-Ottawa scale (NOS)	STROBE guidelines but no scoring mentioned	Newcastle Ottawa Scale
Method opted for publication bias assessment	Begg's funnel plots and Egger's test	Begg's funnel plot	Begg's funnel plots and Egger's test	Begg's funnel plots and Egger's test	Egger's test	Egger's test
Review design method opted	QUOROM (quality of reporting of meta-analyses) guidelines	Preferred reporting items for systematic reviews and meta-analyses (PRISMA)-E 2012 checklist	Not mentioned	MOOSE guidelines	PRISMA guidelines	Not mentioned
Heterogeneity assessment	Not mentioned	Cochran's test and the I ² statistic	∑ ² and I ² statistic	Chi-square and I-squared tests	I-squared test	Chi-square and I-squared tests
Statistical analysis	STATA version 9.2	RevMan 5.2.6 software	RevMan 5.3 software and STATA version.12	RevMan 5.3 software and STATA version.12	STATA version.12	RevMan 5.3
Information of prior protocol publication	No	No	No	No	No	No



Table S3: Study details extracted from the MA selected for critical analysis (supplemental material)

<i>S. no.</i>	<i>Author and year</i>	<i>No. of patients included</i>	<i>Data search timeline</i>	<i>Investigator and year of publication</i>	<i>Type of study</i>
1	Katsnelson ¹⁹ (2012)	52 patients in one group and 39 patients in another group	January 1966 through May 2010	Manganello, 2003	Not mentioned
2	Al-Moraissi et al. ²⁴ (2014)			Balaji, 2003	Not mentioned
				Qudah et al., 2005	Not mentioned
				Tanrikulu et al., 2005	Not mentioned
				Saeed et al. 2002	Retrospective study
				Balaji, 2003	Control clinical trial
				Manganello, 2003	Control clinical trial
				Tanrikulu et al., 2005	Retrospective study
				Qudah et al., 2005	Retrospective study
				Ramezani and Yavary et al., 2006	Control clinical trial
				Vasconcelos et al., 2009	Retrospective study
				Danda et al., 2009	Control clinical trial
				Tang et al., 2009	Retrospective study
				Zhi et al., 2009	Retrospective study
				Elgazzar et al. 2010	Retrospective study
Loveless et al., 2010	Retrospective study				
Mansoor et al., 2013	Control clinical trial				
Shaikh et al., 2013	Control clinical trial				
Mabongo, 2013	Retrospective study				
Holmlund et al., 2013	Controlled clinical trial				
3	Ma et al. ³⁷ (2015)	Reconstruction arthroplasty group 106 and Interposition arthroplasty 92 patients among 6 studies	No time restriction search performed up to October 11, 2014	Balaji, 2003	Retrospective cohort study
				Manganello, 2003	Retrospective cohort study
				Tanrikulu, 2005	Retrospective cohort study
				Qudah, 2005	Retrospective cohort study
				Erol, 2006	Retrospective cohort study
				Loveless, 2010	Retrospective cohort study
				Elgazzar, 2010	Retrospective cohort study
				Sahoo, 2012	Retrospective cohort study
4	Ma et al. ³⁸ (2015)	Total of 272 patients among eight studies divided into two groups	From 1946 to July 28, 2014	Hu, 2005	Retrospective cohort study
				Tanrikulu, 2005	Retrospective cohort study
				Erol, 2006	Retrospective cohort study
				Ramezani, 2006	Retrospective cohort study

Contd...

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<i>S. no.</i>	<i>Author and year</i>	<i>No. of patients included</i>	<i>Data search timeline</i>	<i>Investigator and year of publication</i>	<i>Type of study</i>
				Danda, 2009	Retrospective cohort study
				Zhi, 2009	Retrospective cohort study
				Elgazzar, 2010	Retrospective cohort study
				Holmlund, 2013	Retrospective cohort study
5	De Roo et al. ⁴¹ (2015)	Total of 1,165 patients among 36 studies further divided into five groups consisting of GA-463, IA auto-341, IA allo-68, RA auto-260, and RA allo-33	Up to October 11, 2014	Rajgopal, et al., 1983	Not mentioned
				Chossegros et al., 1997	Not mentioned
				Karaca et al., 1998	Not mentioned
				Chossegros et al., 1999	Not mentioned
				Roychoudhury et al., 1999	Not mentioned
				Erdem and Alkan, 2001	Not mentioned
				Valentini et al., 2002	Not mentioned
				Manganello, 2003	Not mentioned
				Guyen, 2004	Not mentioned
				Dimitroulis, 2004	Not mentioned
				Qudah et al., 2005	Not mentioned
				Tanrikulu et al., 2005	Not mentioned
				Li et al., 2006	Not mentioned
				Huang et al., 2007	Not mentioned
				Guyen, 2008	Not mentioned
				El-Sayed, 2008	Not mentioned
				Krishnan, 2008	Not mentioned
				Mehrotra et al., 2008	Not mentioned
				Bayat et al., 2009	Not mentioned
				Danda et al., 2009	Not mentioned
				Yazdani et al., 2010	Prospective study
				Liu et al., 2010	Not mentioned
				Elgazzar et al., 2010	Not mentioned
				Liu et al., 2011	Not mentioned
				Singh et al., 2011a	Not mentioned
				Singh et al., 2011b	Not mentioned
				Yang et al., 2011	Not mentioned
				Gaba et al., 2012	Prospective study
				Mehrotra et al., 2012	Randomized controlled trial
				Nitzan et al., 2012	Not mentioned
				Sahoo et al., 2012	Not mentioned
				Singh et al., 2012	Prospective study
				Babu et al., 2013	Prospective study
				Jakhar et al., 2013	Not mentioned

Contd...

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S. no.	Author and year	No. of patients included	Data search timeline	Investigator and year of publication	Type of study
6	Mittal et al. ⁵ (2019)	Total of 1,197 patients among 26 studies. Further divided into groups	Searched till April 2018	Karamese et al., 2013	Not mentioned
				Zhu et al., 2013	Not mentioned
				Bhatt et al., 2014	Not mentioned
				Singh et al., 2014	Not mentioned
				Valentini et al., 2002	Nonrandomized controlled trial
				Balaji, 2003	Retrospective study
				Souza and Mariani, 2003	Nonrandomized controlled trial
				Hu et al., 2005	Retrospective study
				Tanrikulu et al., 2005	Retrospective study
				Qudah et al., 2005	Retrospective study
				Ramezani and Yavary, 2006	Nonrandomized controlled trial
				Erol et al., 2007	Retrospective study
				Güven et al., 2008	Retrospective study
				Danda et al., 2009	Nonrandomized controlled trial
				Kummoona et al., 2009	Nonrandomized controlled trial
				Vasconcelos et al., 2009	Retrospective study
				Zhi et al., 2009	Retrospective study
				Elgazzar et al., 2010	Retrospective study
				Loveless et al., 2010	Retrospective study
				Sahoo et al., 2012	Retrospective study
Shaikh et al., 2013	Nonrandomized controlled trial				
Bhatt et al., 2014	Retrospective study				
Kumar et al., 2014	Retrospective study				
Ahmad et al., 2015	Nonrandomized controlled trial				
Bhardwaj and Arya, 2016	Retrospective study				
Denadai et al., 2016	Retrospective study				
Shakeel et al., 2016	Retrospective study				
Dad and Uppal, 2017	Retrospective study				
Jiang et al., 2017	Retrospective study				
Xu et al., 2017	Retrospective study				

Table S4: Spearman's rank correlation between AMSTAR and Glenny et al. scores

	n	Spearman R	t(N-2)	p-level
AMSTA and Glenny et al. scores	6	0.6642	1.7770	0.1502



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Evaluation of Microneedling Therapy in Management of Facial Scars

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Background: Appearance and beauty always have been playing a major aspect of human's lives, regardless of the era. The desire for man to look better had led to various treatment modalities. One among them is microneedling therapy, also called as percutaneous collagen induction therapy or dermaroller therapy. Henceforth, the

aim of the study was to evaluate the efficacy of microneedling therapy in the management of facial scars.

Methods: An observational clinical study was conducted with a total of 14 patients who needed treatment and presented to the out-patient department of Department of Oral and Maxillofacial Surgery. The patients who were cooperative, motivated, and esthetically conscious with facial scars due to trauma, soft-tissue injury following incision and drainage, postsurgical scars, scars following surgery of cleft lip correction, and postacne scars were randomly selected.

Results: In the present study, a statistically significant difference was observed with pre- and postsurgical patient satisfaction scale (PSS) and observed satisfaction scale (OSS; $P = 0.001$). Preoperatively there were 64.3% with depressed scars and 35.7% with elevated scars. The clinical response of height of scar postoperatively was 100% flattening with the surrounding skin surface. Results showed that all atrophic and hypertrophic scars showed improvement, and color of the scar which was darker or lighter than the surrounding skin got the same color as the surrounding skin after microneedling therapy. Pain during the procedure was easily tolerable by the patient and no postoperative pain was noted.

Conclusion: Microneedling therapy can be considered as an effective modality of treatment for facial scars in patients with an added advantage of minimal downtime and effective improvement. It is simple and cost-effective technique in the treatment of facial scars.

Key Words: Dermaroller, microneedling therapy, percutaneous collagen induction therapy, scars

Appearance and beauty have always been playing a major aspect of human's lives, regardless of the era. Skin has a crucial visible element in the social interaction and quality of life, and facial esthetic analysis has a long and fundamental history of cosmetic surgery as the skin being part of body ages proportionately due to direct exposure to the external environment. Even though lifestyle modification and protection of skin from these delay the development of aging in the skin, it is particularly important because of its visibility and social impact. Scarring is a result of abnormal wound healing following damage leading to acute inflammation. This scar tissue lacks the characteristics of normal uninjured skin. Every skin wound will result in scar formation. Scars occurring on the face are challenging to treat. They have impact on the psychological status of

the patient. The desire for man to look better had led to various treatment modalities, one among them is microneedling therapy also called as percutaneous collagen induction therapy or dermaroller therapy. Microneedling therapy is an evolving modality for the treatment of scars. This therapy uses a small hand held device called roller which contains microneedles of various sizes. These microneedles cause microtrauma to the dermis which triggers the healing process ultimately leading to fibrosis. Thus natural tissue remodeling occurs. Therefore, the hypothesis of microneedling therapy is neither effective nor ineffective treatment for facial scars (ie, posttraumatic, postsurgical, postacne scars, and scars following surgical correction of cleft lip scars). The aim of the study was to evaluate efficacy of microneedling therapy in the management of facial scars and the objectives of the study were to compare the pre- and postoperative improvements in grading of scars and patient observer satisfaction with his/her appearance.

METHODS

An observational clinical study was conducted in the Out-Patient Department (OPD), Department of Oral and Maxillofacial Surgery from October 2014 to June 2016 at private dental institute. A total of 14 patients were included in the study. Selection criteria include patients with facial scars, that is, posttraumatic, postsurgical, postacne scars, and scars following surgical correction of cleft lip were included. Patients who are willing for a procedure with facial scars, aged between 15 and 50 years (male and female) and free of systemic conditions (like diabetes, hypertension, and immunocompromised as it affects wound healing resulting in bias) were included in the study. Patients with positive history of keloids, positive history of bleeding/clotting disorders, presence of active bacterial/viral infections, patients with human immunodeficiency virus, HBsAg, and any chronic illness were excluded. This study was approved by scientific and ethical committee of institutional review board. Informed consent was taken. Patients were screened for hemoglobin concentration (Hb), clotting time (CT), and bleeding time (BT).

Procedure

The materials used were roller, topical anesthetic (EMLA), saline pads, and topical antibiotic cream. Area to be treated was anesthetized with topical anesthesia, that is, EMLA and covered with cellophane tape for 45 minutes to 1 hour. The EMLA removed using normal saline. The skin of the face was stretched by one hand while the other is used to roll the instrument over in a direction perpendicular to that of stretching force. The roller was rolled 15 to 20 times in horizontal, vertical and both oblique directions till pin point bleeding occur, which should be from the base of the scar. Saline pads are kept over the treated area. Topical antibiotic cream was applied over treated areas. The whole procedure takes 15 to 20 minutes. Patients were advised to avoid exposure to the sun for 1 week postoperatively and also instructed use cold packs on the face to avoid postoperative redness for 2 days. Likewise, 14 patients with facial scars will be treated with microneedling therapy with a minimum of 3 sittings with an interval of 4 to 6 weeks and at the end the efficacy of the treatment would be assessed.

The appearance of pre- and postoperative scars was measured based on observer satisfaction scale (OSS) and patient satisfaction scale (PSS).¹ Rating above 8 was graded as "excellent improvement" (grade 5), rating between 7 and 8 served as "good improvement" (grade 4), rating between 5 and 6 rated as "fair improvement" (grade 3), rating between 3 and 4 rated as "poor improvement" (grade 2), and rating below 2 rated as "no improvement" (grade 1). Along with OSS and PSS, height of the scar and color of the scar was measured accordingly as follows: height of the

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scar: grade 0 elevated, grade 1 flat, grade 2 depressed. Color of the scar: grade 0 darker than surrounding skin, grade 1 same as surrounding skin, and grade 2 lighter than the surrounding skin.

Statistical analysis was done using statistical package for social sciences (SPSS) software (20.0) version. Comparison between the pre- and postsurgical PSS and OSS was done using *t*-test. The *P* value ≤ 0.05 was considered as a statistically significant.

RESULTS

A sample of 14 patients underwent microneedling therapy out of which 4 were males and 10 were females, with a mean age of 23.4 ± 3.50 years in a range of 19 to 35 years. Among 14 patients, 4 were having postsurgical scars, 4 were having posttraumatic scars, and 6 were having postacne scars with distribution percentage of 28.5%, 28.5%, and 43%, respectively (Supplemental Digital Content, Table 1, <http://links.lww.com/SCS/B66>). All the patients underwent microneedling therapy with a minimum of 2 sittings, which include each sitting with minimum gap of 30 days. Immediate postoperative redness was seen which subsided within 2 days of posttreatment. In terms of clinical patient satisfaction after microneedling therapy, 50% of the patients were satisfied with the treatment and reported good improvement and 21.4% of the patients reported excellent improvement in the scar size reduction. Likewise, 28.4% patients stated with fair (14.2%) and poor (14.2%) improvements. A similar response was observed with clinical observer satisfaction (Supplemental Digital Content, Tables 2 and 3, <http://links.lww.com/SCS/B66>). The mean values have increased when compared with pre- and postsurgical PSS (1 ± 0.00 and 3.7 ± 0.97 , respectively) and OSS (1 ± 0.00 and 3.7 ± 0.97 , respectively), this indicates that the microneedling therapy would help to improve facial aesthetics. A statistically significant difference was observed with pre- and postsurgical PSS and OSS ($P=0.001$) (Supplemental Digital Content, Table 4, <http://links.lww.com/SCS/B66>). Preoperatively there were 64.3% with depressed scars and 35.7% with elevated scars. The clinical response of height of scar postoperatively was 100% flattening with the surrounding skin surface (Supplemental Digital Content, Table 5, <http://links.lww.com/SCS/B66>). Results showed that all atrophic (Fig. 1C-F) and hypertrophic scars (Fig. 1A-B) showed improvement and color of the scar which was darker or lighter than the surrounding skin got the same color as the surrounding skin (Supplemental Digital Content, Table 6, <http://links.lww.com/SCS/B66>) after microneedling therapy. Moreover, pain observed during treatment procedure was easily tolerable in most of the patients. No infection and inflammatory pigmentation were noted.

DISCUSSION

Cosmetology is the study and practice of beautification. The fascination to look beautiful is an age-old obsession with people of all ages as these changes provide insight into the social roles of human, throughout history. Face is the focus of much attention when it comes to physical attractiveness, because it stands for a person's identity and is most exposed to public opinion. Physical attractiveness influences the way people think about themselves and is related to traits such as self-confidence and social acceptance. Henceforth, the present study was conducted to evaluate the efficacy of microneedling therapy in the management of facial scars. Pre- and postsurgical height and color of a scar, patient, and observer satisfaction responses were assessed.

The present study more likely concentrated on formulating the hypothesis that microneedling therapy has been found to be an effective treatment in reducing the scar and a scarred area's resembles a normal adjacent skin due to percutaneous collagen induction.² Hence, in this study, the use of placebo or sham surgeries is not necessary for formulating the hypothesis.

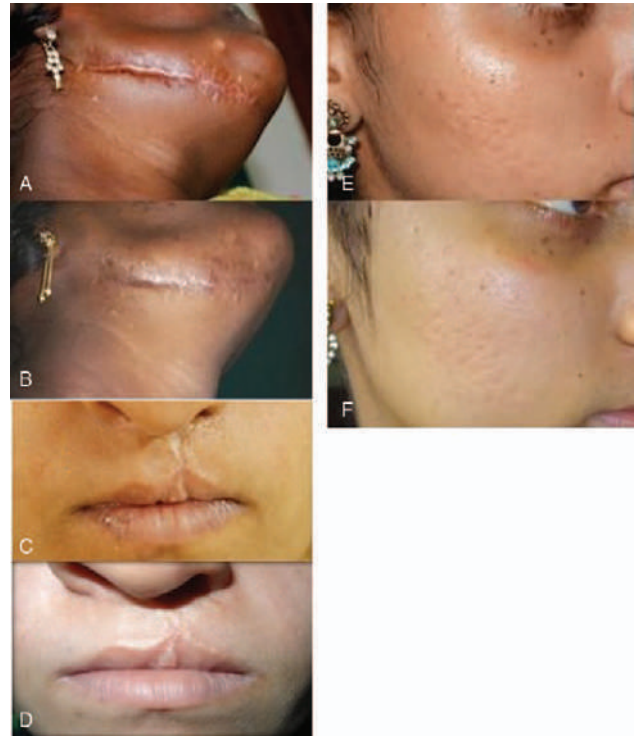


FIGURE 1. (A-B) It is a patient with postsurgical incision scar where patient diagnosed with ameloblastoma underwent segmental mandibulectomy followed by reconstruction plate. This patient underwent microneedling therapy for about 3 sittings and there is improvement of scar after microneedling therapy. (C-D) It is a patient with scar following correction of cleft lip. Patient underwent 4 sittings. Postoperative figure shows improvement in grading of scar after microneedling therapy. (E-F) It is a patient with postacne scar. Here patient underwent 4 sittings. Postoperative figure shows improvement in grading of scar after microneedling therapy.

The results of the present study showed that the scars which were either elevated or depressed got flattened with the skin surface postoperatively and the color of the scar which was darker or lighter than the surrounding skin got same color as the surrounding skin. Patients were satisfied with the treatment and they showed positive improvement scores.

The present study can be comparable to the study done by El-Domyati et al² where he did histologically efficacy of microneedling and established that increased collagens I, III, VII, and tropoelastin. In accordance with the present study Lee et al³ reported the efficacy of microneedling with human stem cell conditioned medium and concluded that the combination better than microneedling. Aust et al⁴ conducted a study, where he noticed the efficacy of microneedling in reducing postburn scars without damaging epidermis. He found 80% improvement in scarring, 45% thickened stratum spinosum, normal rete ridges after 1 year.

In harmony with Safonov⁵ microneedling in postburn scars showed good improvement after microneedling therapy. According to Costa and Costa,⁶ microneedling in varicella scars showed a significant improvement.

Microneedling is an effective modality of treatment, especially in patient with scars of varied etiology. It certainly promises to be a valuable technique with its numerous applications and its ever-expanding modifications as well as feasibility of home use. Though the significant result was achieved in this study, the main limitations of the study were included a less number of patients and short duration of follow-up period. Thus, further work is still required to firmly establish the efficacy of microneedling therapy for facial scars.

CONCLUSION

Microneedling therapy can be considered as an effective modality of treatment for facial scars in patients with an added advantage of minimal downtime and effective improvement. It is simple and cost-effective technique in the treatment of facial scars.

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The Utility of Endoscope-Assisted Rigid Bronchoscopy in Pediatric Airway Foreign Body Removals

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Introduction: Aspiration of foreign bodies is an emergency condition in children and may result in death, especially in children

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under 3 years of age. Therefore, diagnosis and treatment must be made rapidly.

Objective: This study sought to summarize our experience with endoscope-assisted rigid bronchoscopy (RB) in the diagnosis and treatment of pediatric tracheobronchial foreign body emergencies to reduce complications and mortality.

Methods: This was a retrospective cross-sectional study. The medical records of 337 children diagnosed with clinically suspected airway foreign body aspiration in the pediatric emergency department were analyzed retrospectively. The patients were divided into 2 groups with endoscopy used during RB in group 1 whereas group 2 was RB only. The surgeons who performed the bronchoscopies completed a survey on the advantages/disadvantages of these 2 procedures.

Results: All of the patients had a positive history of suspected foreign body aspiration and foreign bodies were identified in 77.1% of the patients during RB. There were 161 (47.8%) patients in group 1 and 176 (52.2%) patients in group 2. In group 2, 5 patients showed transient hypoxia, and 6 patients had an episode of transient bleeding during the operations. These numbers were 3 and 3, respectively, in group 1. One patient in group 2 suffered cardiac arrest and died during surgery. The authors did not see any long-term complications after these operations and the authors did not find any statistically significant differences between the groups for complication rates.

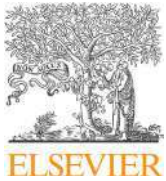
Conclusion: The RB is the gold standard procedure for removal of pediatric airway foreign bodies. The survey used in this study and our extensive experience have shown that the distal bronchi and foreign bodies can be visualized more effectively when using a rigid endoscope during RB, especially in children under the age of 3 years. In order to improve the safety of the surgical procedure, the authors propose that endoscope-assisted RB should be used in emergencies concerning foreign bodies in the airways of children.

Key Words: Aspiration, bronchoscopy, endoscopy, foreign body

Aspiration of foreign bodies is an emergency condition in children and may result in death, especially in children under 3 years of age.¹ Acute pulmonary failure or chronic pulmonary disease may occur in patients who are not treated, therefore, diagnosis and treatment must be made rapidly.^{2,3} In the USA, approximately 600 children per year die as a result of foreign body aspiration.⁴

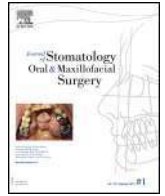
Aspiration of foreign bodies can cause acute asphyxia, cough, cyanosis, or wheezing. Clinical findings depend on the type, size, and location of the foreign body. A chest radiograph (X-ray) may show an unspecific infiltration on the lung, atelectasis, hyperinflation, consolidations, or bronchiectasis.^{5,6} If the foreign body is radiolucent, it cannot be seen on the chest X-ray. For this reason, 33% of children admitted to hospital with foreign body aspiration have normal X-ray results.⁷ Magnetic resonance imaging and computed tomography may show the localization of the foreign body in the lower airways but these procedures take time under emergency conditions.

Although the flexible bronchoscope, suspension laryngoscope, rigid endoscope, and fluoroscopy have all been used in the management of foreign body aspiration, rigid bronchoscopy is the gold standard procedure for removal of foreign bodies. This is an invasive and cost-effective technique but it can cause major complications. Removal of the foreign body using rigid bronchoscopy has a high risk of potential complications, such as complete airway obstruction,



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Case report

Temporomandibular joint alloplastic reconstruction of post-traumatic joint degeneration with Sawhney Type I ankylosis using 3D-custom GD-condylar cap prosthesis to restore condylar form and function

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ABSTRACT

Temporomandibular Joint (TMJ) ankylosis as a sequelae following hemarthrosis from trauma, middle ear infection and progressive debilitating arthritis of various etiologies has been well understood, but challenges always arise in terms of choosing least morbid procedure with maximum functional outcome. Total joint replacement (TJR) is the common final stage correction mandating extensive surgical exposure with good technical expertise with its limitations of risk of failure and complications. A case of post-traumatic TMJ degeneration with ankylosis reconstructed using a customised GD-condylar cap prosthesis is described. The patient had an uneventful post-operative period with an acceptable functional outcome.

Conclusion: The condylar cap prosthesis is a bio-compatible and biomechanically designed in such a way that it can be used for indicated cases by performing minimally invasive surgical technique to achieve an optimal functional and aesthetic outcome.

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1. Introduction

Temporomandibular joint (TMJ) ankylosis is characterised by abnormal immobility and consolidation hindering the normal joint function. There are several etiologic factors but trauma to the TMJ is most common among many others. True ankylosis affects the joint architecture, whereas false TMJ ankylosis is an extra-articular type. Taking into account the severity of TMJ disorganisation, Sawhney divided TMJ ankylosis into 4 radiologically distinguish-

able types [1] with type V being added as a recent modification by Braimah et al. [2].

The management of complete bony TMJ ankylosis is based on Kaban's protocol. The autogenous grafting technique was commonly used but with advancements in material sciences, it is slowly replaced with alloplastic TMJ prosthesis. In the early 1990s, surgeons had experience with stock TMJ devices. However, these had not undergone stringent, pre-market approval for materials testing and/or clinical trials then. The current research and with available bio-materials TMJ-TJR has become an acceptable reconstructive modality. The commonly documented TJR devices include- the Morgan prosthesis, Christensen prosthesis, Kent-Vitek prosthesis, TMJ Concepts, Zimmer biomet, Melbourne prosthesis and DARSN TM Joint prosthesis. Distraction osteogenesis may be considered as a more ideal option, but prolonged activation and consolidation periods lead to compliance issues for neo-condylar regeneration [3–7].

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2. Case study

A 22-year-old male patient reported to the outpatient department of the Facial Surgery Unit, with a chief complaint of progressive restriction of mouth opening for the past four months. Past history revealed trauma to the chin area six months back with a fractured left low condylar neck (Figs. 1 and 2) managed conservatively with three weeks of IMF followed by aggressive mouth opening exercises elsewhere. Extra-oral examination revealed a symmetrical face at rest with deviation of the mandible towards the left side with maximal inter-incisal (MIO) opening at 12 mm. On palpation, pre-tragal inappreciable condylar movement was evident on left side and a click on the right side. Intra-oral examination revealed satisfactory occlusion and hygiene with Class I molar relation.

Open and close mouth TMJ tomogram revealed restricted condylar translation of the right condylar head in relation to the fossa, articular eminence on mouth opening and with no change in position of left condyle in glenoid fossa (Fig. 3). Cone beam computed tomography (CBCT) of the mandible revealed previous fracture line with deviated left condyle along with post-traumatic degenerative changes in the joint architecture.

After complete clinical and radiographical evaluation, a diagnosis of unilateral Sawhney's Type I ankylosis (Turlington and Durr symptomatic Grade 1) [1] of TMJ on the left side was confirmed. Surgical treatment with 3D customised GD-condylar cap prosthesis was planned under general anaesthesia (GA) (Figs. 4–7). The prosthesis computer aided design (CAD) was executed using Geomagic Freeform with the Haptic Touch X software platform. The titanium grade-5 (Ti6Al4V) custom 3D prosthesis was fabricated using direct metal laser sintering technology (DMLS) and was tested for biomechanical parameters using Solidworks finite element analysis model. The standard pre-auricular incision was found sufficient for the surgical implantation of the prosthesis following high condylectomy without altering the lateral pterygoid attachment to the pterygoid fovea.



Fig. 1. Three-dimensional (3D) computed tomographic image showing fracture of the left condylar neck.

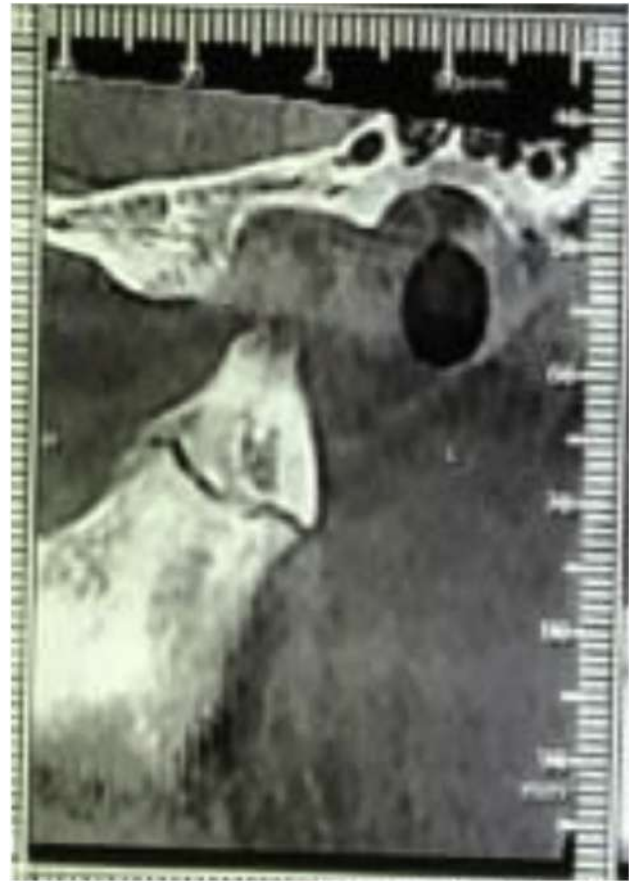


Fig. 2. Radiographic image showing post traumatic degenerative changes in the left temporomandibular joint.

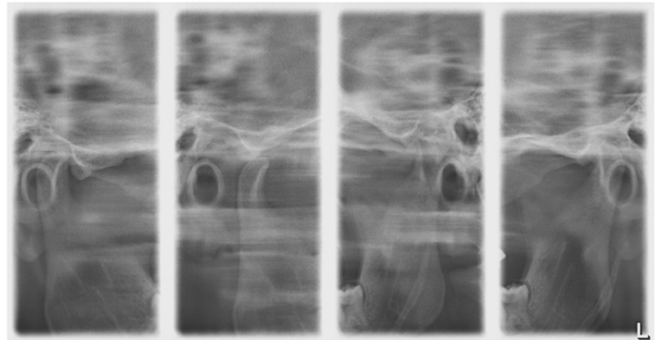


Fig. 3. TMJ tomogram depicting right condylar head translating and is in concurrence with the articular eminence on mouth opening and with no change in position of left condyle.

The postoperative course was uneventful. MIO of 35 mm was achieved post-operatively at six-month follow-up with minimal deviation towards left side (Fig. 8).

3. Discussion

Fixation of low condylar fractures with various mini-plate designs like lambda, delta, trapezoidal, and struts are not only derived out of finite element analysis (FEA) studies for three-dimensional stability but also time tested geometrical designs with

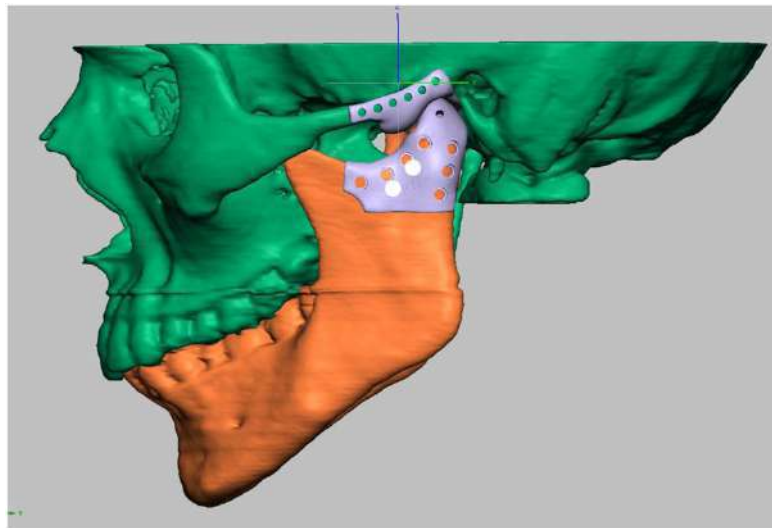


Fig. 4. Computer aided design of the condylar cap prosthesis and UHMWPE fossa component.



Fig. 5. Stereolithographic model showing the UHMWPE fossa component in the glenoid fossa from inferior aspect.



Fig. 6. Stereolithographic model with adapted 3D printed condylar all titanium GD-cap prosthesis.



Fig. 7. Intra-operative image showing the minimal access required to place the fossa as well as the cap prosthesis.



Fig. 8. Post-operative orthopantomogram (OPG) with condylar cap prosthesis in situ.

improved torsional resistance. Incorporating one of these stable fixation geometries into the structural designing of the TMJ prosthesis aids in reducing the self-weight of the prosthesis along with the advantage of drastically reducing the otherwise extensive surgical exposure to single pre-auricular approach. Considering FEA studies on internal condylar fixation implants, the current TMJ cap prosthesis was designed for condylar reconstruction with minimal surgical site exposure and also to reduce disability by not altering the attachment of the lateral pterygoid muscle by virtue of the cap prosthesis design which could not be achieved by the existing commercially available alloplastic TMJ designs.

This case was taken for early definitive surgical intervention with a plan to carefully resect condylar head sparing rest of the bone and securing prosthesis to the neck-ramus region in a lambda plate configuration. Left side ramus height was carefully planned to be 3 mm short as compared to the right with prosthesis in place and a 2 mm high density polyethylene (UHMWPE) slim profile glenoid fossa as an abrasive shield was designed to be anchored to the root of the zygomatic arch. The thickness and length of anchoring bi-cortical screws were well determined with cross sectional CT scan measurements. Surgery was performed via 2.5 cm long pre-auricular incision for the ease of condylectomy and prosthetic implant placement. No post-operative contour discrepancy, facial nerve dysfunction or palpable hardware was noted. With six months of regular follow-up, form and function was found to be satisfactory. Literature evidence documents the existence of customised condylar support prosthesis (CSP) which requires minimal bone removal with the advantage of leaving the residual bone in situ for the revision surgery, if required at a later stage. Such prosthesis design provides more of a physiologic form for load transfer that reduces the stress and strain around the fixation screws [8].

4. Conclusion

Early surgical prosthetic intervention for the above-mentioned case faced considerable criticism and dogma persistently pointing towards autogenous inter-positional arthroplasty. Success rates for autogenous reconstruction of the TMJ vary considerably. Early surgical intervention with micro-detailing into the customised prosthesis helped us achieve near normal form and function.

Funding

Nil.

Ethical approval

Obtained. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from the patient involved in this study.

Declaration

The study was supported by the technical assistance from the TMJ Consultancy Services South East Asia.

Disclosure of interest

None.

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Original Research

Evaluation of Relationship between Bone Density Values and Implant Stability Parameters- In Vivo Study

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ABSTRACT:

The aim of the study was to explore the efficacy of bone density value derived from CBCT by evaluating its correlation with implant stability parameters including insertion torque value [ITV] and resonance frequency analysis [RFA]. A total of 20 patients in the age group of 20- 65 years attending department of prosthodontics, Sibar Institute of Dental Sciences were selected for the study during the period of 2017-2019 with either fully or partially edentulous region. Alpha bio Implant system was used in the present study. Patients were divided into two groups; Group-A and Group-B. In Group- A patients, 10 implants were placed in D1 bone density and in Group B, patients 10 implants were placed in D2 bone density. Osteotomy site was prepared, the implant with dimension 3.75×10mm placed in the osteotomy site. Implant was tightened by torque wrench during placement. Insertion torque values [ITV] were obtained. After placing resonance frequency was checked by osstell mentor. ISQ values were recorded. Implant stability increased as bone density increased. The present study was done to establish the relation between pre-surgical assessment of bone density values derived from CBCT and implant stability parameters and thus estimated the implant survival rate and loading protocol. Within the limitations of the study, bone density showed correlation with implant stability which means as the bone density increases implant stability increases. This suggests that bone quality is one of the factors that require evaluation preoperatively. In the present study the mean ITV and ISQ values were significantly higher in D1 group when compared to D2 group.

Key words: Bone density, primary implant stability, CBCT, Insertion torque, Resonance frequency analysis

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INTRODUCTION:

The success of dental implant treatment is influenced by both the quality and quantity of available bone for implant placement and implant stability. Evolution of CBCT added remarkable changes/advances to the implant dentistry by its way of 3D evaluation which provides accurate measurements and reliable information about bone density, the shape of alveolus,

height & width of proposed implant site. Assessing primary stability immediately after implant insertion is also an important prognostic marker for the success of dental implants. The contact between the implant and the bone is the principle determinant for primary stability which would be estimated by Insertion torque value [ITV] using torque wrench and Resonance frequency analysis [ISQ] using osstell mentor. Hence

this study is being performed to evaluate the presurgical site using CBCT for determination of bone density values and correlating it with implant stability parameters. This assessment would predict the prosthesis loading protocol.

Aims and Objectives:

The aim of the study is to explore the efficacy of bone density value derived from CBCT by evaluating its correlation with implant stability parameters including insertion torque value [ITV] and resonance frequency analysis [RFA].

- To assess the bone density values in the implant recipient site preoperatively.
- To assess insertion torque values [ITV] during implant placement.
- To assess resonance frequency analysis [RFA] immediately after implant placement.
- To compare the bone density values with ITV.
- To compare the bone density values with implant stability quotient [ISQ]
- To predict the possibility of immediate or early loading.

MATERIALS &METHODS:

A total of 20 patients in the age group of 20- 65 years attending department of prosthodontics, sibar institute of dental sciences will be selected for the study during the period of 2017-2019 with either fully or partially edentulous region.

INCLUSION CRITERIA

- D1 & D2 bone density
- Sufficient stable soft tissue architecture at edentulous sites
- Free from infection
- Patients willing to follow recommended plaque control Measures

EXCLUSION CRITERIA

- Uncontrolled diabetes
- Uncontrolled hypertension
- Bisphosphonates⁶
- Osteoporosis

RESULTS:

Table 1: Descriptive statistics

Variable	Bone Density	Sample (n)	Mean	Standard deviation	Range (Min-Max)	Standard Error
ITV	D1	10	49.5	1.58	5 (45-50)	0.50
	D2	10	40	10.54	25 (25-50)	3.33
RFA	D1	10	68.4	4.06	15 (62-77)	1.28
	D2	10	58.6	7.19	26 (40-66)	2.27

- History of radiation therapy in the past 1yr & radiation dosage above 50Gy⁶.
- History of chemotherapy
- Alcohol and smoking
- Tooth extraction in implant recipient site <3 months ago
- Bone augmentation procedures before or during implantSurgery.
- Pregnant and Lactating women
- Under 16 yrs of age
- D3 & D4 bone density

Procedure

- A standardized presurgical CBCT image will be obtained and bone density will be assessed. *Misch bone density classification [Carl. E. Misch, "Contemporary Implant Dentistry", ed 2, St. Louis, 1999, Mosby Inc]*
- A surgical guide or stent is prepared
- After adequate local anesthesia with 2% lignocaine, a midcrestal incision will be given with a No.15 B.P blade. A mucoperiosteal flap elevated and bone will be exposed. A pilot drill of 2mm and sequential drilling will be done with a speed range of 800-1200 rpm.
- Once the osteotomy site is prepared, the implant with dimension 3.75×10mm will be placed in the osteotomy site. Implant will be tightened by torque wrench during placement. Insertion torque values [ITV] will be obtained [*Goswami MM, et al., Evaluation of dental implant insertion torque using a manual ratchet, Medical Journal Armed Forces India (2013)*].
- After placing resonance frequency will be checked by osstell mentor. ISQ values will be recorded [www.osstell.com/scientific-forum].The flaps will be approximated with sutures by No.3.0 silk. And the recorded values will be correlated for a predictable outcome.

Statistical analysis:

Mann Whitney U test was used to assess correlation between Bone density, ITV and ISQ values



Figure 1: Bar graph showing comparison of Mean ITR and RFA values between different bone densities

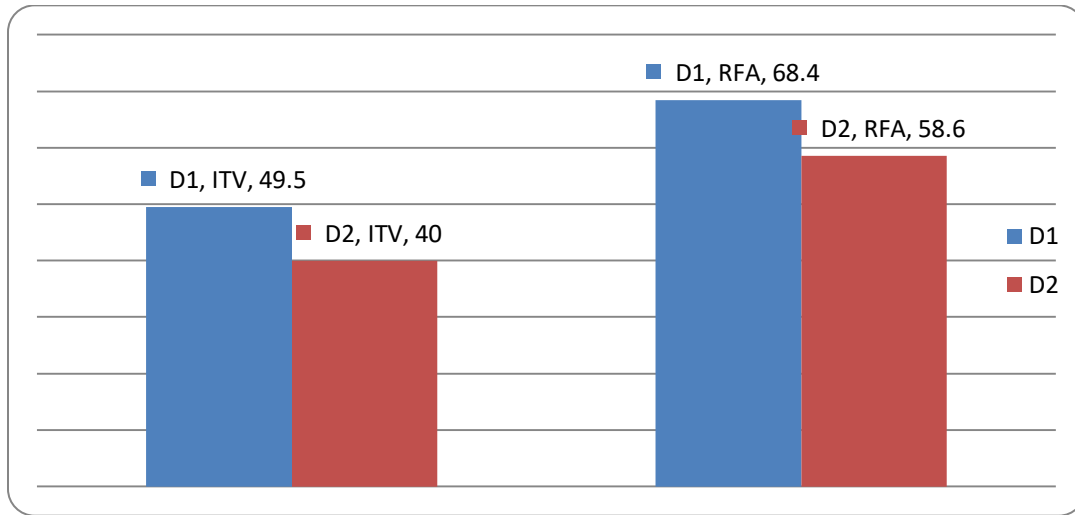


Table 2: Comparison of Mean ITR and RFA values between different bone densities

Variable	Bone Density	Mean (SD)	Mean rank	P value
ITV	D1	49.5 (1.58)	13.25	0.015*
	D2	40 (10.54)	7.75	
RFA	D1	68.4 (4.06)	15.05	0.001*
	D2	58.6 (7.19)	5.95	

Mann Whitney U test; $P \leq 0.05$ considered statistically significant; * denotes statistical significance

It was observed from this study that the mean ITV and mean RFA values were significantly higher in the D1 group compared to D2 group.

DISCUSSION

In the present study, CS3d software has been used to determine bone density preoperatively. Cone-beam computed tomography (CBCT) has achieved its importance in the last few years and has been widely engaged in dentomaxillofacial imaging. CBCT offers various advantages compared to traditional 2D radiography not just in terms of avoiding superimposition but also with its application in multiplanar reformation and face scan marking horizons in real-time imaging. This technique provides relatively high isotropic spatial resolution of osseous structures with a reduced radiation dose compared with conventional CT scans.

The present study was done to establish the relation between pre-surgical assessment of bone density values derived from CBCT and implant stability parameters and thus estimated the implant survival rate and loading protocol.

Implant stability plays a primary role in successful osseointegration, which is a prerequisite for the smooth functioning of dental implants. Implant stability was evaluated at two different stages: Primary and

secondary. Primary stability was obtained from the mechanical engagement of the implant with the cortical bone. Secondary stability developed from regeneration and remodeling of the bone around the implant and was also affected by the primary stability. Measuring implant stability offers better guidance during implant loading.

Ajay Mahajan et al conducted a study to critically evaluate the available scientific data on the influence of insertion torque on implant success. This study includes data on insertion torque, such as primary stability, bone quality and quantity, implant design, and changes in bone-related to high or low torque. At the end of the study, it was found that most of the studies used an insertion torque ranging from 20-45 Ncm. A specific insertion torque value is still challenging to determine as the current evidence suggests the role of various other factors affecting insertion torque during implant placement.

In the present study, the mean primary stability insertion torque values in D1 bone density were 49.5, and D2 bone density was 40. This shows that the mean ITV was significantly higher in the D1 group compared

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to the D2 group.

Lars senner by et al stated that the resonance frequency analysis technique evaluates implant stability as a function of the stiffness of the implant-bone interface and is influenced by factors such as bone density, jaw healing time, and exposed implant height above the alveolar crest. Studies designate that implants with high implant stability quotient values during follow-up examinations are successfully integrated, while low and decreasing implant stability quotient values may be a sign of ongoing implant failure and marginal bone loss. In the present study, the mean primary stability values measured using RFA in D1 bone density were 68.4, and D2 bone density was 58.6. It was observed that the mean RFV was higher in the D1 group compared to the D2 group.

Fuster et al conducted a study to determine bone density value in implant sites using cone-beam computed tomography (CBCT) and to determine possible correlations between age, gender, insertion torque measurements, and resonance frequency analysis (RFA) values. They concluded that bone density measurements using presurgical CBCT might be helpful as an objective diagnostic tool. These values, in conjunction with RFA values and insertion torque measurements, can provide the dentist with an accurate assessment of bone quality and may be especially useful where poor-quality bone is suspected.

Limitations

1. The main limitation of the present study was a smaller sample size.
2. The present study utilized a unified surgical procedure with a single type of implant design. Further studies are required to determine the effects of surgical techniques and implant design, especially the condensing-osteotome method, on primary implant stability.
3. The correlations between follow-up measurements of ISQ and density value from CBCT also need to be addressed.

Potential Risks and Benefits:

The potential risks are comparatively negligible in this study. In this study patient will be exposed to radiation only once as well FOV (Field of view) required is smaller for single implant placement thus radiation exposure is very minimal. Accurate pre-surgical assessment of bone density values, using CBCT and relating with implant stability parameters will provide a better chance for successful implant outcome.

CONCLUSION:

Within the limitations of the study, bone density showed a correlation with implant stability. As the bone density increases, implant stability increases. This suggests that bone quality is one of the factors that


require evaluation preoperatively.

In the present study, the mean ITV and ISQ values were significantly higher in the D1 group when compared to the D2 group.

However, based on available literature and present study evaluation, pre-surgical assessment of bone density values derived from CBCT and implant stability parameters can be considered to estimate the implant survival rate.

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Original Research

Prosthetic Complications in Dental Implants- A Clinical Study

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ABSTRACT:

Background: Complications and failures in dental practice are possible. The mere knowledge of the technique of implant treatment is not sufficient to eliminate all problems. The present study was conducted to assess prosthetic complications of dental implants. **Materials & Methods:** The present retrospective study was conducted on 110 patients who received dental implants of both genders. Type of complications was recorded. **Results:** Out of 110 patients, males were 60 and females were 55. Prosthetic complications associated with dental implants were abutment loosening in 5 cases, abutment fracture in 4 cases, prosthesis screw loosening in 8 cases, prosthesis debonding in 2 cases and ceramic veneer fracture in 9 cases. The difference was significant ($P < 0.05$). **Conclusion:** Authors found that common complication were abutment loosening, abutment fracture, prosthesis screw loosening, prosthesis debonding and ceramic veneer fracture.

Key words: Ceramic veneer fracture, Dental implant, Prosthesis debonding.

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INTRODUCTION

Complications and failures in dental practice are possible. The mere knowledge of the technique of implant treatment is not sufficient to eliminate all problems. The clinical effectiveness of the osseointegration concept introduced by Brånemark and colleagues in the 1960s has revolutionized the clinical practice of dentistry.¹ Dental implants are now the preferred line of treatment for the replacement of missing teeth. Additionally, implant-supported full-mouth prosthesis are a good treatment option for patients who are completely edentulous, achieving a comprehensive and 2 functional oral rehabilitation.²

The failure of dental implants is due not only to biological factors, such as unsuccessful osseointegration or the presence of peri-implantitis, but they also result from technical complications that involve implant body/fixture fracture, abutment screw fracture, abutment fracture, fractured prosthesis, etc.³

The failure of an implant as a single entity, irrespective of its components, may be classified as early or late. Early failures occur shortly after surgery and are characterized by the lack of osseointegration.⁴ In contrast, late failures correspond to those implants that have been regarded as successful for some time, and they occur after prosthetic restoration has been made. There are two main causes for late implant fracture.

Mechanical problems, including fractures— Metal fatigue, due to biomechanical overloading, appears to be the most frequent cause. Loss of supporting tissue secondary to infection or peri-implantitis— The prevalence of peri-implantitis is estimated to be 4-15% among the surviving implant population.⁵ The present study was conducted to assess prosthetic complications of dental implants.

MATERIALS & METHODS

The present retrospective study was conducted in the Department of Prosthodontics, Crown & Bridge including implantology, Sibar Institute of Dental

Sciences, Guntur, Andhra Pradesh, India. It comprised of 110 patients who received dental implants of both genders. Ethical approval from institutional ethical committee was obtained. All were informed regarding the study and written consent was obtained.

Data such as name, age, gender etc. was recorded. A through clinical examination was done in each case on regular recall basis. Type of complications was recorded. Results were tabulated and subjected to statistical analysis. P value less than 0.05 was considered significant.

RESULTS

Table I Distribution of patients

Total- 110		
Gender	Males	Females
Number	60	55

Table I, graph I shows that out of 110 patients, males were 60 and females were 55.

Graph I Distribution of patients

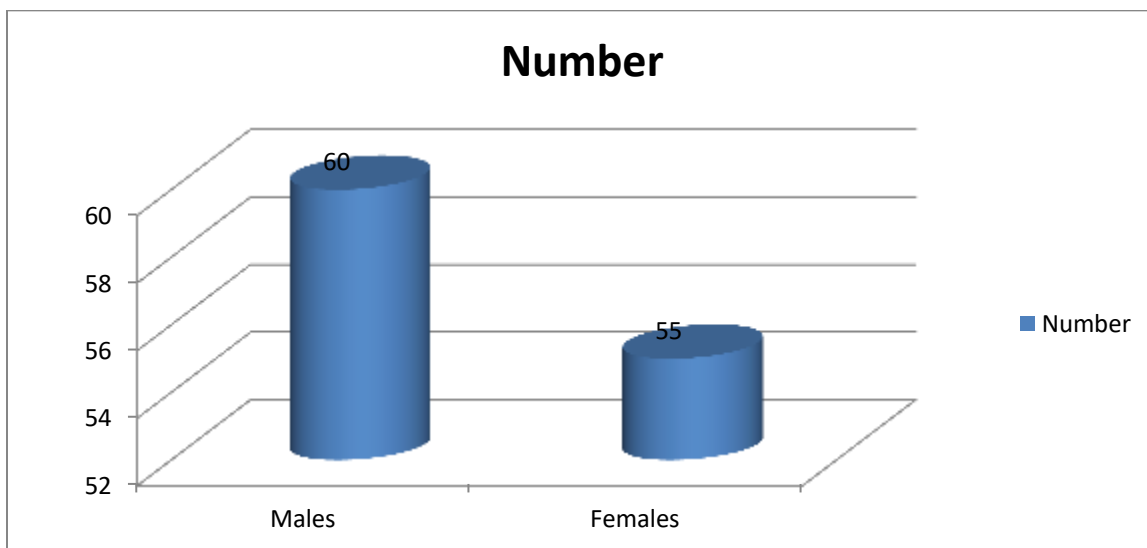



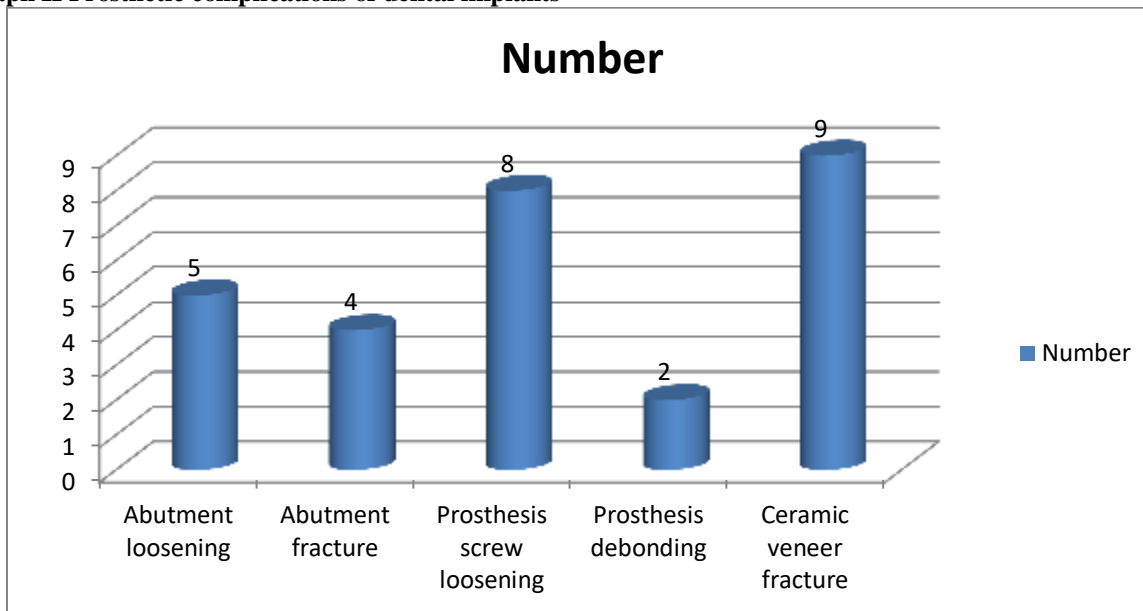
Table II Prosthetic complications of dental implants

Complications	Number	P value
Abutment loosening	5	0.02
Abutment fracture	4	
Prosthesis screw loosening	8	
Prosthesis debonding	2	
Ceramic veneer fracture	9	

Table II, graph II shows that prosthetic complications associated with dental implants were abutment loosening in 5 cases, abutment fracture in 4 cases, prosthesis screw loosening in 8 cases, prosthesis debonding in 2 cases and ceramic veneer fracture in 9 cases. The difference was significant (P< 0.05).


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Graph II Prosthetic complications of dental implants



DISCUSSION

Dental implants are now the preferred line of treatment for the replacement of missing teeth. Additionally, implant-supported full-mouth prosthesis are a good treatment option for patients who are completely edentulous, achieving a comprehensive and functional oral rehabilitation. Implant failures are categorized as primary, when the body is unable to establish osseointegration, or secondary, when the body is unable to maintain the achieved osseointegration and a breakdown process results. The process of osseointegration between the host's bone tissue and the implant is the key to the success of the implant.⁶ The present study was conducted to assess prosthetic complications of dental implants.

In present study, out of 110 patients, males were 60 and females were 55. Sharma et al⁷ classified complications into inflammatory, prosthetic, operative, and major or minor categories. Cox proportional hazards regression models were developed to identify risk factors for complications. The sample was composed of 80 patients. The overall frequency of implant complications was (inflammatory, operative and 3.75% prosthetic), of which were major. The multivariate Cox model revealed that smoking, use of 1-stage implants, reconstructive procedures & placement of dental implant in maxilla were statistically associated with an increased risk for overall complications.

We found that prosthetic complications associated with dental implants were abutment loosening in 5 cases, abutment fracture in 4 cases, prosthesis screw loosening in 8 cases, prosthesis debonding in 2 cases and ceramic veneer fracture in 9 cases. Nancy E. McDermott et al⁸ received Bicon implants (Bicon, Boston, MA) between

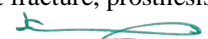
1992 and 2000. The overall frequency of implant complications was 13.9% (10.2% inflammatory, 2.7% prosthetic, 1.0% operative). The present study showed the overall frequency of implant complications occurring after placement of Dental implants was 35% (22.5% inflammatory, 8.75% operative, 3.75% prosthetic). In inflammatory complications, maximum complications were peri-implantitis (10%), followed by impaired wound healing (6.25%), mobility (5%) and pain (1.25%).

Bhagat et al⁹ in their study included a total of 40 subjects. The dental implants were placed by single experienced surgeon so that the surgeon's effect on the rate of complications is minimised. The mean age of the study was 28.34+/- 4.33 years. The study involved 27 males and 13 females. There were 32.5% (n=13) patients in whom 4 implants were placed. In 20% subjects 5 implants were placed. Mucositis were seen in 20% (n=12) subjects. Peri implantitis was seen in 22.5% (n=9) subjects. There were 20% subjects with poor oral hygiene. Crown fracture was seen in 20% (n=8) subjects.

Gallucci GO et al¹⁰ conducted a multicentre prospective study to evaluate the 5 year survival rate and success associated with the use of mandibular implant supported prosthesis. The parameters that were evaluated were Sulcus bleeding index (SBI) at four sites per implant, width of facial and lingual keratinized gingival (mm), peri-implant mucosal level, modified plaque index, mobility and peri-implant radiolucency.

CONCLUSION

Authors found that common complication were abutment loosening, abutment fracture, prosthesis screw


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loosening, prosthesis debonding and ceramic veneer fracture.

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Tooth Implant Supported Fixed Partial Denture

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Abstract

The aim of this review is to summarize and discuss the biomechanical behaviour of the implant and the natural teeth, nature of connection, potential complications associated with splinting of implants and teeth, and guidelines to be followed in fixed partial denture. Implants are connected to the natural teeth in the management of partially edentulous patients. Although implant-supported prosthesis (ISP) has substantial biological and biomechanical advantages, certain potential complications associated with splinting implants to natural teeth were discussed. The articles published only in English, randomized clinical trials, prospective and retrospective clinical studies and computer-generated research were

included. The literature published was searched through PubMed, Medline, Google and indexed journals. The existing studies reveal that there are certain conditions in which this method is applicable. The main advantage of the method based on literature reviewed is reducing the need to the removable prosthesis in patients that otherwise require it. Various complications associated with tooth implant supported prosthesis has been reported with intrusion and implant overloading being the cause of concern. The reports also suggested no significant differences between various types of connections utilized and to use the non-rigid connections with caution. Whenever possible implant supported prostheses should

be the treatment of choice. However, certain clinical situations demand connecting teeth to implants.

Keywords: Fixed partial denture, implant-supported prosthesis, intrusion, nonrigid connection.

Key messages (Provide appropriate messages of about 35-50 words to be printed in centre box):

- The difference in the biomechanical behaviour between Osseo integrated implants and teeth and the efficacy of the different modes of connection that have been employed are explored.
- Evidence based decisions could be made concerning utility of connecting teeth to implants.

Introduction: Implant is connected to natural teeth when there is an anatomic limitation of space for` implants or failure of an implant to Osseo integrate. The advantages of tooth implant supported prosthesis include splinting of a natural tooth to an implant, increased mechanoreception, and additional support for the total load on the dentition. There are methods of attaching natural abutment teeth to an implant. One is rigid connection and the second is non-rigid connection¹. Fixed partial dentures supported solely by implants or by teeth and implants were reported to provide fully satisfactory function and had similarly high levels of predictability¹³.

This review article correlates the studies done on tooth implant connection. Relevant clinical studies written in English were reviewed. **Breeding L, Dixon D, Sadler J, McKay M²** conducted a study on the implant tooth-supported fixed partial denture presents the movement of the natural tooth abutment was not found to change substantially with the fixed partial denture designs tested. **Fugazzotto A, Kirsch A, Ackermann A, Neuendorff G³** conducted a study to examine the incidence of natural tooth intrusion in consecutively placed natural tooth/implant-supported prostheses utilizing screw-fixed attachments over 10 years in 2 practices and concluded

that such a prosthetic design can prevent intrusion of the natural-tooth portion of the prosthesis. **Lindh T, Back T, Nyström E, Gunne J⁴** evaluate the biological and mechanical consequences when implants placed in the posterior maxilla were connected to teeth and concluded that tooth-implant supported prostheses is a safe and predictable treatment. No increased implant failure rate was found for this design. **Block M, Lirette D, Gardiner D, Li L, Finger I, Hochstedler J, Evans G, et al⁵** compared rigidly or non-rigidly connected implant and teeth supported fixed prostheses in a cross-arch model and concluded that the high incidence of intrusion and suggest that alternative treatments without connecting implants to teeth may be indicated. **Gowda S, Quadras D, Sesappa R, Katapadi V, Kumar L, Kulkarni D, et al⁶** evaluate the effect of connector designs on scale and distribution pattern of the stress generated in the supporting bone of implant tooth-supported three-unit fixed partial denture in distal extension situation and recommended that the flexible connector may be placed on the distal aspect of the pontic. **M. Hosny, J. Duyck, D. Van Steenberghe, and I. Naert¹⁴** reported similar levels of bone loss, 1.08mm for the first 6 months and 0.015mm annually, around implants regardless of being connected to teeth or not and regardless of the number of connected teeth or implants.

Discussion

Reasons of connecting tooth to implant:

The reasons of connecting the teeth to the implant are given in four categories:

1. **To maintain proprioception:** Which may help to reduce applied stress to the implants.
2. **The absence of other options:** Because of systemic, local or financial limitations, bone augmentation and insertion of additional implants are not always possible.
3. **To provide stability** against rotational forces.

4. **For aesthetic reasons:** Implants unlike natural teeth always present challenges with regard to aesthetic.

The advantages and disadvantages of connecting the tooth to the implant:

Advantages:

In the literature, the benefits of tooth-implant connection have been listed as follows:

- Broadened treatment possibilities
- Reduced cost (reduction of implant numbers)
- Protective value of proprioception provided by tooth
- Desire to splint a mobile key tooth to an implant
- Additional support for total load on dentition
- Reduction of the need for a cantilever
- Preservation of the papilla adjacent the tooth for aesthetic and phonetic reasons
- More favourable bone reaction when the bridge is connected to both the implant and teeth.

Cavicchia reported that problems such as loosening and fracture of fixation screws and abutments, ceramic fracture and tooth migration seem to occur more frequently in free standing implants compared to the tooth connected restorations. This result can be related to the decrease bite force in tooth-implant supported prosthesis because of tooth related proprioception.

Disadvantages:

- Peri-implantitis
- Tooth intrusion
- Tooth/implant mobility
- Tooth/implant fracture
- Screw loosening⁷.

To avoid this quandary **Clarke et al** has advised:

- i. Selection of the appropriate patient
- ii. The use of rigid connections
- iii. Avoid making coping on teeth which will be used as an abutment

iv. Preparing the abutment to ensure maximum retention and resistance

v. Permanent cementation of prostheses⁸.

The methods of connection of natural teeth and implant are as follows:

Celso Hita-Carrillo has classified the methods of connection into two main groups: Rigid and nonrigid connection. Nonrigid connections could be in the form of attachment or intermobile element (IME).

Types of connection

The type of connection used in tooth implant supported prosthesis is of three types:

1. **Rigid connection:** The tooth is rigidly connected to the implant with a fixed dental prosthesis. (Fig-1)
2. **Non rigid connection:** The tooth is non-rigidly connected to the implant by means of precision attachments, non-precision attachments and telescopic restorations. It acts as a stress breaking element. (Fig-2)

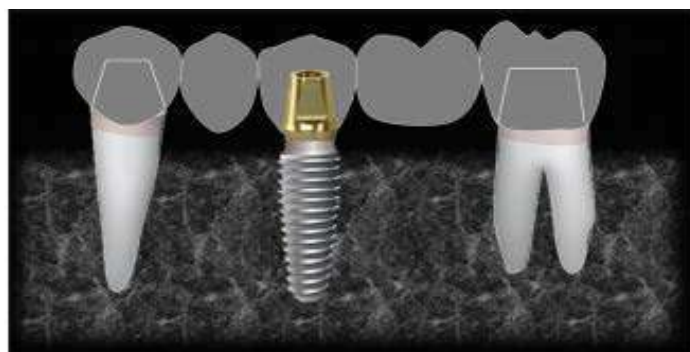


Fig. 1: Coping and superstructure assembly

3. **Resilient connection:** It incorporates a flexible component that simulates the periodontal ligament. It acts as a stress absorbing element.

• Rigid connection

Authors have different opinions about rigid connection. The presented opinions are as follows:

- Some authors believe that rigid connection of the teeth to the implants is not rational due to the adverse effects on the implant in long-term.


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- Rigid connection achieves better outcomes with regard to avoid dental intrusion.
- Finite element analysis showed greater stress concentrations on the neck of the implant and the connector near the tooth.
- Lin CL in 2006 reported micro gap formation between the implant abutment and the fixture under the lateral occlusal forces. The types of such a connection consist of: Rigid screw retained abutments, coping with permanent cement and soldered connectors.

• Nonrigid connection

A. Intermobile elements (IME)

There are few studies about these elements. It has been said that these elements provide flexibility to compensate for the mobility of the tooth.

Uysal in 1996 reported that these elements reduced the strain up to 60% compared to the rigid internal elements. In an in vitro study, it was demonstrated that IME did not contribute to the flexibility of the system and the bending force were transmitted to the retaining screw of the implant abutment.

B. Attachments

It has been mentioned that the attachments reduced the level of stresses in the bone, because it breaks the stress transfer process and more efficiently compensates for dissimilar mobility of the tooth and implant but intrusion in 3 to 4% of the cases has been reported to cause cantilever formation on the implant and increase the unfavourable stress values in the implant and prosthesis.

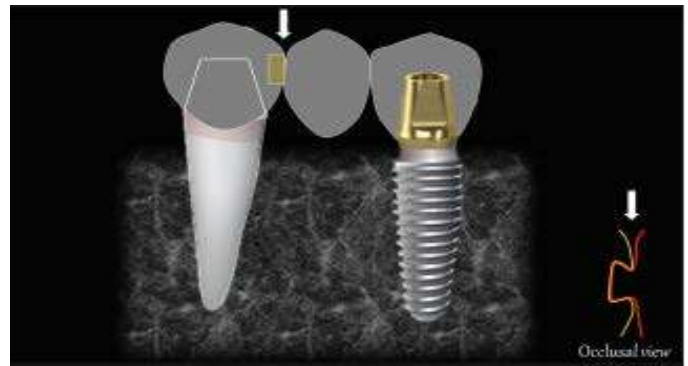


Fig.2: Non rigid key and keyway attachment

Finite element analysis showed stress concentration around the non-rigid connector.

Von Oosterwyck, Naert and Nishimura mentioned that rigid connection compared to free-standing implants or nonrigid connections overstress the implants and result in greater bone loss around the implant; however, along with most of other authors, they expressed their preference for rigid connection over nonrigid connectors.

Hoffmann reported that nonrigid connections drastically reduce the stress on the superstructure while increasing the forces on the supporting teeth and implants¹⁰.

Based on the above studies every surgeon should follow these guidelines for success of the tooth implant supported prosthesis.

The following guidelines can help prevent intrusion of teeth and enhance patient care when contemplating fabricating a TISP:

1. Select healthy teeth—periodontally stable and in dense bone.
2. Rigidly connect the tooth and implant (no stress breakers), employ large solder joints to enhance rigidity, or use one-piece castings. (Fig-3)

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Fig. 3: Metal assemblage of rigidly connected four-unit TISP demonstrating substantial occluso-gingival dimensions of solder joints.

3. Avoid telescopic crowns (no copings). (Fig-4), (Fig-5)

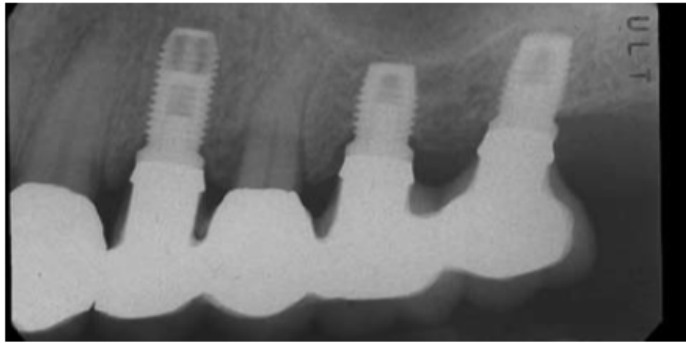


Fig.4: TISP on teeth Nos. 12 through 15. Tooth No. 13 is a natural tooth with a telescopic crown.

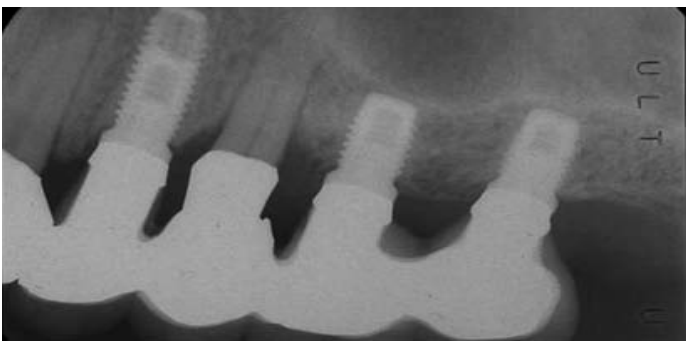


Fig .5: The natural tooth has intruded, and the telescopic crown is now visible beneath the crown.

4. Provide retention form with minimal taper of axial walls on abutment teeth. Enhance resistance form with boxes and retention grooves if the clinical crown is not long. (Fig-6)


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Fig. 6: Intraoral view of four-unit TISP (teeth Nos. 2 through 5); implant is acting as pier abutment No. 4. Note retentive boxes placed on tooth abutment No. 2.

5. Parallel the implant abutment to the preparation of the tooth and use a rigid connection.

6. Use permanent cementation (no screw retention or temporary cementation).

7. The bridge span should be short. Preferably, place one pontic between two abutments. However, with additional tooth or implant support or cross-arch stabilization, additional pontics can be used.

8. Occlusal forces should be meticulously directed to the opposing arch.

9. In general, do not use TISPs in patients with parafunctional habits. If they are treated with TISPs, overengineer the case by maximizing the number of implants and splinting.

10. Cantilever extensions should be used cautiously; however, they may be employed when tooth or implant support is adequate, e.g., cantilever-implant-implant pontic-tooth-tooth. (Fig-7)



Fig.7: Cantilever TISP (teeth Nos. 8 and 9, No. 10 is a natural tooth).

pontic, Nos. 11 and 12 are implants, and No. 13 is a cantilever) with intact interim cement seal at 3 months.

11. TISPs in patients with uncontrolled caries should be avoided; ISPs are preferred. (Fig-8)

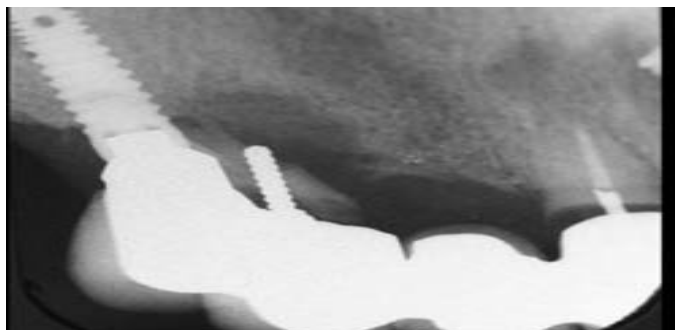


Fig. 8: Patient with TISP at 5 years who demonstrates extensive recurrent caries. Patients with high caries rates would benefit from restoration with an all-implant supported prosthesis.

12. Pulp less teeth with extensive missing coronal tooth structure or root canal anatomy that is inadequate to predictably retain a core or post and core should not be used in a TISP.

13. High-risk TISPs (e.g., multiple adjacent pontics, double cantilevered pontics) or prostheses with minimal abutment support should be expected to have a higher failure rate even though these treatment plans may benefit certain patients. (Fig-9), (Fig-10)

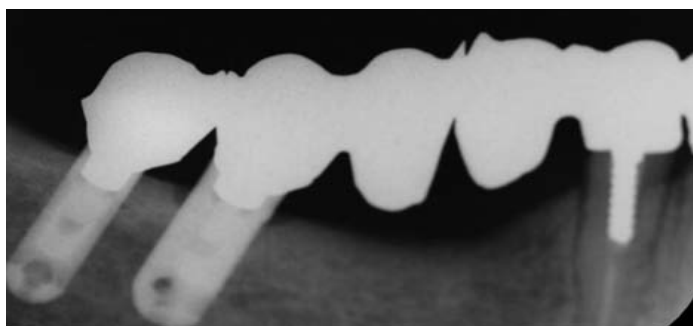


Fig. 9: Radiograph in 1998. A TISP was created with two pontics because the implants used as terminal abutments were placed too posteriorly.


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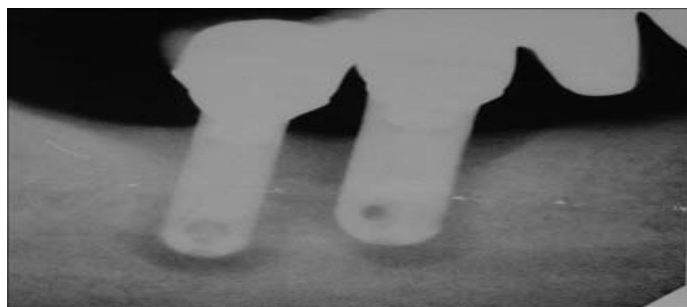


Fig. 10: Radiograph in 2002 demonstrating deosseointegration of both implants supporting the TISP. The patient had parafunctional habits.

14. In the aesthetic zone, if a papilla or papillae is crucial for aesthetics or function (e.g. Phonetics), consider using natural teeth (TISPs) because the supracrestal gingival fibres associated with healthy teeth will provide interproximal soft-tissue support. (Fig-11)



Fig. 11: TISP after 5.5 years where the papilla between tooth No. 11 and No. 12 implant has been preserved by the presence of the tooth. Distal buccal recession around the implant is evident between the implant and cantilever pontic No. 13. The supracrestal gingival fibres of the tooth are a benefit to preservation of the papilla while the implant has no such effect.

15. If appropriate case selection principles are applied (e.g. minimal caries rate, good root anatomy, minimal tooth mobility, adequate retention and resistance form, rigid prosthesis design, adequate overall abutment support for the prosthesis), then combining implants and natural teeth may permit segmentation of a prosthesis into smaller sections, which may provide an alternate treatment plan to a large one-piece bridge¹⁰.

Limitations: Connecting teeth to Osseo integrated implants presents a biomechanical challenge. This is due to the implant being rigidly fixed to the bone with a periodontal ligament. This contributes to a greater mobility of teeth than Osseo integrated implants. In this type of restoration, because of the physiological movement of natural tooth, some amount of movement is expected from within the implant system.

In addition, the amount of support offered by a natural tooth will also be altered. To reduce these torquing forces on the implant, different attachment mechanisms have been proposed by various authors. These methods include:

- a. Key and key-way type attachments (semi-rigid)
- b. Rigidly connected implant and tooth-supported segments
- c. Telescopic attachments¹¹

Conclusion

Joining teeth and implants during the rehabilitation of partial edentulism is indicated to provide clinicians with more treatment options where proprioception and bone volume are maintained and distal cantilevers and free end saddles are eliminated. Whenever suitable and justified, such treatment option becomes a valid alternative especially if it makes the treatment less complex, of less cost, and more acceptable for the patient. This treatment paradigms associated with some risks and complications including loss of osseointegration, periapical tooth infection, tooth intrusion, ceramic fracture, prostheses decementation, and screw loosening. In order to improve treatment success rate, it is better to avoid using short implants, poor bone quality, and endodontically treated teeth when this treatment paradigm is considered. Also, using rigid connection and permanent cementation are associated with less tooth intrusion and less complications. Further research is still required on many aspects of this treatment paradigm¹².

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Original Research

An Evaluation of Stress Distribution in Implant VS Tooth-Supported Mandibular Overdentures - A Finite Element Analysis Study

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ABSTRACT:

The aim of the study is to appraise the stress distribution in tooth-supported and implant-supported mandibular overdentures with different attachment systems. The objective is to evaluate & compare stress distribution between 1) Tooth-supported and implant supported overdentures with the ball attachment 2) Tooth-supported and implant supported overdentures with bar attachments 3) Implant supported overdentures having ball and bar attachments. A three-dimensional finite element solid model of the human mandible was constructed based on CT data. Two mathematical models were created which include the tooth-supported overdenture, implant-supported overdenture that was supported by two mandibular canines and two implants respectively and then the denture was retained using ball attachment and bar attachment. These were done with the help of software ANSYS version 15.0 and hardware of Intel Pentium 4 and Windows XP. The axial 100N loading conditions will be introduced on the anterior region between canines and in the posterior region at molar areas. Then the calculation of Von Mises stresses and comparison of stress distribution between tooth-supported overdenture and implant-supported overdenture retained by ball attachment, tooth-supported overdenture and implant-supported overdenture retained by bar attachment, implant-supported overdenture retained by ball and bar attachment will be done. This study concluded that implant-supported overdentures, the magnitude of stress was higher compared to tooth-supported overdentures. Meanwhile, Ball attachments demonstrated greater stress compared to bar attachment. However, there was no significant difference between the stress magnitudes in both the groups; hence either of them can be used based on the clinical scenario.

Key words: Finite element analysis, Overdentures, Ball and Bar attachments, Von Mises stress.

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INTRODUCTION:

The prime objective of prosthetic rehabilitation is to improve and maintain the quality of life in the patients. This can be accomplished by preventing diseases, improving mastication, relieving pain, enhancing speech and improving aesthetics. A logical method for the dentist in preventive prosthodontics is the usage of overdentures. Treatment of partially edentulous mandible with overdenture supported by one or two remaining teeth has been the treatment modality for many years. In the completely edentulous mandibular arches, treatment with implant-retained overdenture has become a routine therapy particularly in patients who have relentless problems with conventional mandibular denture. Use of this implant-

retained overdenture has advantages like improvement in retention and stability of the denture, preservation of alveolar ridge, increased chewing efficiency and enhanced quality of life with greater satisfaction. Different engineering tools are used for evaluating the stress on implants as it is still not yet possible to assess the stress distribution clinically at bone level. Finite element analysis is a proficient method of providing detailed qualitative data within the model at any location and has become an important analytical tool in dentistry. The proper selection of an attachment system is also a significant characteristic in the success of overdentures. This finite element study aims to compare and evaluate the stresses


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between tooth-supported and implant-supported overdentures retained by ball and bar attachments

Aim: To assess the stress distribution in tooth-supported and implant-supported mandibular overdentures with different attachment systems

Objectives: To evaluate & compare stress distribution between

- 1) Tooth-supported and implant supported overdentures with the ball attachment
- 2) Tooth-supported and implant supported overdentures with bar attachment
- 3) Implant supported overdentures having ball and bar attachments

Materials and methodology:

This study was conducted in the Department of Prosthodontics, Sibar institute of dental sciences with the help of the institute CAD-CAM experts, Vijayawada.

Mandibular bone construction:

A three-dimensional finite element solid model of the human mandible was constructed based on CT data (Fig 1). Numerous investigations state that to assess the stress distribution around the tooth or dental implants, it is not necessary to build a finite element model of the entire jaw structures.

In this study mandibular jaw was created only up to the position where the complete denture extends i.e ramus part of the mandibular jaw was extruded in the design.

Finite element models:

Two mathematical models were created which include

1. Tooth-supported overdenture that was supported by two mandibular canines and the denture was retained using ball and bar attachment

2. Implant-supported overdenture that was supported by two implants and the denture was retained using ball and bar attachment.

Tooth model construction:

Mandibular canines were considered for the study. These were modelled based on the patient's original data obtained from the CT data set. Dimensions of the canines include- 11mm root portion height and as it was an overdenture abutment, the height of the clinical crown was taken only about 2mm. The periodontal membrane width was taken as 0.2mm.

Implant model construction:

Two implant models were created with dimensions of 3.5mm width and 11.5mm length. Solid, Screw type commercially pure titanium implants were selected for the study. They were placed in the mandibular bone at the region of canines 8mm from the midline.

Attachment models:

Ball type- Overdenture was connected to the teeth in the tooth-supported prosthesis and to the implants in implant-supported overdenture by two ball abutments which were modelled with a diameter of 2.25mm and length 3mm.

Bar type- Overdenture was connected to the teeth in the tooth-supported prosthesis and to the implants in implant-supported overdenture by two bar abutments which were modelled with a diameter of 2mm.

Prosthesis model construction:

The denture contour was obtained from a photographic image of a demonstration model of mandibular complete denture and then it was modified and modelled accordingly.

All materials used in the models were considered to be isotropic, homogenous, static and linearly elastic.

Table 1: Elastic properties of the members:

Material	Elasticity(MPa)	Poisson's ratio
Dentin	18.600	0.31
Periodontal ligament	2	0.45
Cortical bone	13.700	0.30
Cancellous bone	1.370	0.30
Titanium	103.400	0.35
Co-Cr alloy	21800	0.33
Acrylic resin	26.500	0.35

Table 2: Elements and nodes:

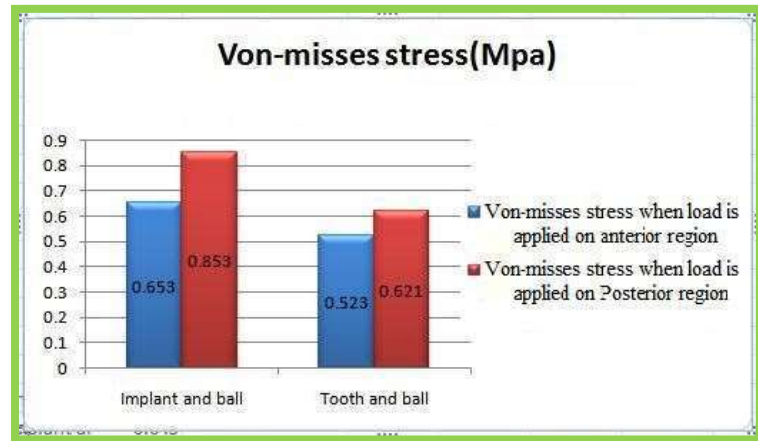
Model	Elements	Nodes
Tooth and ball	12492	23252
Tooth and bar	12625	23246
Implant and ball	12918	23542
Implant and bar	13380	24675

- For the implant-supported models, the conventional implants were virtually placed in the mandibular canine regions 8mm from the midline.
- All the models were converted into four nodes of the tetrahedral element type in finite element analysis ANSYS software.

- The friction coefficient, μ , for all contacting surfaces was set at 0.3 to simulate an immediate loading condition. The threaded part of the implant body was simulated via contact properties accordingly and was assigned a friction coefficient of 0.5 to represent the strong attachment to the bones.
- The axial 100N loading conditions are introduced on the anterior region between canines and in the posterior region at molar areas followed by the calculation of Von Mises stresses and comparison of stress distribution between tooth-supported overdenture and implant-supported overdenture retained by ball attachment, tooth-supported overdenture and implant-supported overdenture retained by bar attachment, implant-supported overdenture retained by ball and bar attachment.

Results:

- 1) The magnitude of stress in tooth-supported and implant-supported overdenture retained by ball attachment (fig12, 16)



Graph 1

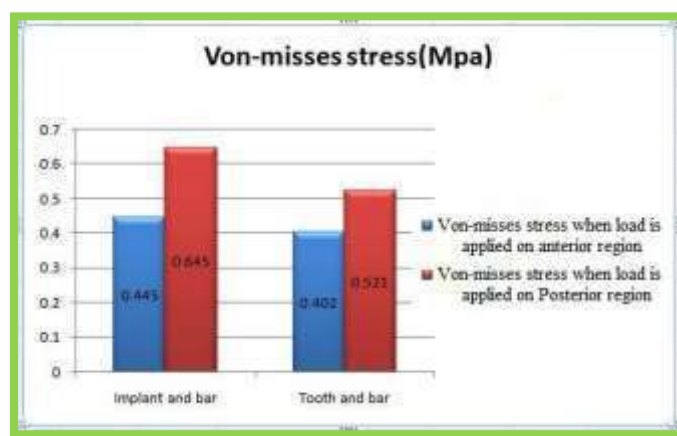
Order of magnitude of stress in overdenture when the load is applied anteriorly

Implant-Ball > Tooth-Ball

Order of magnitude of stress when the load is applied posteriorly

Implant-Ball > Tooth-Ball

- 2) The magnitude of stress in tooth-supported and implant-supported overdenture retained by bar attachment



Graph 2

Order of magnitude of stress in overdenture when the load is applied anteriorly

Implant-Bar > Tooth-Bar

Order of magnitude of stress when the load is applied posteriorly

Implant-Bar > Tooth-Bar


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TABLE 1: Comparison of the Magnitude of stress (Mpa) for tooth-supported and implant-supported overdentures retained by ball and bar attachments

	Anterior region	Posterior region	Mean
Tooth-Ball	0.52	0.62	0.83
Tooth-Bar	0.41	0.52	0.67
Implant-Ball	0.65	0.85	1.07
Implant-Bar	0.44	0.64	0.76

Results showed that there was an increase in the magnitude of stress when overdentures were supported by implants rather than natural tooth. Whilst for retention mechanisms, ball attachments showed greater stress compared to bar attachment

Discussion:

Preventive Prosthodontics accentuates the importance of any procedure that can delay or eliminate future Prosthodontic problems. The overdenture is a reasonable method for the dentist to use in Preventive Prosthodontics. They are mainly classified as tooth supported and implant supported overdentures.

A range of new prosthetic anchoring options will be available for the removable prosthesis by the placement of implants in the most favourable strategic position in the oral cavity. It offers a better alternative for elderly patients especially with resorbed ridges in whom the retention and stability of conventional dentures may be hampered. This concept has been effectively used for about 30 years.

Many number of implants can be used to retain overdentures either by splinting or freestanding. Various studies showed higher implant survival rates when mandibular overdentures were retained by either two or four implants. A study conducted by Galluci et al. states that retaining mandibular overdentures by two unsplinted implants in canine regions is as successful as four splinted implants regardless of immediate loading or delayed loading.

Naert stated that when ball, bar, and magnet attachments were compared regarding the soft tissue complications and patient satisfaction, ball attachment was considered to be the best of them.

Van Kampen in 2003 affirmed that ball and socket attachment had higher retention when compared to bar/clip and magnet attachment.

Implant failure always presents a certain amount of disappointment for both the patients and dentists despite its high success rate. Many biomechanical evaluations state that implant overload is the main factor for cortical bone loss. Stresses that are generated from these loads are transmitted from attachments to natural tooth, implants and supporting tissues. The amplitude and also the intensity of bone loss are determined by means of stress distribution and transmission from each attachment system. A positive prognosis requires correct selection of attachments not only based on retention but also in the biomechanical aspect.

Different bioengineering studies have verified the biomechanical characteristics of prosthesis and

implants and many engineering tools have been used to assess and quantify the implant stresses and deformation of the components. Clinically, it is not possible still to evaluate the stress distribution of implant retained overdentures at bone level but only at abutment level through strain gauge analysis.

FEA provides an important contribution to clinical safety when the bone anchored prosthesis is used as it explains the mechanism and safety margins of transfer of load at the interface with emphasis on the actual anatomical situation. The finite elemental analysis was chosen for the present study as it has proved to be a useful tool in estimating stress distribution in the bone.

This study was conducted to gain more insight into the stress distribution on tooth supported overdentures and implant retained overdentures with different attachment systems by using FEA.

Four finite element models were created which include tooth supported overdentures retained by ball attachment and bar attachment; implant retained overdentures retained by ball and bar attachment. Axial loads of 100N were applied in the anterior region between the canines and in the posterior area at the region of molars.

According to the study, stress around the tooth supported overdentures retained by ball attachment was about 0.83 and of bar attachment was 0.67. In the case of implant-retained overdentures, stress for ball attachment was 1.07 and for bar attachment 0.76. These results showed that highest peaks of stress were observed in implant-ball model followed by tooth-ball, implant-bar, and tooth-bar.

A rigidly anchored implant overdenture which was assumed to be 100% osseointegrated provides a hard over denture support foundation compared to a healthy periodontal ligament supporting natural teeth which has a cushion-like effect. It explains why the stress contour was less in tooth-supported prosthesis compared to the implant prosthesis. The viscoelastic properties of periodontal ligament also play a crucial role.

This result was consistent with the study done by Paek J-H et al. in 2011 to evaluate stress distribution in mandibular implant-supported overdentures and tooth-supported overdentures with telescopic crowns. Results showed that the implant group had more stress than natural teeth.

A consistent amount of stress was observed with ball attachment compared to bar attachment. So, bar attachment may be considered to be a favourable attachment system due to its potential to dissipate the

stresses uniformly between both the implants with its splinting effect. This result was consistent with some of the studies done previously.

Menicucci G in 1998 did a study to relate peri-implant bone stress and reaction forces on the edentulous ridge mucosa to two types of anchorage- ball and clips/bar. Stress in the peri-implant bone was seen in cortical layers around the neck and bottom of implants. Working side implant showed less stress than non-working side implant. Ball anchorage showed greater stress than clips/bar anchorage. In case of clips/bar anchorage, stress was high at cortical bone, but with ball anchorage, it was within the central part of the mandible between implants.

Certain limitations of the finite element study should be taken into consideration. Viz., material properties used in the analysis was simplified and assumed to be homogeneous, isotropic and linearly elastic. The resultant stress values obtained may not be accurate quantitatively but are generally accepted qualitatively. Due to the limitations pertaining to the study, further research should be done biomechanically combined with long-term clinical evaluation.

Conclusion:

The following conclusions were drawn from the study:

- ✓ For implant-supported overdentures, the magnitude of stress was higher compared to tooth-supported overdentures
- ✓ Ball attachments demonstrated greater stress compared to bar attachment.

But there was no significant difference between the stress magnitudes in both the groups; hence either of them can be used based on the clinical scenario.

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Comparative Evaluation of Microleakage In Full Veneer Metal Crowns Luted With Various Glass Ionomer Based Cements – An Invitro Study.

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Abstract

Aim: The aim of the study was to assess, compare and evaluate the microleakage at the margins of full veneer metal crowns cemented with two luting agents.

Materials: (A) Human mandibular third molars, normal saline solution, (B) Medium inlay casting wax, die spacer— die: master gold Renfert GmbH, Germany, (C) Phosphate-bonded investment material- BEGO, DIN EN ISO 15912, Germany, nickel-chromium base metal alloy, (D) Auto mix glass ionomer cement (GIC) G-CEM Link Ace (G.C. Corporation Tokyo, Japan) Manual mixing

luting and lining glass ionomer cement (GIC) (G.C. Corporation Tokyo, Japan),(E) 5% basic fuchsin dye, and (F) Acrylic resin, Cast metal cutting device with diamond disc and external water spray, Stereo microscope with the image analyzing unit.

Methods: A comparative study was done to evaluate the ability of two contemporary luting agents to resist microleakage in cemented nickel-chromium complete metal copings cast on prepared mandibular third molars. The copings were placed back on the respective prepared teeth to check the fit and marginal adaptation was

observed under an stereo microscope. The castings were cemented with the two luting agents under study, namely, Manual mixing glass ionomer cement, G-CEM Link Ace under ideal conditions. Cemented specimens were thermocycled after 24hrs between 60° (± 2°) C . After thermocycling, the teeth they were treated with 5% basic fuschin dye solution for 60 minutes, embedded in clear acrylic resin and sectioned buccolingually.

Results: It was concluded from the study that the Auto mix glass ionomer cement showed less microleakage than the manual mixing luting and lining glass ionomer cement.

Keywords: Luting agents, microleakage

Introduction

Dental luting cements form the link between a fixed restoration and the supporting tooth structure. Luting cements play a pivotal role in sealing the margins and prevent marginal leakage. Unfortunately, most of the dental cements available cannot guarantee continual impermeability because of their relatively high solubility in oral fluids. A gap thus created at the restoration margin may become a repository for microorganisms that release toxic products. These toxins may eventually cause gingival and pulpal inflammation, leading to secondary caries.(1)

Microleakage is defined as the seepage of oral fluids containing bacteria and debris between a tooth and its restoration or cement layer. Microleakage is of concern because of the effect bacteria may have on the remaining tooth structures and the pulpal tissues. The process of microleakage can affect the tooth- cement interface associated with a crown restoration as well as the tooth foundation. Different luting agents vary considerably in solubility, strength and ability to adhere to tooth structures, therefore marginal gaps alone may not be the most important cause of microleakage.

Over the last two decades, a lot of interest has been focused on the selection of good adhesive luting agents for the cementation of fixed restorations. Investigators and researchers have advocated different materials and methods to improve the retention of the fixed prosthesis in the oral cavity. Considerable evolution has taken place from ionomer-based luting agents to resin-based adhesive luting agents. With wide acceptance of base metal alloys, numerous luting agents have been used for the cementation of fixed restorations.

Therefore, it is important to assess and evaluate microleakage at the margins of cast metal copings so that it may be prevented.

Materials and Methods

A comparative study was done to evaluate the ability of two contemporary luting agents to compare and evaluate the marginal leakage under cemented nickel- chromium complete metal copings cast on prepared mandibular third molars. Tooth preparation was carried out on the mounted molars by following standardized tooth preparation procedures with help of aerator. An assembly with the airtor mounted on the dental model surveyor was used to achieve a uniform taper of six degrees. The prepared teeth were cleaned with pumice and water. Wax patterns were to be prepared for each prepared tooth. Three coats of the die spacer were applied on the tooth to provide space of 24-25µm for the cement layer. Care was taken to keep it short of the margins by 1 mm. The wax patterns for the copings were fabricated using the dip wax technique to get a close adaptation of the wax to the tooth surface. The casting was carried out in the casting machine with nickel-chromium base metal alloy.

The copings were placed back on the respective prepared teeth to check the fit and the marginal adaptation was observed under an stereo microscope. The castings were cemented with three luting agents, namely, a manual mix

glass ionomer cement, a G-CEM LINK-ACE Automix GIC (Figure 1,2). The cemented specimens were thermocycled after 24 hours between 60° (± 2°) C. After thermocycling, the teeth were treated with 5% basic fuschin solution for 60 minutes. The samples were embedded in clear acrylic resin (Figure 3) and allowed to set for 24 hours. After sectioning of the samples the sections were observed under the stereomicroscope (figure 5) and stain penetration was recorded at the tooth-cement interfaces.

The marginal leakage (amount of dye penetration) observed for each sample is scored 0,1,2&3, respectively, based on the following criteria.

Score	Criteria
0	No evidence of stain penetration at the interface of the crown and tooth surface.
1	Evidence of slight stain penetration-less than half the height of the axial wall of the preparation.
2	Evidence of stain penetration equal to half the height of the axial wall of the preparation.
3	Evidence of stain penetration above half the height of the axial wall and extending to the occlusal aspect of the preparation.

Thus obtained score for each sample is noted and then statistically analyzed by the Mann Whitney U test for the comparative evaluation of the microleakage.

Results

It was concluded from the study that the Auto mix glass ionomer cement-GCEM LINK-ACE (figure-1) showed less microleakage than the manual mix glass ionomer cement scores were tabulated from table 1-3.

Table 1: Microleakage mean scores, standard deviation (S.D.), standard error (S.E.), and median of the study samples

Statistic	Auto mix GIC		Powder form GIC	
	Buccal	Lingual	Buccal	Lingual
Mean	0.76	0.72	1.44	1.52
SD	0.72	0.45	0.917	0.918
SE	0.145	0.092	0.183	0.184

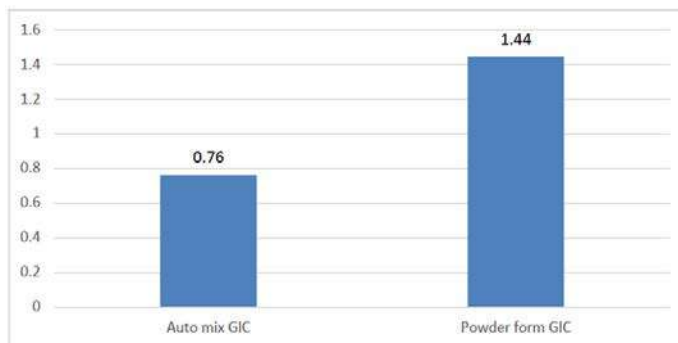
Table 2: Comparison of microleakage between the two groups on the buccal aspect

Group	Mean	SD	Mean Rank	Test statistic	P-value
Auto mix GIC	0.76	0.72	20.42	185.5	0.001*
Powder form GIC	1.44	0.917	30.58		

Table 3: Comparison of microleakage between the two groups on the lingual aspect

Group	Mean	SD	Mean Rank	Test statistic	P-value
Auto mix GIC	0.72	0.45	19.62	165.5	0.001*
Powder form GIC	1.52	0.918	31.38		

Graph 1: Table 2: Comparison of microleakage between the two groups on the buccal aspect



Graph 2: Comparison of microleakage between the two groups on the lingual aspect

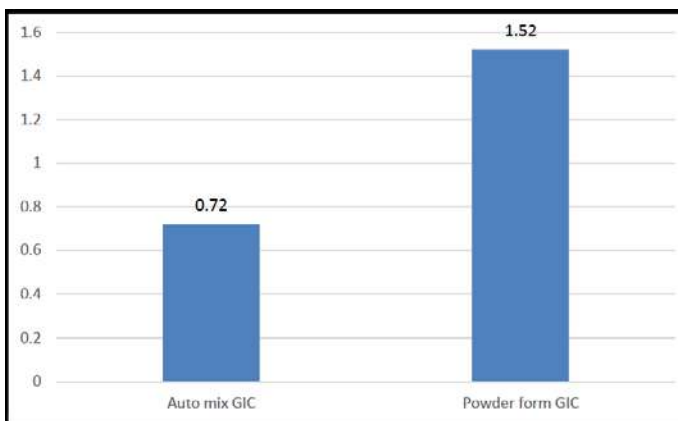


Figure 1: Auto mix GIC



Figure 2: Manual mix GIC

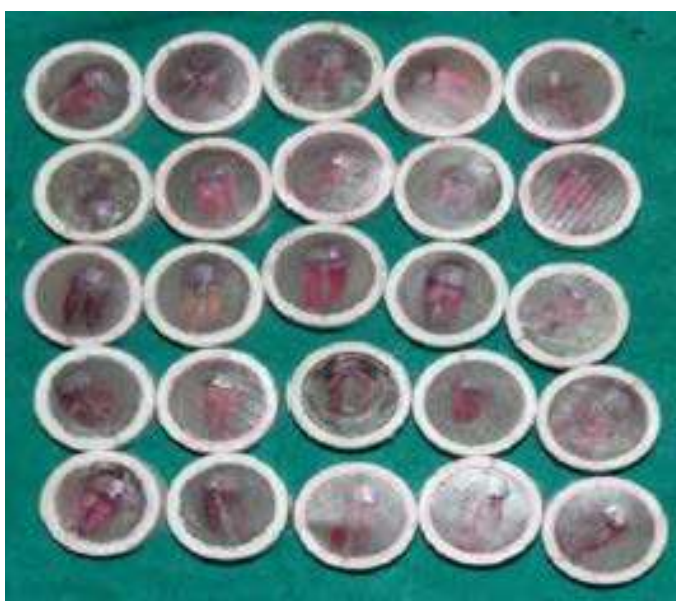


Figure 3: stained samples embedded in clear Acrylic resin

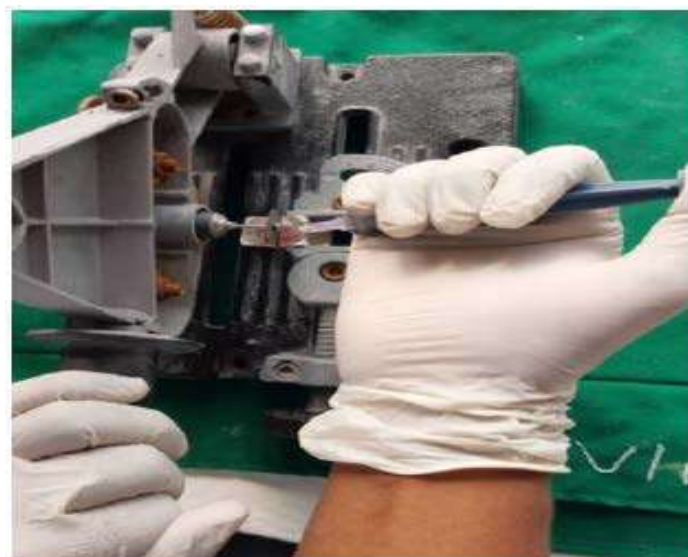


Figure 4: Sectioning of samples with diamond disc

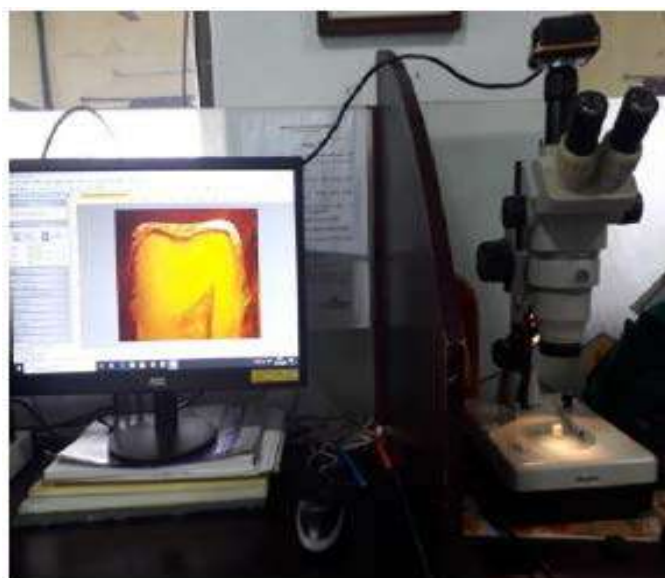


Figure 5: Sectioned samples observed under stereomicroscope

It was found in the present study that the microleakage was significantly higher in manual mix GIC (figure-2) group on the buccal and lingual aspect compared to Auto mix GIC (figure-1) group. Mann Whitney U test was employed as the data violated the assumption of normality as assessed from Kolmogorov Smirnov test and the difference between the groups was found to be statistically significant.

Discussion

Proper selection of a luting agent is an important decision in a series of steps that require meticulous execution and will determine the long-term success of fixed restorations. Previously only one luting agent is available for restoration and luting procedure, i.e. zinc phosphate cement, nowadays plenty of luting agents are available. The proper cement selection should be based on knowledge of physical properties, biological properties and other attributes of both restorative materials and luting agents.

This study aims at providing an overview of currently available luting agents (cement) and discusses their properties, advantages and disadvantages.

In the search for a material that bonds chemically to the tooth structure, it is essential to investigate its leakage-inhibiting potential. In most of the studies evaluating microleakage beneath full-cast crowns, investigators have used organic dyes, chemical tracers or radioactive isotopes.

The use of organic dyes as tracers has been one of the oldest and perhaps the most commonly used method in microleakage detection. However, it is highly technique-sensitive and the assessment of results requires careful standardization. The choice of whether to use a stain, dye or isotope should be based on the preference of the investigator.(4) All methods require meticulous technique and standardized criteria for evaluating and scoring the degree of marginal leakage. Radioactive isotopes require special handling and authorization for use. The use of a stain or a dye as a tracer may be more convenient for the researcher because no authorization is required for their possession and use. Therefore, 5% basic fuschin dye was used in this study.

This study describes the two different forms of glass ionomer cements Auto mix glass ionomer cement, Manual mixing luting and lining glass ionomer cement (GIC)). Manual maxing GIC cement possesses advantages of Chemical bonding, Sustained fluoride release and ability to absorb fluoride from the oral environment (fluoride recharge) makes it the cement of choice in patients with high caries rate.

Disadvantages include Initial slow setting and sensitivity to early moisture contamination and desiccation, Modulus of elasticity lower than zinc phosphate, so the potential of elastic deformation in areas of high masticatory stress.

Initial low setting pH was assumed to be associated with post cementation sensitivity. Dentin desiccation, thin cement mix together with an excessive hydraulic force,

and microleakage may sometimes be responsible for the sensitivity, Insufficient wear-resistance.

The greater leakage of the manual mix GIC compared to the Auto mix glass ionomer cement (G-Cem LINK-ACE) might be thus attributed to the coefficients of thermal expansion of the material involved (*i.e.*, tooth substance, cement, metal crown).

One possible reason for the reduced microleakage with the glass ionomer cement can be its low solubility and disintegration in oral fluids.

To better correlate thermocycle testing in *in vivo* conditions, the system used in this study used a short exposure time to the extremes of temperature with an adequate intervening period for the specimens to return to body temperature. Previous studies have generally used immersion times, which allow both the tooth and the restoration to stabilize at the extreme temperatures, which does not occur *in vivo*. The rationale for the cycling sequence used in this study is that the maximum *in vivo* exposure time of a tooth to an extremely hot or cold material is considered to be two to five seconds after which the tooth returns to the oral temperature.

There is, as yet, no ideal dental cement. Each material is to be used depending upon its merits, demerits and limitations. It has long been recognised that in general, dental materials do not bind or adapt to tooth structure well enough to provide a perfect seal and there is always a pathway for penetration of various solutes and solvents.

Conclusions

Within the limits of this study, the following conclusions can be drawn

1. No strong correlation was found between margin fit parameters and microleakage
2. None of the cementing agents investigated in this study yielded a perfect seal at the bonding interface in enamel or dentin.

3. Auto mix glass ionomer cement (GIC) G-CEM Link Ace revealed the smallest degree of microleakage both in enamel and in dentin when compared to manual mixing luting and lining glass ionomer cement.
4. Under a stereomicroscope, it was observed that the microleakage of basic fuchsin dye was between the cement layer and the tooth surface.
5. Marginal microleakage is only one among the various factors, which influence the success of the cemented restoration.
6. The choice for a particular cement depends entirely on the judgment of the clinician based on the appropriate oral environment, biological and mechanical factors, which the clinical situation demands.

Limitations

- 1) In this study, only two types of dental luting cements were evaluated.
- 2) Microleakage was not observed between the crown and the cement interphase.
- 3) Only the sealing property of the luting cement is evaluated with no relation with the marginal fit of the crown
- 4) To better correlate thermocycler testing in *in-vivo* conditions. The system used in this study had short exposure time with an adequate intervening period for the specimens to return to body temperature.

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Maintenance of Gingival Profile in the Esthetic Zone using Natural Tooth Pontic

Abstract

The extraction of periodontally compromised teeth in the anterior esthetic region is a challenging situation due to patients' psychological and esthetic demands. Irrespective of the replacement of missing teeth with the final prosthesis, the first line of management would be to provisionally restore the teeth at the earliest. Routine treatment options for replacement are time-consuming and expensive. Using the patient's natural tooth as a pontic offers the benefits of the same size, shape, color, and preservation of the gingival architecture. Using the patient's platelet concentrate (platelet-rich fibrin) facilitates early wound healing and preservation of the alveolar ridge shape following tooth extraction. With minimal or no preparation, the technique can be completed at the chairside, thereby avoiding laboratory costs. This case report details the procedure with a follow-up of a case where the natural extracted tooth of the patient was used as pontic to replace a missing anterior tooth.

Keywords: Esthetics, gingival contour, natural tooth pontic, periodontitis, platelet-rich fibrin, resin-bonded bridges

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Introduction

Advanced periodontal disease is one of the reasons for the removal of teeth in the anterior region. Restoration of these lost teeth in the anterior region is a prime concern due to esthetics and maintenance of gingival contour.^[1] Cast partial dentures, removable partial dentures, fixed partial dentures, and implants may be planned as a permanent replacement after the tissues have healed. Limited options are available for an excellent esthetic temporary prosthesis. Removable partial dentures delay wound healing due to bulky nature if placed right away after tooth extraction.^[2] Immediate implants can be chosen whenever there are good quality bone and no residual infection after extraction.^[3]

Considering all the possibilities, natural tooth pontics (NTP) are the best alternatives to maintain soft-tissue contour during healing, and due to the perfect color match, size, and shape. NTP satisfy the esthetic and psychological demands of the patient.^[4] With the emergence of various composite materials, NTP can be modified and bonded to the adjacent natural teeth using these composite materials solely or in combination with orthodontic wires.^[5]

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Case Report

A 39-year-old female patient with complaints of the loose tooth and swollen gums in the upper front tooth region associated with difficulty while mastication reported to the Department of Periodontics. On examination, Grade III mobility with a probing pocket depth of >10 mm and suppuration was observed in relation to the maxillary right central incisor [Figure 1a]. Orthopantomogram (OPG) revealed an extensive bone loss in relation to 11, suggesting a hopeless prognosis [Figure 1b]. Both clinical and radiographic examination indicated an extraction of 11. Scaling and root planing was done and the patient was recalled after 1 week for extraction of 11 as the patient was not willing for any endodontic treatment. Before the procedure, the position of the tooth in the arch and its relationship with the adjacent teeth was evaluated. Atraumatic extraction of the tooth was done under local anesthesia. The extraction socket was curetted and irrigated with antiseptic povidone-iodine to remove the granulation tissue [Figure 1c]. Care was taken to remove the granulation tissue completely and to create fresh bleeding in the socket. Five milliliter venous blood was collected from the antecubital fossa, platelet-rich fibrin (PRF) membrane was

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prepared using a centrifuge machine, and this membrane was placed in the extraction socket [Figure 1d].

Preparation of the extracted tooth

The extracted tooth was cleansed using an ultrasonic scaler to remove any surface debris and adherent granulation tissue. The root portion of the tooth was reduced using a rotor to the desired length [Figure 2a]. An access cavity was prepared, and all the pulpal remnants were removed and irrigated using a sodium hypochlorite solution. Then, the orifice was closed with composite resin. At the cervical region of the extracted tooth modified ridge lap pontic was designed to facilitate self-cleansing and appearance of an emergence profile.

On the lingual side, at the level of cingulum, a groove was created with the bur to accommodate the orthodontic wire. The NTP and abutment teeth were etched with 37% phosphoric acid for 30 s and washed off [Figure 2b]. Then, the bonding agent was applied to the tooth and splinted the NTP to the adjacent teeth using light-cured composite resin [Figure 2c]. The following finishing and polishing the occlusal relationship was evaluated, and occlusal interferences were removed if any.

Oral hygiene instructions were reinforced to the patient and followed up for 1 year [Figure 3a and b].

Discussion

Tooth loss due to trauma, periodontal disease, and failed endodontic therapy in the esthetic zone creates a challenging situation to the dentist and requires immediate treatment due to the psychological and esthetic demands of the patient.^[6] When the tooth is planned for extraction due to periodontal disease in the anterior region, the primary concern is esthetics. Due to increased esthetic demands from the patient, the dentist relies on esthetic principles while operating in esthetic areas considering patients' economic status.^[7] Quirynen *et al.* conducted a study by comparing the acrylic tooth pontics and NTP placed after the loss of tooth due to periodontal disease and followed for 5 years. They concluded that NTP can be regarded as a semipermanent solution for the replacement of the lost natural tooth.^[8] The major concern of placement of NTP is the psychological advantage of the patient and maintaining the soft-tissue profile for further permanent restoration.

In the present case, a NTP was planned as an immediate replacement for the missing anterior tooth because of patient esthetic demands and considering her socioeconomic status. Immediately after extraction, the NTP was splinted to the adjacent teeth for the preservation of gingival architecture. Composite resin and orthodontic wire were used to splint the pontic to the abutment teeth. The rationale for placing PRF membrane in the extracted socket is that platelet growth factors in PRF exhibit chemotactic and mitogenic properties, which promote cellular functions involved in

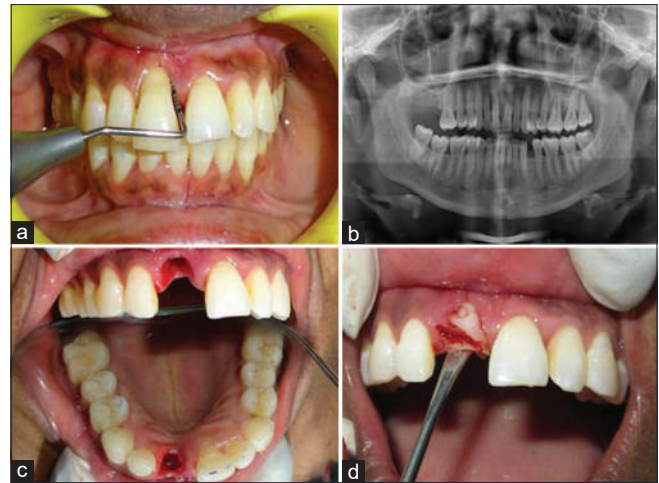


Figure 1: (a) Preoperative clinical view. (b) Preoperative radiographic view. (c) Atraumatic extraction of tooth done and the socket is curetted. (d) Platelet-rich fibrin membrane placed in the extraction socket

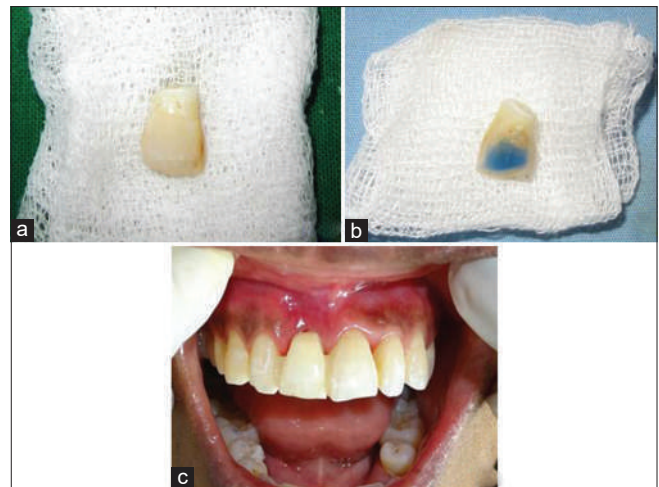


Figure 2: (a) Preparation of natural tooth pontic. (b) Etching of pontic with 37% phosphoric acid. (c) Splinting of natural tooth pontic to abutment tooth by composite resin

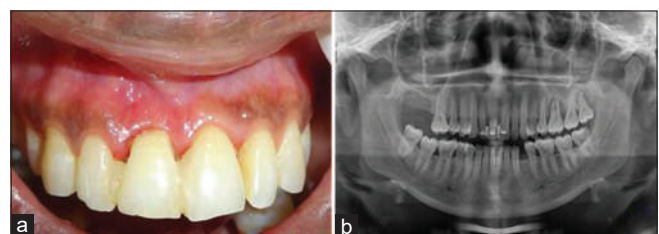


Figure 3: (a) Postoperative clinical view after 1 year. (b) Postoperative radiographic view after 1 year

tissue healing and cell proliferation. The patient reported again after 1 year with no complications. Bhandari *et al.* conducted a study in 15 patients, in which they have placed NTP soon after extraction of periodontally compromised teeth and concluded that NTP serve as the better option for immediate replacement, provides esthetic, and psychologic benefit to the patient.^[9] Walsh *et al.* reported a case using NTP and concluded that NTP provides an excellent

psychological benefit, with less treatment cost, maintenance of gingival profile, and no extensive surgical procedures.^[10]

Conclusion

NTP serves as an excellent replacement immediately after extraction in the esthetic zone. Even though good oral hygiene maintenance and appropriate case selection are needed for this treatment option, NTP provides good psychological benefits following extraction and also provides time for permanent treatment options.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

Comparison of stress distribution in teeth restored with fiber post and dentin post by applying orthotropic properties: A three-dimensional finite element analysis

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Abstract:

Aim of the Study: We aimed to compare stress distribution in a tooth restored with fiber post and dentin post after applying the orthotropic properties using three-dimensional finite element analysis (3D-FEA).

Materials and Methods: Two 3D-FEA models were constructed. The material properties were assigned, and a load of 100 N was applied at 45° to the long axis of the model onto the lingual surface incisal to the cingulum. The FEA was done by applying orthotropic properties of dentin and fiber post. The maximum stresses produced in the tooth and post referred to as von Mises stress were recorded. The Ansys software was used which depicts the stress concentrations.

Results: Von Mises values showed that glass fiber post (331 MPa) and dentin post (338 MPa)-restored tooth models presented similar stress values.

Conclusion: Although both fiber post and dentin post presented similar von Mises stress values, the pattern of stress distribution is more favorable in dentin post. More favorable fracture could be expected in case of dentin post. Thus, the dentin post is a promising alternative post material for rehabilitating endodontically treated teeth.

Keywords: Dentin post; fiber post; finite element analysis; stress pattern evaluation; von Mises stress

Clinical Significance: Ascribing the orthotropic properties in a FEA study means that the computational simulation was similar to that of clinical scenario, and hence simulates the dynamic intraoral conditions, thereby giving the accurate results.

INTRODUCTION

The longevity of endodontically treated teeth predominantly relies on the amount of residual tooth structure and the type of postendodontic restoration.

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Teeth with inadequate coronal tooth structure are to be restored with posts for better retention of a core.^[1] In the modern era, the focus of interest has shifted from metal post systems to fiber posts because of the quest for esthetic materials and also simple chairside technique.^[2] Naumann *et al.* conducted a prospective observational clinical study on glass fiber-reinforced postendodontic restorations for 10 years and stated that the yearly failure rate was high in relation to the anterior teeth.^[3] Hence, there is a need for

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Tamineedi, *et al.*: FEA analysis of the teeth restored with fiber post and dentin post

a material that has better mechanical properties as well as esthetics.

Factors like the post material, and its mechanical properties such as Young's modulus, compression strength, and coefficient of thermal expansion, play a critical part in the biomechanical behavior of root canal-treated tooth. Ideally, the post material should have these properties analogous to dentin, and a good bonding is anticipated between post and the root canal dentin. To date, human dentin is the only structure that essentially satisfies all these biomechanical requirements. Various authors reported dentin posts that were prepared from extracted human teeth.^[4,5] However, the studies evaluating the stress distribution of the dentin posts are limited. Hence, the current study aims to assess and compare the distribution of stress in teeth that were restored with dentin post and the fiber posts.

Finite element analysis (FEA) is a modus operandi to analyze stress distribution. In this technique, the actual structure is visualized as an assembly of a finite number of elements. The problem domain is divided into a collection of smaller parts (elements), and an overall approximated solution to the original problem is determined. In earlier studies, fiber post and dentin were assumed to be isotropic (i.e., the material has the same physical property when measured in different directions), which may not simulate the clinical condition. According to Grzebieluch *et al.*, the application of anisotropic properties to the dentin during FEA resulted in a reduction of the displacements and strain. They proposed that the application of anisotropy of the dentin should be considered in FEA studies.^[6] Therefore, the current study was conducted to evaluate the stress concentration in a tooth restored with dentin post and fiber post using three-dimensional FEA by applying the orthotropic (subset of anisotropy) properties of fiber post and dentin.

MATERIALS AND METHODS

Three-dimensional geometrical model design of maxillary central incisor

A human extracted maxillary central incisor with average dimensions was selected. The tooth was sectioned to a level of 3 mm coronal to dentino-enamel junction. The tooth was subjected to cone-beam computed tomography (CBCT) imaging. The CBCT images in Digital Imaging and Communications in Medicine format were imported into AutoCAD software (Catia V5R22) and outline of each layer (i.e., dentin, alveolar bone, gutta percha, post, and core) were traced. A 0.25-mm thick periodontal ligament and 0.25 mm thick lamina dura outlines were developed. In both the preliminary designs, post length, width of 1.0 mm and 1.2 mm, respectively, and apical gutta-percha of 4 mm were designed. The thin luting cement sandwiched

between the dentin and post material was difficult to replicate, and it is treated as a part of dentin. No significant error was anticipated by the exclusion of this layer in the model.^[7] In order to eliminate the exterior reinforcing effect on the post and core, models were not restored with any prosthesis.^[8]

Defining the simulation and loading conditions

The preliminary designs of post and core were imported to Hypermesh software (ABAQUS 2016), and a Hypermesh assembly is formed [Figure 1]. After converting the completed model into an Ansys input file, it was then brought into Ansys software. Mechanical properties such as modulus of elasticity and Poisson's ratio were assigned to all of the constituents [Table 1].^[9-14] The models had only one variation, which was the post material, i.e., Model A as fiber post and Model B as dentin post. All materials except dentin and fiber post were assumed to be homogeneous and isotropic. Fiber post and dentin post were considered to be orthotropic [Tables 2 and 3].^[6,15]

Loading

The models were fixed at the bottom to create a stress model. For the simulation of load conditions in the oral cavity, an arrow is used 45° at the level of the cingulum. A progressive load of 100 N was applied to both models. The greatest stresses developed in the models were referred to as von Mises stress. The Ansys software also has a color-coding system, which indicates the stress distributions.



Figure 1: Hypermesh assembly

Table 1: Mechanical properties of materials in finite element analysis model

Materials	Young's modulus (Mpa)	Poisson's ratio
Gutta-percha ^[9,10]	0.69	0.45
Periodontal ligament ^[11]	6.9	0.45
Cortical bone ^[12]	13,700	0.30
Spongy bone ^[12,14]	1,370	0.30
Composite resin ^[13]	16,600	0.24

RESULTS

The results in each model were presented in terms of the von Mises stress values, which gives the failure predilection of the material analyzed. When the models were subjected to analysis, both showed maximum stresses at the cervical third and least forces at the apical third of the tooth model. Model A (331 MPa) and Model B (338 MPa) were not significantly different in performance, but the pattern of stress distribution is different in both the models. In Model A, more stresses are observed at the cervical and middle third, whereas it is at the cervical level in Model B [Figure 2]. The color-coding system indicates the stresses generated. Maximum stresses were indicated in red color, and minimum stresses were indicated in blue color. Minimal change in the color at the apical third means the complete dissipation of occlusal load, thereby decreasing the chance of vertical fractures.

DISCUSSION

FEA is the recent method to measure and analyze the stress distribution. Various other methods, such as the strain

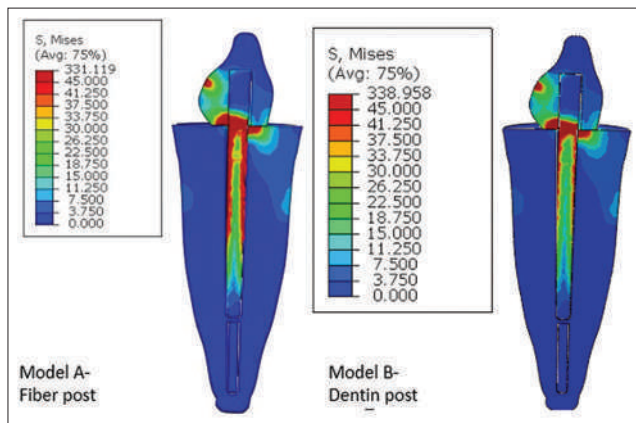


Figure 2: Stress distribution in the models

Table 2: Mechanical properties of fiber posts^[15]

Elastic moduli (E: Mpa), Shear moduli (G), and Poisson's ratio (n)	Glass fiber post
E11: Longitudinal modulus of elasticity (MPa)	4000
E22: Longitudinal modulus of elasticity (MPa)	11000
E33: Longitudinal modulus of elasticity (MPa)	11000
G12: Cross-sectional modulus of elasticity (MPa)	4200
G13: Cross-sectional modulus of elasticity (MPa)	4200
G23: Cross-sectional modulus of elasticity (MPa)	4100
n12: Poisson's coefficient	0.26
n23: Poisson's coefficient	0.26
n13: Poisson's coefficient	0.32

Table 3: Material properties of dentin tissue used for analysis^[6]

Material designation Orthotropic dentin	Exx (GPa)	Eyy (GPa)	Ezz (GPa)	Vxy (-)	Vyz (-)	Vxz (-)	Gxy (GPa)	Gyz (GPa)	Gxz (GPa)
Top	23.1	23.1	26.0	0.31	0.3	0.3	8.9	9.3	9.3
Middle	19.1	19.2	24.1	0.3	0.3	0.3	7.3	8.1	8.1
Bottom	15.7	15.7	22.0	0.31	0.3	0.3	6.0	7.0	7.0

gauge method and the photoelastic method, were also used in the past for the same. Hrennikoff and Richard Courant were the first persons who used FEA in civil and aerospace engineering to resolve the difficulties in the analysis of elastic properties and structural investigations.^[9] Davy *et al.* used FEA to study the post and core restorations.

FEA is applied to dental biomechanics as it is a popular numerical method to precisely evaluate the intricate biomechanical performance of nonhomogeneous structures in a nondetrimental, reproducible fashion. The model is fragmented into numerous tiny parts or components, every single component with specific physical properties. Then, the operator utilizes a computer platform to attain a prototypical model of stresses made by different loads. Modulus of elasticity and Poisson's ratio of the modeled structure are quantified for every component. Unlike other testing methods, FEM analysis can provide 100% reproducible results, even after repeating any number of times. Hence, conventional statistical analysis is not generally included in these studies.^[16] FEA permits investigators to overcome certain restrictions in terms of ethics and methodology and permits them to demonstrate the modes of stress transmission throughout the materials. FEA also permits the analysis of a single variable in a multifaceted configuration. This infers that the procedure is time saving as it does not have any standardization issues and there is no need to prepare numerous test samples.^[9]

Most of the studies conducted using FEA modeled the tooth components as isotropic and not orthotropic. Such models denoted a static condition during mechanical loading and not representing a clinical scenario, which is dynamic and cyclic. Accurate measurements could be attained if the anisotropic material properties are used, although it demands the use of arduous mathematical calculation. Orthotropic (subset of anisotropic materials) materials have properties that vary in three orthogonal two-fold axes of rotational symmetry. Because of the orientation of dentinal tubules and glass fibers, both the post systems need to be considered as anisotropic. Ascribing these orthotropic properties meant that the computational simulation was similar to that of a clinical scenario. Hence, the present *in vitro* study was assumed to evaluate the distribution of stress when fiber post and dentin acts as a post material after applying orthotropic properties.

In the current study, both the fiber post and dentin post showed similar stress values. This is contrary to the study

Tamineedi, *et al.*: FEA analysis of the teeth restored with fiber post and dentin post

conducted by Memon *et al.*, which showed that the dentin post exhibited less stress than a fiber post.^[17] The difference in the results could be attributed to the application of orthotropic properties to dentin post and fiber post. No other FEM studies compared stress distribution in dentin and fiber post using orthotropic properties. Hence, direct comparisons could not be made. The maximum stress development was concentrated at the cervical area in the case of dentin post, whereas it is mainly concentrated at the cervical third and slightly extended into the middle third for fiber post. If the tooth restored with dentin posts were to fracture, failure probably occurs at the core/root intersection and is easily repairable compared to fiber posts. This is in agreement with the study conducted by Ambica *et al.* and Kathuria *et al.*^[18,19] Better results of the dentin post in their study could be because of the similarity in modulus of elasticity with surrounding dentin leading to homogeneous stress distribution. Clinically, adhesion between the fiber post and a luting agent is feeble.^[20] However, adhesion to a dentin post is highly anticipated. In a clinical scenario, better properties of dentin posts can be expected because of better bonding with the luting agent, as reported by Ambica *et al.* However, future investigations are essential to assess the safe and effective use of dentin posts for restoring endodontically treated teeth. Long-term clinical studies are also needed to evaluate the longevity of the dentin posts.

Limitations of the study

- Properties applied for the materials and structures were obtained from previously published data
- FEM study can predict stress concentration, but direct extrapolation to clinical scenario may not be precise as the mechanism of bonding, surface treatments, and residual stresses come into play. Hence, rigorous experimental validation is needed.

CONCLUSION

Although both fiber and dentin posts presented similar von Mises stress values, the pattern of stress distribution is more favorable in dentin posts. A more favorable fracture could be expected in the case of dentin post. Thus, the dentin post is a promising alternative post material for rehabilitating endodontically treated teeth.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

Comparative evaluation of apical extrusion of intracanal bacteria using ProTaper Next, Mtwo, and ProTaper rotary systems: An *in vitro* study

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Abstract

Aim: The aim of this study was to evaluate the number of intracanal bacteria extruded apically after instrumentation with three different nickel–titanium rotary instruments.

Materials and Methodology: Forty freshly extracted mandibular premolars were selected, access cavities were prepared, and the teeth were mounted in the bacterial collection apparatus. Root canals were contaminated with a suspension of *Enterococcus faecalis* and incubated for 24 h at 37°C. The contaminated teeth were divided into four groups of 10 teeth each according to the rotary system used for instrumentation: Group 1: ProTaper universal files, Group 2: MTwo files, Group 3: ProTaper Next files, and Group 4: Control group (no instrumentation). Bacteria extruded after preparations were collected into vials. The number of colony-forming units (CFUs) was determined for each sample.

Statistical Analysis: The data obtained were analyzed using the one-way analysis of variance followed by *post hoc* Tukey's test with a $P = 0.05$ as the level for statistical significance.

Results: The results suggested a statistically significant difference in the number of CFUs between four experimental groups ($P < 0.001$).

Conclusion: Least amount of bacterial extrusion was seen in ProTaper Next Group while more bacterial extrusion was seen in MTwo Group.

Keywords: Apical bacterial extrusion; *Enterococcus faecalis*; MTwo files; ProTaper files; ProTaper Next files

INTRODUCTION

The successful endodontic treatment must be directed toward the elimination of bacteria, their products from the root canal system, and this objective can be achieved by thorough chemo-mechanical preparation. Despite strict length control, almost all instruments

and preparation techniques used for the root canal treatment are associated with debris extrusion, which contains dentin chips, pulp tissue, microorganisms and/or irrigants, into the periradicular tissue. This extrusion may potentially result in postoperative flare-ups.^[1] Bacteria extruded mainly include Gram-positive, Gram-negative bacteria, and obligate anaerobes. *Enterococcus faecalis* (*E. faecalis*) has been identified as a species most commonly recovered from the posttreatment diseases.^[2]

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In recent times, various rotary systems with varying instrument designs have been introduced and thus differences exist in terms of apical extrusion of debris. Previous studies reported that ProTaper Universal (DENTSPLY Maillefer) (PTU) rotary files extruded greater amounts of debris that has been attributed to their aggressive cutting ability.

Recently, the ProTaper Next system (DENTSPLY Maillefer) with M-wire nickel–titanium (Ni-Ti) configuration has been introduced. M-wire technology helps to improve file flexibility and resist cyclic fatigue while retaining cutting efficiency of the instrument. Moreover, also, this rotary system ensures only two-point contact with the canal wall at a time, assuring efficient cleaning of the canal.

MTwo endodontic instruments (VDW, Munich, Germany) are newer generation rotary files with an italicized “S” transverse section and two cutting edges with a noncutting tip are designed with minimum radial contact that permits for continuous upward shifting of dentin chips.

The aim of this *in vitro* study was to compare the apical extrusion of *E. faecalis* using three rotary Ni-Ti instruments (ProTaper Next, MTwo, and ProTaper).

MATERIALS AND METHODOLOGY

Forty freshly extracted human, single-rooted mandibular premolar teeth with mature apices and root curvatures between 0° and 10° were selected. All teeth were analyzed radio graphically in bucco-lingual and proximal directions to ensure for the presence of single and noncalcified canals. The teeth were cleaned of debris and soft-tissue remnants and endodontic access cavities were prepared with endo access bur No: 2 (DENTSPLY Maillefer) using a super torque high-speed hand piece and the pulp remnants were extirpated with a fine-barbed broach. A model system described by Myers and Montgomery 1991 *et al.*^[3] was used to evaluate bacterial extrusion [Figure 1].

The teeth were inserted under pressure into perforated rubber stoppers of 10 ml glass vials. Two coats of nail varnish were applied to the external surface of all roots to prevent bacterial microleakage through the lateral canals. The rubber stopper with the tooth was then fitted into the opening of the vial. The vial was vented with a 27 G hollow needle alongside the rubber stopper which served two purposes: (1) Equalize the air pressure and (2) act as an electrode for the electronic working length (WL) determination during canal instrumentation.

A sterile, 10 K-file was placed 1 mm beyond the foramen to remove the nail varnish that covered the apical foramen., with the same 10-k file, 1 mm short of the file



Figure 1: The experimental model system

penetration was considered as WL. In this way, standard size of foramen and apical patency was achieved. The WL determined manually was again confirmed with an electronic apex locator (DENTSPLY Maillefer Propex Pixi) after fitting the tooth with rubber stopper into the vial. The entire model system was then sterilized in ethylene oxide gas sterilizer (3M, USA) for a 12-h cycle.

A suspension of pure culture of *E. faecalis* (ATCC-29212) was prepared by adding 1 mL pure culture of *E. faecalis* to freshly prepared brain–heart infusion broth and incubated for 24 h. Then, McFarland standard no. 0.5 was used to evaluate the broth to ensure that the number of bacteria was 1.5×10^8 colony-forming units (CFUs)/ml. Each root canal was filled with the *E. faecalis* suspension using sterile pipettes in a Class II vertical laminar airflow cabinet to prevent any airborne contamination and a size 10 K-file was used to carry the bacteria down the length of the canals. The contaminated root canals were placed in an incubator at 37°C for 24 h for bacterial multiplication.

Before the experiment, sterilized glass vials were filled with 0.9% NaCl (saline) solution. The tooth-rubber stopper-needle unit was then fixed into the mouth of the vials. The specimens were then divided into four groups of ten specimens each ($n = 10$). The root canals in three experimental groups were instrumented with three different rotary endodontic systems while in the fourth group which acted as a control, no instrumentation was attempted.

The rotary instruments were used with a 16:1 gear-reduction, contra-angle hand piece powered by a torque controlled electric motor (X-Smart DENTSPLY). The total of 8 ml volume of saline was used as irrigant in conventional manual mode intermittently with 27-G side vented needle (Max-i-Probe, DENTSPLY Maillefer) placed passively down the canal up to 3 mm short of WL without binding and the needle was constantly pulsed 1–2 mm in

the apical to coronal direction during irrigation. The flow of irrigant was standardized to a constant speed of 1 mL/4 sec controlled by a chronometer.

Group I – ProTaper universal

ProTaper rotary files were used in a crown-down manner according to manufacturer’s instructions using a gentle in-and-out motion. An SX file was used at half of the WL, S1, and S2 files were carried to 2/3 of the WL, and F1, F2, F3 sequentially to full WL.

Group II – MTwo

All MTwo instruments were used to the full length of the canal (single-length technique) at a rotational speed of 280 rpm according to the manufacturer’s instructions using small, stroking brushing movements. The instrumentation sequence was as follows: Size 10/.04 taper, size 15/.05 taper, size 20/.06 taper, size 25/.06 taper, and size 30/.05 taper.

Group III-ProTaper next

All ProTaper Next files were used in a crown-down manner according to the manufacturer’s instructions with a gentle in-and-out motion at 300 rpm and 2 Ncm torque. The instrumentation sequences were X1 (17/04), X2 (25/06), and X3 (30/07).

Group IV (control)

All the samples in the control group were kept under laminar airflow cabinet for a time period of 1 min, without attempting instrumentation and were taken out.

Single operator under aseptic conditions carried the canal preparation, whereas bacterial extrusion was assessed by another investigator who was blinded regarding the experimental groups. Prior to and at the end of canal preparation, 0.01 mL of solution was collected from all the forty experimental vials to count the bacteria. Using a bacterial inoculation loop, the bacterial suspension was placed on brain–heart agar plates. All the agar plates were placed in an incubator at 37°C for 24 h. After 24 h, colonies of bacteria were counted using the classical bacterial counting technique (Collins *et al.* 2004),^[4] and they were counted as number of CFUs.

RESULTS

The soft ware used was statistical package for the social sciences soft ware version 24.0IBM Corp. Armonk, NY, USA was used for the statistical analysis. Mean and standard deviation were estimated from the sample for each study group. The mean values were compared by the one-way analysis of variance appropriately followed by *post hoc* Tukey test. *Post hoc* Tukey test was employed to identify the significant groups. In the present study, the level of significance was set at $P = 0.05$.

Table 1 shows the mean counts, standard deviation, and *post hoc* Tukey test of extruded bacteria (*E. faecalis*) between different experimental groups. Table 2 and Graph 1 show mean comparison between the groups.

Mean CFU was compared in all the groups, and there was a statistically significant difference among the groups ($P < 0.001$). The mean amount of extruded bacteria ranged between 0 CFU/ml and 13.56×10^3 CFU/ml. *Post hoc* test suggested that Group 4 (Control) had significantly lower mean CFU than all the groups.

The highest amount of bacterial extrusion was seen in Group 2, and the least amount of bacterial extrusion was observed with Group 3.

DISCUSSION

The primary goal of the root canal treatment is to eliminate microorganisms, thereby reducing the periradicular inflammation. Hülsmann and Hahn^[5] stated that in any endodontic treatment, an instrument used in an apical direction or an instrument acting as a plunger may result in apical extrusion of material such as dentin chips, pulp tissue, microorganism, and irrigation solution. Most NiTi rotary instruments work in the coronal direction

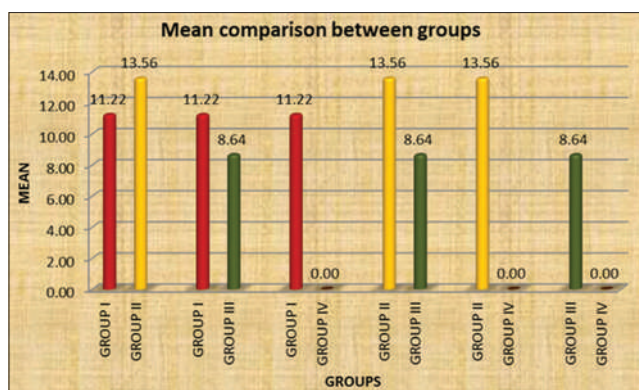
Table 1: Comparison of mean values among experimental and control groups

Groups	n	Mean×10 ³	SD×10 ³	F	P	Post hoc test
Group I	10	11.22	1.25	431.922	<0.001	1>3, 4
Group II	10	13.56	0.91		significant	2>1, 3, 4
Group III	10	8.64	0.92			3>4
Group IV	10	0.00	0.00			-

SD: Standard deviation

Table 2: Mean comparison between the experimental groups and control groups using the *post hoc* tukey te

Groups	Mean difference	Std error	P	95%confidence interval	
				Lower bound	Upper bound
GROUP I					
GROUP II	-2.34	0.403	<0.001 S	-3.425	-1.255
GROUP III	2.58	0.403	<0.001 S	1.495	3.665
GROUP IV	11.22	0.403	<0.001 S	10.135	12.305
GROUP II					
GROUP I	2.34	0.403	<0.001 S	1.255	3.425
GROUP III	4.92	0.403	<0.001 S	3.835	6.005
GROUP IV	13.56	0.403	<0.001 S	12.475	14.645
GROUP III					
GROUP I	-2.58	0.403	<0.001 S	-3.665	-1.495
GROUP II	-4.92	0.403	<0.001 S	-6.005	-3.835
GROUP IV	8.64	0.403	<0.001 S	7.555	9.725
GROUP IV					
GROUP I	-11.22	0.403	<0.001 S	-12.305	-10.135
GROUP II	-13.56	0.403	<0.001 S	-14.645	-12.475
GROUP III	-8.64	0.403	<0.001 S	-9.725	-7.555



Graph 1: Comparison of mean values among all the groups

with push-pull rotation filing movements during the canal preparation.^[6]

The present research used single-canaled mandibular premolar teeth; this is to minimize nonstandardized preparations that are more likely to occur in curved canals and to ensure that bacterial extrusion is due to instrumentation and not due to the tooth morphology.

Myers and Montgomery^[3] showed that debris extrusion can be minimized when WL was taken 1 mm short of the canal length. Beeson *et al.*^[7] reported that significantly less debris extrusion when instrumentation done 1 mm short of apex compared to the instrumentation performed till apical foramen. The present study used electronic apex locator (DENTSPLY Mallifer Propex Pixi) to make sure that WL of the canals was maintained 1 mm short of the apex, so that variables which may affect results could be minimized.

E. faecalis was used as the bacteriological marker as it is implicated in persistent root canal infections and is identified as the species most commonly recovered from the root canals of teeth with posttreatment disease.^[8] The apical diameter of master apical instruments in all the groups was standardized at ISO size 30 to avoid any variations in the amount of extruded bacteria due to the size of apical enlargement.^[9] Therefore, any bacteria extruded apically could be attributed to the design of the rotary file and instrumentation technique used in that particular group.

Irrigation is an important phase in cleansing the canal. In the present study, saline was used as irrigant as it has no antibacterial effect, and the irrigation was done passively to ensure that bacterial extrusion is only due to the mechanical action of the rotary instruments being tested. Irrigant was delivered with 27 G side vented at a constant rate of 1 ml/4 s.^[10] Abou-Rass and Piccinino^[11] recommended the use of small diameter (27 G or 30 G) needles for irrigation because they provide sufficient volume of irrigant for efficient flushing of debris by fitting at depths just short of the physiological terminus. Various studies have shown that

apical extrusion of irrigant is less when irrigation done with side-vented needles.^[12] In this study, during the root canal irrigation, the needle was gently inserted up to apical 3 mm of WL because it ensures adequate irrigant flow through the root canal, thereby creating less apical pressure and maintaining minimal extrusion of the irrigant.^[13]

In the present study, ProTaper Next extruded significantly least amount of bacteria compared to MTwo and ProTaper rotary file systems. Koçak *et al.* in their study found that bacterial extrusion was comparatively less when instrumented with ProTaper Next Ni-Ti instruments.^[14] The current study used rotary instruments with different cross-sectional design: ProTaper Next-rectangular cross section, ProTaper-convex triangular cross section, and MTwo-S-shaped cross section. ProTaper Next instruments have M-wire technology with off-centred cross section and generates snake-like swagging movement along the active part of the file, thus reducing the screw effect, the unwanted taper lock,^[15] thereby decreasing the file-root dentin contact and provides more cross-sectional space for enhanced cutting, loading and allowing the debris to travel in apico-coronal canal direction when compared to the file systems with center of mass and axis of rotation in line with the center of axis of the file.

The common design feature of the ProTaper Next and PTU systems is the presence of progressive and regressive percentage tapers on a single file. However, the PTU F2 instrument has a 0.08 taper at the apical 3 mm, whereas the ProTaper Next X2 instrument has a 0.06 taper at the apical 3 mm. The large apical taper may result in more aggressive preparation of the root canals, which could have led to a larger quantity of debris being extruded apically.^[16]

Another reason for this finding may be the number of instruments from each system used to prepare a canal. Specifically, the number of instruments used was three files for the ProTaper Next group, five files for the MTwo group, and six files for ProTaper group. In addition, a reduction in the number of instruments decreases the number of walls played during instrumentation, one of the reasons why systems with smaller numbers of files result in less extrusion of debris.^[17]

In the present study, MTwo Rotary Ni-Ti instruments extruded a greater amount of bacteria in comparison with ProTaper and ProTaper Next. The results of this study were in correlation with the study done by Garlapati *et al.*^[18] This may be attributed to the standardized length preparation technique (single-length technique) and also to the s-shaped cross section with double-cutting edge geometry of MTwo instruments. Because of these design characteristics, they remove an adequate amount of dentin in a short period of time, thereby unable to displace the debris coronally, leading to significantly greater amount of apical extrusion

Polineni, *et al.*: Apical extrusion of intracanal bacteria using rotary instruments

of bacteria. Early coronal flaring with the crown down technique used with ProTaper and ProTaper next rotary files, helped in removing majority of microorganisms and reduced the possibility of debris packing, thereby allowing deeper penetration of irrigating solutions and clearing debris from the apical area,^[19] reducing the potential of extruding material from the root apex.

The present research was an *in vitro* study, and its results should not be extrapolated to clinical situations, because different results may be achieved *in vivo* as the periapical tissues may serve as a natural barrier, inhibiting bacterial and debris extrusion and also their pressures might differ at the apex with normal or pathological tissues.

CONCLUSION

ProTaper Next rotary instruments extruded less number of bacteria where instrumentation was performed by the crown-down technique. MTwo rotary instruments extruded more number of bacteria where instrumentation was performed by the single-length technique.

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Conflicts of interest

There are no conflicts of interest.

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Case Report

Rehabilitation of a mutilated maxillary central incisor using autogenous dentin post

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Abstract

Ideal coronal restoration that provides satisfactory esthetic and functional outcome for endodontically treated and extensively damaged teeth is still an arduous task for restorative dentistry. None of the available post systems have all the ideal biological and mechanical properties. Biological dentin posts are considered as a good alternative to conventional post systems as they preserve dentin walls, which results in better distribution of forces along the root surfaces in the compromised tooth. This case report is an attempt to detail "autogenous dentin post" which serves as a homologous unit for the reinforcement of endodontically treated tooth by virtue of its biomimetic property.

Keywords: Autogenous; biomimetic; dentin post; endodontically treated tooth

INTRODUCTION

Restoration of endodontically treated teeth helps in maintaining function and esthetics. Despite having various commercially available posts such as metal, fiber, and ceramic, none of them meet all the ideal physical, mechanical, and biological properties.^[1] The biological post serves as a viable substitute owing to the resiliency of tooth structure, excellent adhesion, and preservation of the internal dentin walls and decreases the stresses applied on dentin. This case report employs the use of autogenous dentin post for the restoration of a fractured maxillary central incisor. The preparation and adhesive cementation of a "biological post" prepared from a freshly extracted grossly fractured lateral incisor of the same individual is discussed.

CASE REPORT

A 25-year-old male reported to the Department of Conservative Dentistry and Endodontics, with the chief

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complaint of fractured upper front teeth and history of fall 10 days before the visit. The patient had severe localized pain immediately after the trauma lasting for 3 days, which aggravated upon eating and relieved upon medication. Dental history revealed a trauma pertaining to anterior teeth in childhood, which did not lead to fracture but resulted in pain for a week which subsided after few days. Medical history was noncontributory. The extraoral examination did not reveal any significant findings. Clinical examination revealed crown fracture extending below the level of the gingiva with respect to 11, crown loss with only a small mobile fragment of crown attached to the soft tissue with respect to 12 [Figure 1a], and crown fracture involving pulp with respect to 21. Periodontal probing of 12 revealed the cervical third root fracture. There was slight tenderness to percussion and palpation, and the mobility of teeth was in normal physiological limits. The intraoral periapical radiograph revealed a slight widening of periodontal ligament space with respect to tooth #11. Cervical third root fracture was observed in relation to tooth #12 [Figure 1b]. Cold testing with Endo-ice (Coltene) elicited no response, which was confirmed with electric pulp testing. Based on clinical and radiographic findings, diagnosis of crown/root fracture (WHO 873.64) with

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Figure 1: (a) Preoperative clinical picture. (b) Preoperative radiograph showing cervical root fracture with respect to 12, crown/root fracture with respect to 11. (c) Root canal treatment performed with respect to 11. (d) Clinical picture after extraction of 12. (e) Post space preparation done with respect to 11. (f) Dentin post prepared from extracted 12. (g) Intermittent trial verification of dentin post. (h) Flap elevation with respect to 11 to expose fracture line. (i) Postoperative radiograph. (j) Suturing done with respect to 11. (k) Postoperative clinical picture. (l) 1-year follow-up radiograph

respect to 11, root fracture (WHO 873.63) with respect to 12 [Figure 1a], and crown fracture with pulp exposure (WHO 873.62) with respect to 21 was made.

The following treatment options were explained to the patient:

- Root canal treatment (RCT) followed by orthodontic extrusion with respect to 12, RCT with respect to 21, RCT followed by post placement with respect to 11, and fixed partial denture (FPD) with respect to 11, 12, 21
- Extraction followed by implant placement with respect to 12, RCT with respect to 21, RCT followed by post placement with respect to 11, and FPD with respect to 11, 21
- Extraction of 12, RCT with respect to 21, RCT followed by post placement with respect to 11, and FPD with respect to 11, 12, 21
- Extraction of 12, which is used as a post material for 11. RCT with respect to 21 and FPD with respect to 11, 21.

The patient wanted the treatment to be completed at the earliest. Hence, orthodontic extrusion was ruled out. He was not affordable for implant placement, and he was willing to get the tooth 11 restored with a biological post made from the patient's extracted lateral incisor. Endodontic treatment was initiated with respect to 11. All the instruments which were used for the treatment were soaked in 2.5% NaOCl for 1 h and autoclaved before the treatment. Under aseptic conditions, 1 ml of lidocaine

2% with epinephrine 1:100,000 was administered using anterior superior alveolar nerve block and incisive nerve block. Rubber dam isolation was done. Access cavity preparation was done from the incisal aspect using a safe end tapered carbide fissure bur to conserve the coronal dentin. Working length of 18 mm was determined using apex locator (Canal pro, Coltene) and k-hand files (MANI, INC Japan) were used in a step back technique till size 120. A chelating agent, Glyde (Dentsply), was used during biomechanical preparation. Intermittent recapitulation and irrigation with 3% sodium hypochlorite and normal saline was performed. A master cone of size 70/2 was verified, and cold lateral condensation was done using gutta-percha, AH Plus sealer (Dentsply, DeTrey, Konstanz, Germany) [Figure 1c].

In the next appointment, 12 was extracted [Figure 1d], adequately cleaned, and sterilized by autoclaving at 121°C for 15 min. Meanwhile, the root canal of 11 was prepared until size 2 peeso reamer (MANI, INC Japan) [Figure 1e]. The sterilized extracted tooth was then split into two halves vertically, and one half is shaped into dentin post [Figure 1f]. An inlay wax impression of the post space was taken, which acted as a guide for shaping the lateral incisor as a post.^[1] Fit of the post to the canal was checked intermittently after minor adjustments [Figure 1g]. After radiographic evaluation of the satisfactory fit of dentin post, it was again subjected to autoclaving (134°C, 18 min) to minimize contamination. Local anesthesia was administered and internal bevel, crevicular, interdental incisions were given with a 15c blade, and a full-thickness conventional flap was

raised to expose the fracture line of 11 [Figure 1h]. After achieving adequate hemostasis with the use of Surgicel, the canal was irrigated with normal saline and dried with a paper point. Etching of both root canal and dentin post was done with 37% phosphoric acid for 15 s for smear layer removal followed by rinsing with distilled water and blot dried. According to the manufacturers' instructions, etching is not required when using Rely X U100. Since the procedure of trimming, the dentin post according to the canal anatomy involves massive smear layer production, to ensure better bonding etching for 15 s was done. Dual-cure self-adhesive resin cement (Rely X U100, 3M ESPE) is injected into the prepared post space, and the dentin post is completely coated with the resin cement and pushed gently to the full length of the post space and held firmly during the initial curing period to compensate the curing shrinkage. The light-curing is done for 40 s. Core buildup is done with composite (Z250, 3M ESPE, USA). The crown form was shaped with composite material, an X-ray was taken to verify the adaptation of composite material, and the flap was sutured back [Figure 1i and j]. After 1 week, sutures were removed. RCT was performed with respect to 21. Fixed prosthesis was given for rehabilitation of these teeth [Figure 1k]. The patient's occlusion was assessed to investigate the presence of any premature contacts and adjustments were done accordingly. Postoperative instructions to the patient were given regarding diet and special attention to hygiene and dental care (not to open any bottle caps or wedging of any foreign objects) to avoid excessive pressure on the teeth, which could in turn predispose the tooth to fracture. On 1-year follow-up, radiograph revealed no significant changes, and the patient was asymptomatic [Figure 1l].

DISCUSSION

Increased emphasis on the maintenance and preservation of natural dentition combined with an increase in the predictability and effectiveness of endodontic therapy has made the postendodontic restoration a great challenge.^[1] The recent progress in restorative materials coupled with advances in adhesive protocols many times turn out to be expensive and technique sensitive and also require the proficiency of the operator. Advancements in adhesive technology and restorative armamentarium have resulted in the use of natural tooth fragments for the management of fractured anterior teeth. These restorations are commonly known as biological restorations. When a patient's own tooth fragment is bonded to provide optimal function and esthetics, it is called as autogenous bonding.^[2]

The advantage of autogenous dentin post is that it preserves root canal dentin and results in better distribution of forces along the root surface in compromised teeth, which shows excellent outcomes in terms of its functional value.

Another advantage is its low cost.^[2] The physical properties of dentin post such as modulus of elasticity, viscoelastic behavior, compressive strength, and thermal expansion closely resembles root dentin. A dentin post forms a micromechanical homologous unit with the root dentin resulting in uniform stress distribution. The dentin post acts as a shock absorber because of its similarity in the elasticity to that of root dentin which allows post flexion to mimic tooth flexion and allows only a fraction of the stresses transferred to the tooth.^[3]

Dual-cure self-adhesive universal resin cement (Rely X U100) eliminates procedural technique sensitivity owing to its single-step luting process.^[4] It has the properties similar to conventional cements in terms of ease of handling, as well as the mechanical properties, dimensional stability, and micromechanical retention of resin cements.^[5] Its adhesion to the dentin and various restorative materials was found to be satisfactory. In a study by Bharali *et al.*, resin cement showed the highest resistance to solubility and sorption, followed by RMGIC and conventional GIC.^[6] Hence, self-adhesive resin cement was selected to lute the dentin post in this case.

Ambica *et al.* and Kathuria *et al.* in their study reported that teeth restored with dentin posts demonstrated higher fracture resistance than those restored with carbon fiber posts and glass fiber posts.^[7,8] Henrique *et al.* performed a finite elemental analysis study to compare stress distribution in teeth restored with fiber post, dentin post, and concluded that both the posts presented a similar biomechanical performance.^[9] The polymer matrix in the prefabricated fiber posts is highly cross-linked and, therefore, less reactive. This makes it difficult for these posts to bond to resin luting agents.^[10] The resin bonding to the dentin is more predictable. In case of dentin posts, since both the post material and surrounding tooth structure is dentin, better bonding can be seen compared to fiber post.^[7] Hence, the use of a dentin post is considered as a better treatment option for the restoration of endodontically treated teeth compared to fiberposts.

The use of natural exacted teeth for restoration does present limitations such as patient acceptance, tooth bank for availability, difficulty in retrieval, availability of teeth with similar structure, and tooth color. By utilizing the dentin post prepared from the same patient, we can overcome many of these limitations. The biomechanical properties of the dentine would be well conserved as the freshly extracted tooth was used in this case report. Another concern is the prion infection. The pulp tissue exposed by trauma might be an entry point for various microorganisms of which prion infection is difficult to handle.^[11] Prions are the proteinaceous infectious materials. Hence, they cannot be inactivated using sterilization procedures such

Kakollu, *et al.*: Autogenous dentin post: A case report

as autoclaving (121°C, 15 min), ultraviolet radiation or gamma radiation, and ethanol treatments.^[12] To inactivate prions, use of an autoclave under severe condition (134°C, 18 min), NaOH (1 N, 20°C, 1 h), sodium dodecyl sulfate (SDS) (30%, 100°C, 10 min), and NaOCl (20000 ppm, 20°C, 1 h) is recommended.^[13]

The various practical methods for prion inactivation are^[12]

- Wash with appropriate detergents + SDS treatment (3%, 3–5 min),
- Rinse with alkaline detergents (80°C–93°C, 3–10 min) + autoclaving (134°C, 8–10 min),
- Rinse with appropriate detergents + autoclaving (134°C, 18 min) and
- Wash with alkaline detergents + vaporized hydrogen peroxide gas plasma sterilization.

The present case report presented the successful use of dentin posts, however further studies are obligatory to measure the adhesion, function, and long-term behavior of these biological posts. Adaptation of the post to the root canal configuration may be time taking. Use of advanced technology such as computer-aided designing and machining may be used in future research work to achieve the accurate dimensions of biological posts to that of post space.^[2]

CONCLUSION

This case report has demonstrated a morphofunctional rehabilitation of a severely mutilated tooth using an autogenous dentin post, which serves as a viable substitute owing to the resiliency of tooth structure, excellent adhesion, preservation of dentin, and not promoting dentin stresses.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and

other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Oral hygiene habits, oral health status, and oral health care seeking behaviors among spinning mill workers in Guntur district: A cross-sectional study

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ABSTRACT

Introduction: Though the oral health status of workers from different industries was reported in literature, there is little information with regard to spinning mill workers. The aim of this study was to document the oral health status, oral hygiene routine, and frequency of utilization of oral health care services among spinning mill workers in Guntur district. **Materials and Methods:** 458 spinning mill workers in Guntur district participated in this study. Data on hygiene practices, self-reported dental problems, past dental visits, type and place of availed treatments, and barriers for utilizing dental services were recorded. Oral health status was examined using Simplified Oral Hygiene Index, DMFT index, and Community Periodontal Index. **Results:** Female participants were found to have better oral hygiene status compared to males, which is partially significant. Similar was the scenario when caries experience was considered. Majority of the study subjects (74%) have a DMFT score of 1–6. There were 86 participants without any coronal caries experience. The mean coronal caries experience was more among older spinning mill workers compared to the younger workers. The difference in DMFT scores between males and females was not significant. Majority of the participants (46.3%) were with CPI score 2, while only 10.2% were observed to have all healthy sextants. 136 subjects (30.15%) demonstrated loss of attachment of some severity. **Conclusion:** Though the oral hygiene habits reported by the spinning mill workers were fair, oral health care seeking behaviors were found to be less informed. There is a serious need to improve the oral health awareness and care seeking behaviors among these workers.

Keywords: Care seeking behaviors, industrial workers, oral health status

Introduction

India is the third largest producer of cotton in the world and second largest producer of cotton yarns and textiles. Owing to the domestic and multilateral policy reforms, India was expected to play an important role in global textile market. High

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profitability associated with commercial crops like cotton drives cultivation of cotton in unfriendly agro-climatic conditions. The demand for cotton in India was exclusively generated by the domestic market until the liberalization of industrial policies in 1990s, but the scenario has changed since then with a substantial growth in textile exports.^[1] However, recent reports suggest that a third of spinning mills across India have shut down in light of the drastic decline in export and domestic sales.^[2] India's textile industry is the second largest employment generator with around 100 million workers finding employment in this sector. The reports by Directorate General of Commercial Intelligence and Statistics (DGCI and S) suggest that the cotton yarn export in India fell by 34.6%, and this has resulted in a huge loss of jobs.^[3] Guntur is one of the leading cotton producers in the state of Andhra Pradesh, and textile industry is one of the focus sectors in this district along with cement industry and agro and food processing.^[4]

Workers comprise a huge and valuable population. In 2007, WHO estimated that the global labor volume would be about half of the global population. 60%–70% of males and less than half of females across the globe are officially registered working labor. According to the joint committee of WHO and International Labor Organization (ILO), occupational health is defined as the promotion and maintenance of the highest degree of physical, mental, and social well-being of workers in all occupations.^[5] Occupational environment constitutes the extraneous states and drivers existing at the workplace which would have an influence on the health of the workers. The incidence of occupational diseases in India is a matter of concern with India contributing 20% to the world's occupational disease burden.^[6] Working environments are often regarded as important risk factors for oral health problems and in light of the established negative influence of oral health problems on the quality of life of people, poor working environments frame a vicious cycle where the quality of life of the stake holders keeps compromising with time.

Though the oral health status of workers from different industries like cement,^[7] marble,^[8] battery,^[9] fireworks,^[10] bakery,^[11] etc., was reported in literature, there is little information on the oral health status, oral hygiene behaviors, and dental care seeking patterns of spinning mill workers. With this background and given the reputation of spinning mill industry in Guntur district of Andhra Pradesh, the aim of this study was to document the oral health status, oral hygiene routine, and frequency of utilization of oral health care services among spinning mill workers in Guntur district.

Materials and Methods

This cross-sectional study was conducted among spinning mill workers in and around Guntur city, Andhra Pradesh. The ethical approval for this study was obtained from the Institutional Ethical Committee of the teaching dental institution the investigators are affiliated with (54/IEC/SIBAR/2018). Five spinning mills from this geographical area were randomly selected and all the

industrial workers were invited to participate in the study. Of the 642 workers employed in the mills, 39 were not present on the days when the study was conducted. The response rate was 92.7% with 559 workers demonstrating interest to take part in the study. Subjects with self-reported systemic diseases and experience of less than 3 months as a spinning mill worker were excluded from the study. The final sample constituted 458 subjects and the study purpose was explained to all the participants; informed consent was obtained prior to the collection of data. Data on gender, age, education, income, tobacco and alcohol habits, oral hygiene practices, self-reported dental problems, past dental visits, type and place of availed treatments, and barriers for utilizing dental services were recorded. Oral health status was examined using Simplified Oral Hygiene Index (OHI-S),^[12] Decayed, Missing, Filled Tooth Index (DMFT),^[13] and Community Periodontal Index (CPI).^[13] Two investigators took part in the data collection process after demonstrating good interexaminer reliability [intraclass correlation coefficient ranged from 0.84–0.87; weighted Kappa for CPI was 0.91 (95% CI 0.84–0.97)]. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20 software (IBM SPSS statistics for Windows version 20, Armonk, NY, USA) and descriptive statistics, independent samples t-test, one-way analysis of variance, and Fisher's exact test were employed to analyze the data. The significance level was set at 5%.

Results

Of the final sample of 451, 229 (50.77%) were males and 222 (49.23%) were females. Majority of the participants (33.7%) belonged to the age group of 31–40 years. 41.01% of the study subjects were illiterate, while 33.03% did not complete primary school education. The prevalence of tobacco use in any form was 32.15% and such use was reported only among males, which implies that 63.6% of male spinning mill workers have the habit of tobacco consumption. Nearly 60% of the male spinning workers reported alcohol use. Descriptive statistics of the background characteristics of the study population is reported in Table 1.

More than 90% of the study participants reported using toothbrush and toothpaste. Greater than 50% of those reported using toothbrush responded that they change the brush once in a year. Only 31.5% of the study participants reported availing oral health services in the past, and a significant difference was noted between the age groups in this regard, with considerably higher percentage of older subjects reporting past dental visits compared to younger age groups. No differences were noted between males and females in the proportion of participants reporting past dental visits. Almost 50% of those who reported availing dental services in the past sought dental care more than 12 months from the time of data collection. Nearly 80% of the study subjects have experienced some sort of oral health problems in their lifetime, and toothache was the most common problem reported accounting for 57.14% of all the problems reported, followed by dental caries (24.3%), loose teeth (21.56%), bleeding

gums (16.8%), and missing teeth (13.16%). Among those who sought dental care in the past, extraction was the most common treatment sought (64.78%), followed by restorations (22.53%). Majority of the participants reported availing dental care from private clinics (40.14%), and consulting a registered medical practitioner and quacks in the locality for oral health problems was found to be not uncommon among the study population. High costs associated with dental treatment, lack of time, other expenses to meet, and the perception that dental pain will go away in some time even left unattended were the major reasons for not availing oral health care. Gender wise and age group wise

differences among study population with regard to oral hygiene habits, previous dental visits, dental problems experienced in the past, type of care sought, and barriers for utilization of dental services are presented in Table 2.

Female participants were found to have better oral hygiene status compared to males, which is partially significant. Similar was the scenario when caries experience was considered. Majority of the study subjects (74%) have a DMFT score of 1-6. There were 86 participants without any coronal caries experience. The mean coronal caries experience was more among older spinning mill workers compared to the younger workers. The difference in DMFT scores between males and females was not significant. Majority of the participants (46.3%) were with CPI score 2, while only 10.2% were observed to have all healthy sextants. 136 subjects (30.15%) demonstrated loss of attachment of some severity. The age and gender wise differences in the oral health status of the study participants are presented in Table 3.

Table 1: Descriptive statistics of the background characteristics of study population

Variable	Category	Number (n)	Percentage (%)
Gender	Male	229	50.77
	Female	222	49.23
Age group	18-30 years	84	18.62
	31-40 years	152	33.7
	41-50 years	135	29.93
	51 years and above	80	17.73
Education	Illiterate	185	41.01
	Not completed primary education	149	33.03
	Primary education	85	18.84
Tobacco habit	Yes	145	32.15
	No	306	67.85
Alcohol habit	Yes	137	30.37
	No	314	69.63

Discussion

The study data shows that there is near equal employment from both genders in the spinning mills considered for this study. Majority of the participants were middle-aged adults aged between 31 and 50 years. One of the reasons for this observation could be the tendency to explore other livelihood options among the younger age groups.

In a study conducted by Sherley *et al.*^[10] toothbrush was used among 61% of workers, while 29% used fingers and 7% used

Table 2: Age and gender wise differences in oral hygiene habits and oral health care service utilization among the study subjects

Variable	Category	Gender		P	Age group				P
		Male n (%)	Female n (%)		18-30 n (%)	31-40 n (%)	41-50 n (%)	51 and above n (%)	
Cleaning aid	Brush	202 (88%)	209 (94%)	0.04*	82 (97%)	148 (97%)	124 (91%)	57 (71%)	0.03*
	Finger	23 (10%)	9 (4%)		2 (2%)	4 (2%)	8 (5%)	18 (22%)	
	Twig	4 (1%)	4 (1.8%)		0	0	3 (2%)	5 (6%)	
Cleaning material	Paste	214 (93%)	212 (95%)	0.38	84 (100%)	151 (99%)	128 (94%)	63 (78%)	0.041*
	Powder	13 (5%)	7 (3%)		0	1 (0.6%)	6 (4%)	13 (16%)	
	Others	2 (0.8%)	3 (1.3%)		0	0	1 (70%)	4 (5%)	
Frequency of changing toothbrush	3 months	37 (18%)	68 (32%)	0.003*	23 (0.28%)	36 (0.24%)	26 (20%)	20 (12.7%)	0.036*
	6 months	44 (21%)	43 (20%)		17 (0.20%)	23 (0.15%)	24 (19%)	23 (40.3%)	
	1 year	121 (59%)	98 (46%)		42 (51%)	89 (60%)	74 (59%)	14 (24.5%)	
Past dental visit	Yes	63 (27.5%)	79 (35.5%)	0.06	24 (28.5%)	31 (20.3%)	49 (36.2%)	38 (47.5%)	0.001*
	No	166 (72.4%)	143 (64.4%)		60 (71.4%)	121 (79%)	86 (63.7%)	42 (52.5%)	
Last dental visit	6 months	19 (30.1%)	23 (29.1%)	0.18	6 (25%)	6 (19.3%)	17 (34.6%)	13 (34.2%)	0.001*
	12 months	9 (14.2%)	21 (26.5%)		3 (12.5%)	5 (16.1%)	14 (28.5%)	8 (21%)	
	>1 year	35 (55.5%)	35 (44.3%)		15 (62.5%)	20 (64.5%)	18 (36.7%)	17 (44.7%)	
Facility where dental care was sought	Government hospital	7 (11.1%)	11 (13.9%)	0.02*	2 (8.3%)	2 (6.4%)	3 (6.1%)	11 (28.9%)	0.001*
	Private clinic	35 (55.5%)	22 (27.8%)		7 (29.1%)	22 (70.9%)	17 (34.6%)	11 (28.9%)	
	Dental college	7 (11.1%)	14 (17.7%)		9 (37.5%)	4 (12.9%)	6 (12.2%)	2 (5.2%)	
	Quacks	5 (7.9%)	13 (16.4%)		2 (8.3%)	2 (6.4%)	8 (16.3%)	6 (15.7%)	
	RMPs	9 (14.2%)	19 (24%)		4 (16.6%)	1 (3.2%)	15 (30.6%)	8 (21%)	

Chi-square test; $P \leq 0.05$ considered statistically significant; * denotes statistical significance

Table 3: Age and gender wise differences in the oral health status of the study participants

Variable	Category	Gender		P	Age group				P
		Male n (%)	Female n (%)		18-30 n (%)	31-40 n (%)	41-50 n (%)	51 and above n (%)	
Subject wise	0	17 (7.4%)	29 (13%)	0.013*	25 (29.7%)	11 (7.2%)	8 (5.9%)	2 (2.5%)	0.001*
CPI SCORE [†]	1	51 (22.2%)	67 (30.1%)		44 (52.3%)	39 (25.6%)	29 (21.4%)	6 (7.5%)	
	2	113 (49.3%)	96 (43.2%)		12 (14.2%)	88 (57.8%)	62 (45.9%)	47 (58.7%)	
	3	48 (20.9%)	30 (13.5%)		3 (3.5%)	14 (9.2%)	36 (26.6%)	25 (31.2%)	
Mean OHI-S (SD) [‡]		2.41 (0.83)	2.29 (0.64)	0.086	1.7 (0.31)	1.93 (0.54)	2.68 (1.05)	3.27 (1.81)	0.001*
Mean DMFT (SD) [‡]		4.73 (2.1)	4.6 (2.26)	0.52	3.08 (0.93)	3.76 (1.62)	5.41 (2.18)	6.75 (4.80)	0.001*

[†]Chi-square test; [‡]Independent samples t-test for dichotomous independent variable and One-way analysis of variance for multichotomous independent variable; P≤0.05 considered statistically significant; * denotes statistical significance

neem stick to clean their teeth. In our study, 91.74% reported using toothbrush, 7.14% used fingers, and 1.16% used neem stick. Though the percentage of population using toothbrush were observed to be comparatively more, close to 10% of study subjects still rely on cleaning aids like neem twigs and fingers. In spite of using toothbrush daily, the results showed that the subjects had only fair oral hygiene. Sharma *et al.*^[7] showed similar results in which despite daily tooth brushing by 86.67% of participants, 60% of the population had poor oral hygiene. Although greater number of workers used toothbrush to clean their teeth, this habit failed to show its effect on periodontal status of spinning mill workers.

The overall mean DMFT score of the study participants in our study was higher when compared to the study conducted by Raj *et al.*^[9] where the overall mean DMFT score was 3.1. The results of the study revealed that mean DMFT score of the workers increased with age, which is similar to the study conducted by Dileep *et al.*^[11] and is in contrast with the study conducted by Duraiswamy *et al.*^[8] where no such trend was observed. International experiences suggest poor oral health status among spinning mill workers.^[14] A recent study conducted in the same geographical location as the present study reported relatively better oral health status and oral health related quality of life among spinning mill workers compared to workers from tobacco industry.^[15]

Majority of the workers sought their dental care through private dental clinics and least number of workers through government hospitals and by the quacks. This can be due to their financial background which and one of the reason could be due to the lack of facilities in the government hospitals. Most of the spinning mill workers had not undergone previous dental visits which can be supported by the fact that there is a lack of knowledge regarding their oral health and the timing of the work could be one of the reasons and their negligence can be one of the contributing factors.

Primary care physicians and oral health care professionals play a key role in informing and enabling the spinning mill workers to realize the importance of oral health care in safeguarding general health and maintain quality of life. In addition, oral health is increasingly being emphasized as an essential component of primary care.^[16] Often oral health care facilities serve as the

primary contact points for people in the diagnosis of general health conditions and in engaging the subjects to seek requisite medical care.^[15] However, people from lower socioeconomic strata neglect dental care for a variety of reasons.^[17] It is, therefore, important that primary care physicians play a proactive role in emphasizing on the maintenance of good oral hygiene, screening for basic oral health problems, and referring to oral health care professionals in case of necessity, especially for those from lower socioeconomic strata. This study highlights the increased normative dental needs among spinning mill workers and calls for the need for awareness programs among the study population.

Conclusion

It is to be understood that workers comprise a valuable sector vastly contributing toward the comfortable living of the concerned populations. In this context, it is important to safeguard the health and maintain the quality of life of the working sector which consequentially improves the quality of life of the beneficiaries. The documentation of oral hygiene behaviors, status, and oral health care seeking behaviors in this study identifies the areas which demand focus while aiming to improve the oral health status and related quality of life of spinning mill workers.

Declaration of patient consent

The authors certify that they have obtained all appropriate participant consent forms. In the form, the participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Mapping the link between cardiac biomarkers and chronic periodontitis: A clinico-biochemical study

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Abstract:

Background: Various risk factors are coupled with atherosclerotic complications, such as myocardial infarction and stroke. Periodontitis is considered one of them. **Aims and Objectives:** The objective of the study is to compare and correlate the occurrences of periodontitis with serum levels of cardiac-biomarkers in patients with coronary heart-disorders. **Materials and Methods:** Of 70 individuals diagnosed with coronary artery diseases, 32 patients with chronic periodontitis constituted the test group, 31 without chronic periodontitis constituted the control group. Cardiac-biomarkers analyzed were Troponin T, Troponin I, Myoglobin; low density lipoprotein (LDL), high-density lipoprotein, very LDL (VLDL), total cholesterol (TC), and highly sensitive C-reactive protein (Hs-CRP). Periodontal characteristics were drawn from the plaque index (PI) and gingival index, probing depth (PD), clinical attachment loss, and periodontal inflammatory surface area (PISA). **Statistical Analysis:** In order to separate any association between cardiac biomarkers and clinical parameters of periodontitis, detailed statistical analysis through independent *t*-test and Pearson test of correlation was done. **Results:** Statistically significant differences were seen not only in PI, PD, and PISA between both the groups ($P < 0.05$), but also between various cardiac parameters of test and control groups ($P < 0.001$). Positive relations were seen in the test group, between cardiac biomarkers such as TC, VLDL, Hs-CRP, and Troponin T with periodontal parameters such as PD and PISA. **Conclusion:** The study reveals, a strong association between periodontitis and diseases of cardiovascular nature, highlighting the need for awareness and timely medical interventions to prevent periodontitis from scaling up and interfering with the risk of cardiovascular problems.

Key words:

Cardiac biomarkers, chronic periodontitis, coronary artery disease, lipid profile, troponin and myoglobin

INTRODUCTION

Periodontal medicine, a term first coined by Offenbacher refers to an area that analyzes a host of all-new data. It points to a binding relation between periodontal diseases and systemic health conditions. Being a critically important constituent of overall health, oral health in patients with periodontitis is a potential risk leading to other diseases. Worth noting is that the infections on account of periodontitis are considered chronic with occasional acute bursts. Periodontitis can trigger systemic inflammations by actuating the acute hepatic phase response owing to the possible bacteremia which is temporary but recurrent in nature.^[1]

Coronary artery disease, also known as coronary heart disease is the culmination of atheromatous plaques formed within the arterial walls. Severe periodontitis patients are twice at the risk of a fatal heart-attack, and three times prone to a stroke compared to those without periodontitis; and this is true for cases known through cardiovascular risk, such as, lipid profile, cholesterol readings, body mass, conditions

associated with diabetes and smoking habits.^[2] The fact that periodontal pathogens have been observed in the atherosclerotic plaques of humans establishes that infections caused by periodontitis can develop bacteremia and accelerate accumulation of atherosclerotic plaque.^[3]

Biomarkers play a very critical role in drawing conclusions for a diagnosis. Owing to highly sensitive (Hs) nature, preciseness and level of availability, analysis of cardiac troponins is

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currently considered gold standard for diagnosing myocyte necrosis bio-chemically. Myocardium releases two types of troponins, namely I (cTnI) and T (cTnT) which can be diagnosed within 2–4 h, but an increase which is helpful to diagnosis often does not happen until 4–6 h of the observed event due to gradual nature of discharge from necrotized cardiomyocytes. This also answers why plasma of cTn can remain at elevated levels of duration as lengthy as 14 days in the presence of extensive myocardial necrosis. In the event of injury to myocytes, myoglobin (Myo), another major marker by virtue of it is comparably low-weight in the presence of myocyte injury, skips into and persists in circulatory system for its short (half) life of just 10–30 min. Increase in plasma levels become apparent within an hour of the start of the event. Similar increase is helpful in early diagnosis of acute myocardial infarction (AMI), say between 2 and 12 h of the event. Nevertheless, it should be noted that Myo is not myocardium specific.^[4]

The analysis of cross-sectional evidences denotes that periodontitis causes only little elevation in markers of acute phase responses from liver like, C-reactive protein (CRP), haptoglobin, α -antitrypsin, and fibrinogenin in response to virulence factors like lipopolysaccharides of blood borne oral bacteria, which also triggers discharge of cytokines such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF- α).^[5] Hs-CRP is a precursory indicator of atherothrombosis, thus being a strongest marker of inflammation in the event of cardiovascular diseases.^[6] Thus, the aim of this study in this background has been to analyze the instance of chronic periodontitis in patients having cardiac biomarkers in serum levels of patients pointing to coronary heart problems. Also to deduce a probable association between both chronic periodontitis and coronary heart disorder by correlating both cardiac biomarkers and periodontal clinical as well as inflammatory parameters.

MATERIALS AND METHODS

Study population and design

This analytical cross-sectional study attempts to check the differences in various cardiac parameters between cardiac patients with periodontitis and without periodontitis. Seventy patients diagnosed with coronary artery disease(s) marked by cardiac necrosis, coronary angiography, and characteristic changes in electrocardiogram (ECG) and clinically established symptoms were short-listed for double-blinded cross-sectional analysis. From this, those who did not match the eligibility criteria were seven in number and were excluded. Thus, the final number of patients was 63. After a round of interview to assess their suitability for including in the study, these volunteers were subjected to periodontal tests, conducted while patients were in-hospital, or shortly after they were discharged.

Patients diagnosed with chronic periodontitis who had bleeding while probing; patients with clinical attachment loss (CAL) ≥ 1 having minimum 20 natural teeth with at least two probing pockets having a depth more than 3 mm;^[7] those who had not had any medical care for periodontitis in the previous 6 months, and those who had not used any systemic antibiotics in the preceding 6 weeks of the study, constituted the test group ($N = 32$). Thus, the test group comprised of patients having coronary artery disease

with chronic periodontitis; and subjects without chronic periodontitis formed the control group ($N = 31$). Participants found to have any other systemic health concerns, conditions of compromised immune system, auto-immune disorder, having prolonged antimicrobial therapy, recent treatment with corticosteroids (since corticosteroids may mask the immune response of the host being an immunosuppressant) or nonsteroidal anti-inflammatory drugs (NSAIDs), (as NSAIDs may façade inflammatory reaction of the host, leading to misinterpretation of the inflammatory markers/parameters), or presently receiving any treatment for periodontitis were excluded.

Ethical parameters for clinical researches, mentioned in the Declaration of Helsinki 2008 were adhered to during the study. Protocol was approved of by the Scientific and Ethical Committee of the institution from December 2017 to June 2018. After being thoroughly educated about the study protocol, the volunteers' willingness was obtained in writing, duly signed.

A periodontal specialist (B. R) singly performed the examination, remaining blinded to clinical, cardiac, and hematological information of patients. In order to gauge the probing pocket depth and loss of clinical attachment at six sites per tooth, excluding the third molars, a properly calibrated UNC-15 probe, (Hu-Friedy, Chicago, IL, USA) was put in use. Using parameter of periodontal inflammatory surface area (PISA), periodontal disease characterized by inflammation was assessed, which was the sum of periodontal probing depth (PD) of bleeding on probing-affected areas, for the complete dentition.^[8] PISA also referred to the extent of inflammation that could be quantitatively assessed to separate subjects with active inflammation from those having noninflamed or healed tissues. Also considered were the plaque index (PI) (O'Leary *et al.*, 1972) and gingival index (GI) (Loe and Silness, 1963). The consort flow chart for the study population and design is given in Figure 1.

Cardiology study and hematologic measurements

In order to diagnose the presence of coronary artery diseases, ECG and coronary angiography were resorted to. The cardiac biomarkers were assessed by withdrawing patient's blood on his/her arrival at the emergency section, and at 6, 8, 12, and 18 h, together with bio-medical criteria of lipid profile. Besides low-density lipoprotein (LDL), high density lipoprotein (HDL), very LDL (VLDL) and total cholesterol (TC), inflammatory marker, Hs CRP, were recorded and assessed through the blood. The blood drawn was collected; it was transferred into ethylenediaminetetraacetic acid – containing tubes to assess cardiac biomarkers such as peak cTn, TcI, and Myo readings by chemiluminescence immunoassay from Beckman Coulter Inc. (Brea, CA, USA). This brings out the sensitivity and specificity percentages of 96 and 94 respectively for a cutoff point of 0.50 ng/mL, where Myo escalation is pointed to, by an increase of $>70\%$ ng/mL at any point of the time measurement. Blood serum was separated by centrifuging at 13,000 rotations per minute before performing the chemiluminescence assay. Samples along with control serum were diluted to the ratio of 1:50. After 100 μ l of standardized solution and serum were transferred to the wells, 50 μ l of immune-reagent was added, followed by incubating and shaking at 300 rpm for an hour. 3,3',5,5'-Tetramethylbenzidine (TMB) substrate 100 μ l was

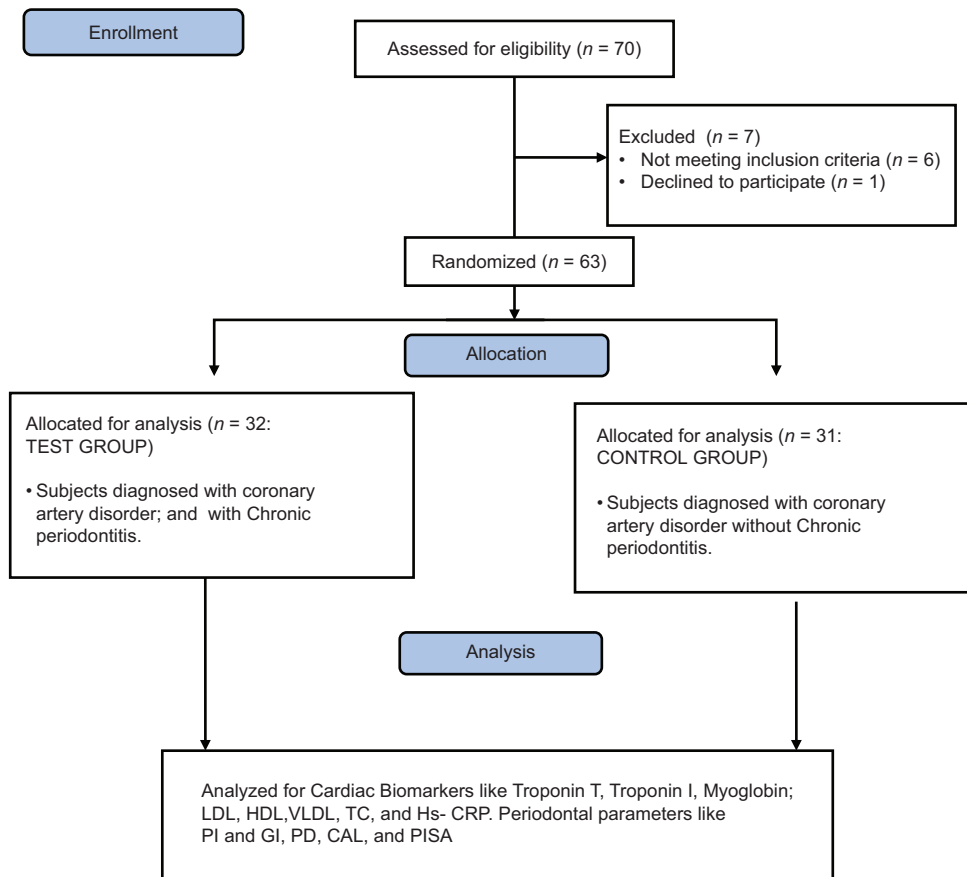


Figure 1: Consort flow chart. n – number; LDL – low-density lipoproteins; HDL – high-density lipoproteins; VLDL – Very low-density lipoproteins; TC – Total count; HS-CRP– High- sensitivity C-reactive protein; PI – Plaque index; GI – Gingival index; PD – Probing depth; CAL – Clinical attachment loss; PISA – Periodontal inflammatory surface area

added, followed by incubating on a shaker at 300 rpm for 25 min at room-temperature. After the buffer was aspirated away; 25 µl of TMB stopper solution was added specifically to each well on finding sufficient color, which was again incubated for 1 min.^[9]

Statistical analysis

For analysis of data, SPSS version 23 (IBM Inc. Chicago, Illinois, USA) was used. To ascertain associations between cardiac biomarker indicators and periodontal clinical parameters, independent *t*-test and Pearson correlation tests were done. Kappa statistics assessed inter and intra testing reliability, which was found 0.77 and 0.81, respectively, showing a high degree of conformity in the findings.

RESULTS

Table 1 and Graph 1 represents the Intergroup comparison of mean age in years. While the mean age was 54.92 years for the test group, in control group, it stood at 51.16 years. There were no statistically significant differences between the two groups in age ($P = 0.113$). This finding ensures matching between the two groups, as age could be a possible confounding factor in this association. The fact that individuals with other systemic diseases and those who have been under treatment for periodontal disease were excluded in this study also adds value in the quest of eliminating intrusion of bias. As for comparison of PI, PD, and PISA between the two groups ($P < 0.05$), as

Table 1: Intergroup comparison of mean age in years

Group	n	Minimum	Maximum	Mean±SD	P
Test	32	39	65	54.92±6.825	0.113 (NS)
Control	31	30	65	51.16±9.450	

NS – Notsignificant ($P > 0.05$); n – Sample size; SD – Standard deviation; P – Probability value

shown in Table 2 and Graph 2, the variations were significant, where mean PI of 2.24 ± 0.59 , 1.89 ± 0.40 ; mean PD value of 6.83 ± 0.99 , 5.63 ± 0.60 ; and mean PISA of 31.9 ± 3.7 , 27.48 ± 4.22 were statistically analyzed in the respective order of test groups and control groups. While not much difference was noticed between GI and CAL in the groups, significant variations were noted between different cardiac parameters among test and control groups ($P < 0.001$), as shown in Table 3 and Graph 3. Comparison between cardiac biomarkers and periodontal parameters in test group was captured in Table 4 and Graph 4, and that of control group in Table 4 and Graph 5, indicated statistically positive and measurable similarities among TC, VLDL, Hs-CRP and Troponin T with PD and PISA in patients having congestive cardiac disorder and chronic periodontitis.

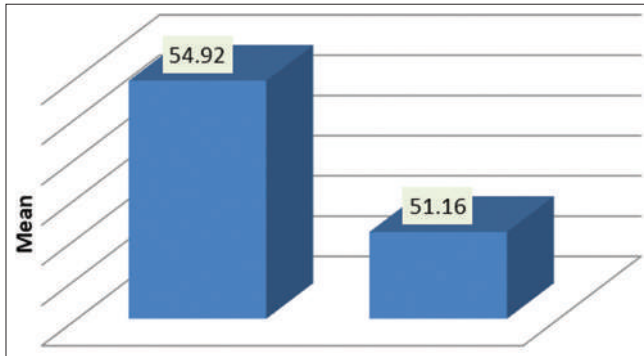
DISCUSSION

When systemically challenged with gram-negative bacteria or virulence factors such as lipopolysaccharide (LPS) or endotoxins, blood vessels show inflammatory cell infiltration that subsequently leads to vascular smooth muscle

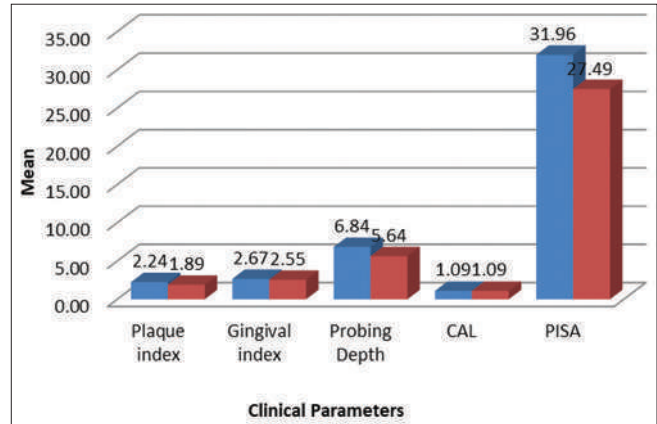
Table 2: Intergroup comparison of periodontal clinical parameters

Parameter	Mean±SD		Mean difference	P
	Test group	Control group		
Plaque index	2.2408±0.59228	1.8948±0.40816	0.34600	0.020*
Gingival index	2.6680±0.40360	2.5500±0.17678	0.11800	0.187 (NS)
Probing depth	6.836±0.9995	5.636±0.6048	1.2000	<0.001**
CAL	1.092±0.6582	1.092±0.6582	0.0000	1 (NS)
PISA	31.9568±3.74697	27.4852±4.22111	4.47160	<0.001**

*Significant ($P < 0.05$); **Highly significant ($P < 0.001$). NS – Not significant ($P > 0.05$); CAL – Clinical attachment loss; PISA – Periodontal inflammatory surface area; SD – Standard deviation; P – Probability value



Graph 1: Intergroup comparison of mean age



Graph 2: Intergroup comparison of periodontal clinical parameters

proliferation and fatty degeneration with intra-vascular coagulation.^[10]

The current study has shown statistically significant differences not only in periodontal parameters such as PI, PD, and PISA between both the groups ($P < 0.05$), but also between various cardiac parameters of test and control groups ($P < 0.001$). This correlation was seen indicative of the inflammatory background in both coronary artery diseases as well in chronic periodontitis. However, logistic regression analysis by Mattilla et al., who analyzed that the factors influencing dental health and coronary heart disease were not dependent on age, TC, HDL, triglycerides (TGs), C peptide, diabetes, hypertension, and even smoking.^[11]

In the current study, positive relations were seen in the test group, between cardiac biomarkers like TC, VLDL, Hs-CRP, and Troponin T with periodontal parameters such as PD and PISA. These findings were in accordance with the studies by DeStefano F, et al. who showed a relation between periodontal diseases and coronary heart disease citing linkage between periodontitis, AMI and/or atherosclerosis.^[12] The elevated levels of inflammatory parameters in patients with coronary artery disease with chronic periodontitis could be attributed to the systemically hyper inflammatory monocyte phenotype that discharges inordinately higher levels of inflammatory cytokines indicating high risk of atherosclerosis and emboli formation due to underlying M0 + phenotype.^[13]

Atherosclerosis that leads to cardiovascular diseases is marked by dyslipidemia, bio-marked by abnormal levels of TG, cholesterol or lipoproteins in plasma, although other confounding factors like chronic inflammation also contribute to its pathogenesis. This probably explains the reason for the statistically significant elevated levels of lipid parameters like VLDL in test group, having positive correlation with

periodontal parameters.^[14] LDL accelerates the onset of atheroma caused by accelerating monocyte discharge of IL-1 β , TxA2, and TNF- α and exaggerates response to LPS.^[15] The demonstration of lipid deposition in descending aorta of a rat where in periodontitis was induced by ligatures, pointed that diet high in cholesterol could exacerbate periodontitis in those animals.^[16]

Periodontal epithelium and connective tissues can be attacked by anaerobic bacteria such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Aggregatibacter actinomycetemcomitans*. These were shown to be viably persistent in the coronary endothelium and atherosclerotic plaque as well.^[17] *P. gingivalis in vitro*, in the presence of LDL-induced macrophages to change into foam cells and the measure of tissues damaged by periodontitis is directly linked to the proportion of macrophages activated by LDL.^[18]

A study establishing role of *P. gingivalis* in aggression, degradation, inactivation of proteins and dysfunctions at cellular levels, by modification of oxidative phosphatidylcholines in vascular LDL, arrived at the conclusion that *P. gingivalis* can change both protein expression and LDL's potential to proliferate, besides playing a critical role in periodontitis-related atherosclerosis.^[19]

According to recent literature, CRP and its peptides influence processes such as shedding of cellular adhesion molecules, which has intrigued the researchers regarding its role in influencing adhesion and trans-migration of leukocytes around the endothelial wall. What is hypothesized here is that an inflammation, even low grade, identified by CRP contributes to an evidence of chronic infection.^[20]

Cardiac troponins T and I, the myofibrillar proteins, have been recognized as specific and sensitive myocyte injury markers, thereby fortifying stratification and substantially helping the diagnosis, in patient care of coronary syndromes.^[21] The relation of periodontitis with serum LPS/endotoxin, and raised levels of troponin have been observed in the studies conducted by Goteiner et al.^[22]

Taking into consideration elevated ST in ECG and typical AMI aspects, it was seen that the total count of leukocytes correlated the linkage between chronic periodontitis and cTnI; and also between cerebral palsy and Myo.^[23] Similarly, the extremity level of periodontitis has a direct impact on the size of AMI, as counted by serum troponin I and Myo levels.^[24] Likewise, other markers

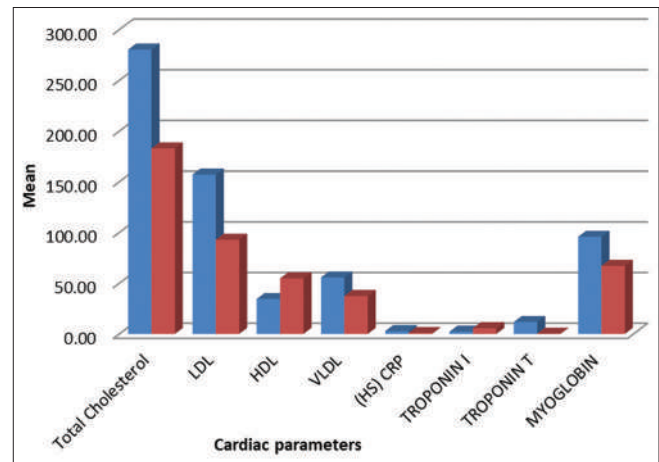
showing cardiac lesions in chronic patients with inflammation are troponin T, troponin I, pro-BNP, LDH, and high sensitivity CRPs.^[25] Myo is considered a less effective marker with respect to specificity compared to troponin in investigating myocardial necrosis even though both are used in the present analytical study due to their better prediction capabilities in people with coronary artery disorder, and their relation with final peak of troponin.^[26]

The limitations of this study are its small sample size which raises questions on the power to detect significant differences between the groups. Nevertheless, this is one of

Table 3: Intergroup comparison of cardiac parameters

Parameter	Mean±SD		Mean difference	P
	Test group	Control group		
Total cholesterol	280.32±47.20	182.84±23.55	97.480	<0.001**
LDL	157.04±19.73	92.76±9.81	64.280	<0.001**
HDL	34.44±4.33	54.72±8.19	-20.280	<0.001**
VLDL	55.67±9.18	37.37±2.27	18.30	<0.001**
HS CRP	2.77±0.74	0.87±0.71	1.90	<0.001**
Troponin I	2.22±0.27	5.58±1.11	-3.36	<0.001**
Troponin T	11.72±2.12	0.26±0.14	11.46	<0.001**
Myoglobin	95.69±14.66	67.00±12.72	28.69	<0.001**

**Highly significant (P<0.001). There is statistically significant difference present between various cardiac parameters between test group and control group (P<0.001). LDL – Low-density lipoprotein; HDL – High-density lipoprotein; VLDL – Very LDL; HS CRP – Highly sensitive C-reactive protein; SD – Standard deviation; P – Probability value

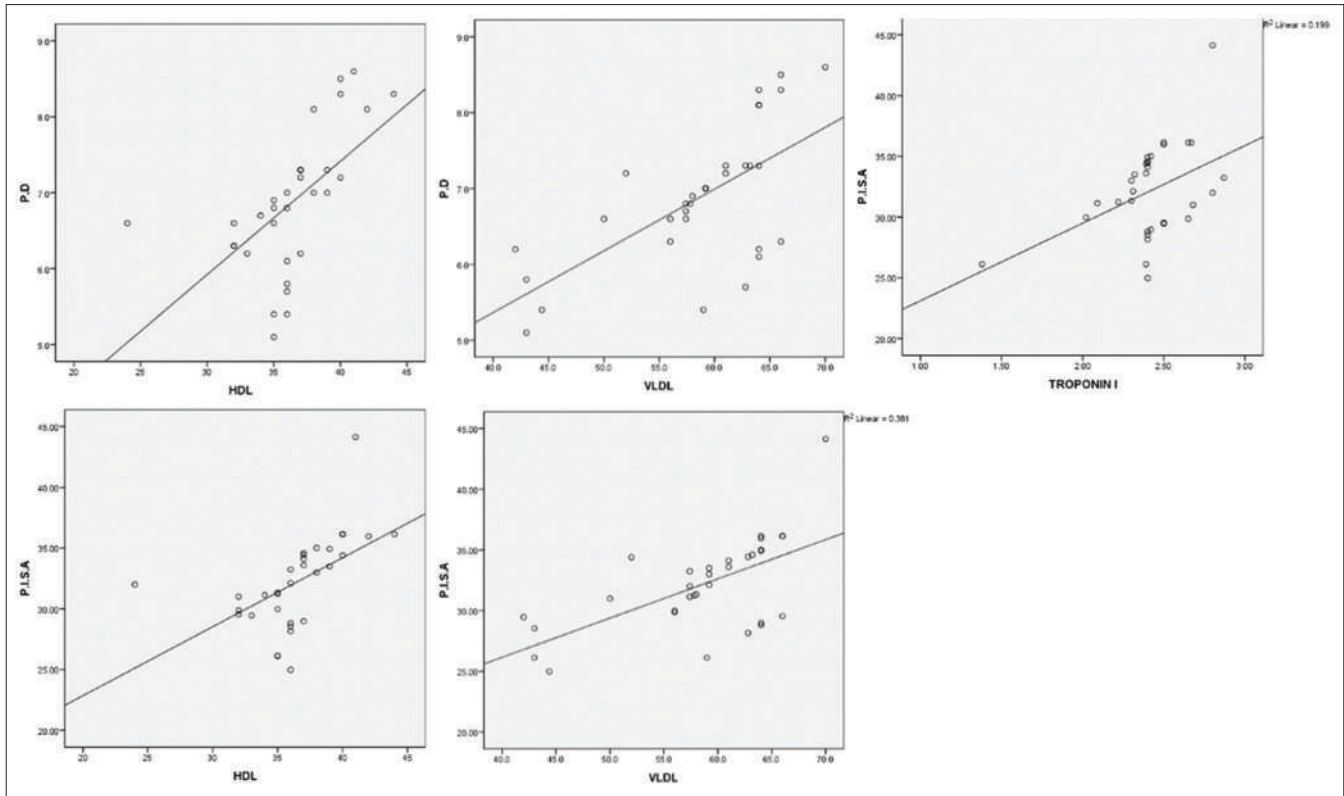


Graph 3: Intergroup comparison of cardiac parameters

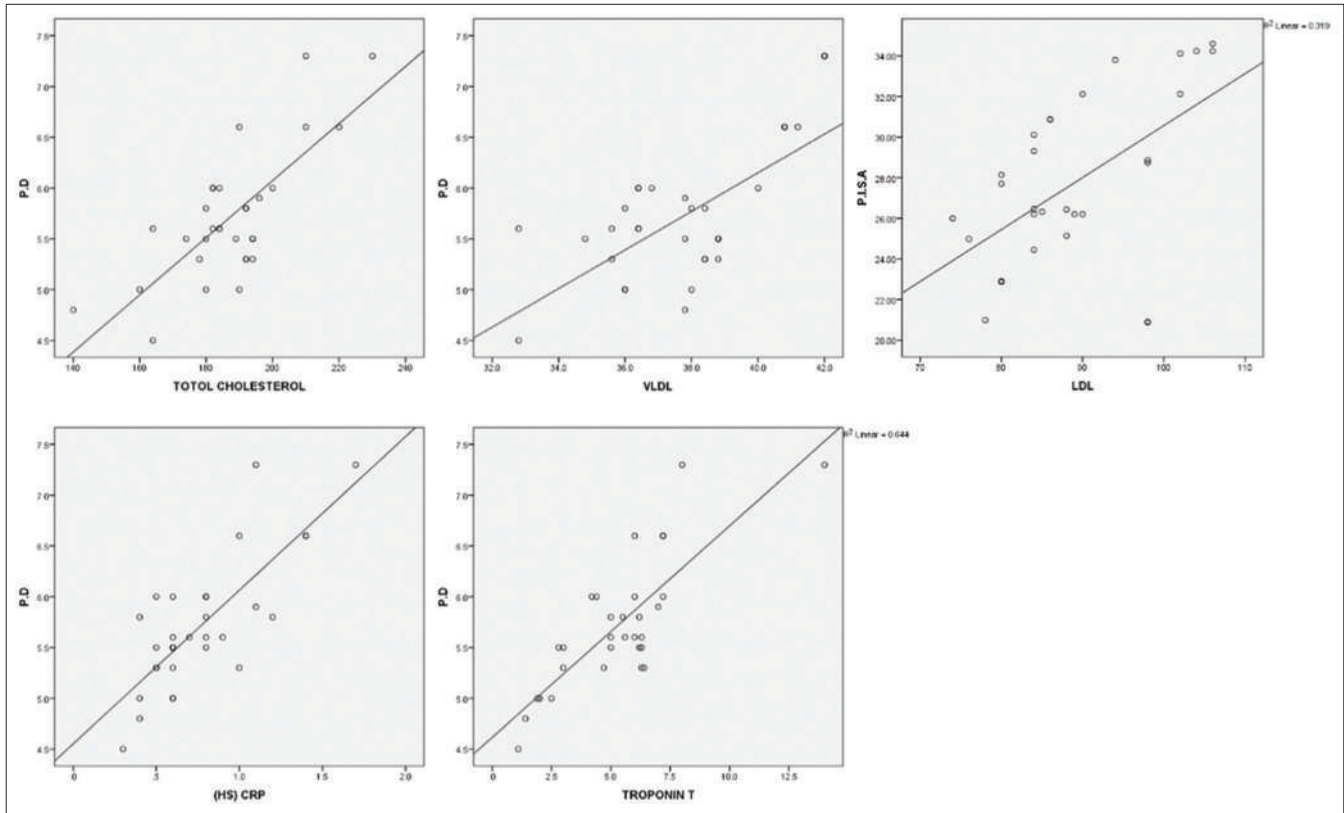
Table 4: Correlation between clinical and cardiac parameters

Group	Parameter	Statistics	Total cholesterol	LDL	HDL	VLDL	HS CRP	Troponin I	Troponin T	Myoglobin
Control	PI	Pearson correlation	0.068	0.246	-0.193	0.058	0.183	0.267	0.123	0.389
		P	0.746	0.236	0.355	0.781	0.381	0.197	0.557	0.055
		n	25	25	25	25	25	25	25	25
	GI	Pearson correlation	0.177	0.183	-0.068	0.155	0.026	-0.169	0.215	-0.038
		Significant (two-tailed)	0.399	0.382	0.747	0.459	0.902	0.418	0.303	0.858
		n	25	25	25	25	25	25	25	25
	PD	Pearson correlation	0.752**	0.279	-0.318	0.699**	0.793**	0.246	0.825**	0.148
		Significant (two-tailed)	<0.001	0.177	0.122	<0.001	<0.001	0.236	<0.001	0.480
		n	25	25	25	25	25	25	25	25
	CAL	Pearson correlation	-0.291	0.152	-0.045	-0.308	-0.008	-0.167	-0.273	0.094
		Significant (two-tailed)	0.158	0.468	0.829	0.134	0.968	0.425	0.187	0.656
		n	25	25	25	25	25	25	25	25
	PISA	Pearson correlation	0.390	0.563**	-0.317	0.361	0.362	0.212	0.389	0.360
		Significant (two-tailed)	0.054	0.003	0.122	0.076	0.075	0.308	0.054	0.077
		n	25	25	25	25	25	25	25	25
Test	PI	Pearson correlation	0.070	0.067	-0.087	0.155	-0.621**	0.173	0.133	-0.263
		Significant (two-tailed)	0.738	0.750	0.680	0.461	0.001	0.408	0.527	0.204
		n	25	25	25	25	25	25	25	25
	GI	Pearson correlation	0.040	0.061	0.197	-0.022	-0.163	-0.335	0.166	0.063
		Significant (two-tailed)	0.851	0.771	0.345	0.916	0.435	0.101	0.427	0.767
		n	25	25	25	25	25	25	25	25
	PD	Pearson correlation	0.210	0.365	-0.591**	0.647**	0.045	0.290	0.217	-0.068
		Significant (two-tailed)	0.313	0.073	0.002	<0.001	0.830	0.160	0.297	0.746
		n	25	25	25	25	25	25	25	25
	CAL	Pearson correlation	0.119	0.343	-0.212	0.381	-0.022	0.265	0.047	-0.216
		Significant (two-tailed)	0.571	0.093	0.308	0.061	0.917	0.201	0.824	0.300
		n	25	25	25	25	25	25	25	25
	PISA	Pearson correlation	-0.085	0.236	-0.550**	0.423*	-0.021	0.417*	0.311	-0.309
		Significant (two-tailed)	0.685	0.256	0.004	0.035	0.919	0.038	0.130	0.133
		n	25	25	25	25	25	25	25	25

**Correlation is significant at the P<0.01 level (two-tailed); *Correlation is significant at the P<0.05 level (two-tailed). LDL – Low-density lipoprotein; HDL – High density lipoprotein; VLDL – Very LDL; HS CRP – Highly sensitive C-reactive protein; PI – Plaque index; GI – Gingival index; PD – Probing depth; CAL – Clinical attachment loss; PISA – Periodontal inflammatory surface area; n – Total number; P – Probability value



Graph 4: Correlation between periodontal and cardiac parameters in the test group. PD – Probing depth; PISA – Periodontal inflammatory surface area; HDL – High-density lipoproteins; VLD L– Very low-density lipoproteins



Graph 5: Correlation between periodontal and cardiac parameters in the control group. PD – Probing depth; PISA – Periodontal inflammatory surface area; HS-CRP – High-sensitivity C-reactive protein; VLDL – Very low-density lipoproteins; LDL – low-density lipoproteins

the first attempts to correlate between cardiac parameters and periodontal health of subjects in the study area and offers important insights for future health promotional initiatives. Although statistically significant, the variability of analyzed markers has been low; hence, there is a need to interpret the results with care and in terms of their clinical cardiac relevance. In addition, in order to interpret these findings, more studies on gingival crevicular fluid-markers associated with chronic periodontitis will be warranted in future.

CONCLUSION

This study significantly contributes to further data for research highlighting the jurisdiction and severity of periodontitis and its linkage to coronary artery diseases, by way of cardiac bio-markers, such as cardiac troponin I, cardiac troponin T, Myo, lipid profile, and chronic periodontitis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Reflex Gastroesophageal Disorders and Functional Dyspepsia: Potential Confounding Variables for the Progression of Chronic Periodontitis: A Clinical Study

Abstract

Aim: To probe into the possible connection between gastroesophageal reflux disorders (GERDs) and functionally occurring dyspepsia as a factor raising the risk of chronic periodontitis.

Materials and Methods: A cross-sectional study was carried out on 40 patients with chronic periodontitis with age group between 40–60 years. The test group included 20 people diagnosed with gastroesophageal reflux disease (GERD), according to the Montreal Definition and Classification agreement, and chronic periodontitis. Symptomatic diagnoses were done to confirm functional dyspepsia. The control group comprised 20 systematically healthy people suffering from chronic periodontitis. Indices measured included flow-rate of saliva, repetitive saliva swallowing test for swallowing function, papillary marginal attachment index of gingiva, oral hygiene index-simplified and decayed, missing, filled teeth index. Data was analyzed using SPSS version 22 (IBM Inc. Chicago, USA). Descriptive statistics, such as mean and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables were determined. T test was performed for intergroup comparison and Pearson correlation test was done for evaluating correlation between various parameters. $P \leq 0.05$ considered as significant. **Results:** Statistically significant differences were observed between the test and control groups with regard to all the clinical parameters of interest. Pearson's correlation test revealed a strong negative correlation between salivary flow rate and OHI-S and DMFT scores. The RSST swallow function values demonstrated a moderate negative correlation with OHI-S scores, while OHI-I scores and DMFT scores were observed to be strongly correlated in a positive direction. A statistically significant difference was present in the probing depth and CAL levels between both the groups with higher levels in test group. **Conclusion:** GERD was linked to incremental incidences of chronic periodontitis and was established as an independent risk-raising factor.

Keywords: Chronic periodontitis, functional dyspepsia, gastroesophageal reflux, risk factor

Introduction

Periodontitis is an inflammatory oral disease that causes swelling in the tissues around the teeth. It also causes raised inflammatory biomarkers, resulting in tooth loss, disarrayed esthetics, impaired oral-functioning and poor quality of life caused by compromised oral health.^[1]

In addition to being resistant to antibiotics, bacterial biofilms are usually linked to a variety of chronic infections compared to nonattached bacteria.^[2] Bacteria like *Helicobacter pylori* are found in large numbers in periodontal pockets and can result in gastroesophagitis and gastric conditions that could be precancerous.^[3] Increased growth of free radicals, apoptotic

or necrotic cell destruction, and augmented proliferation of cells are considered procancerous. Periodontal pathogens can cause inflamed conditions stimulated by incidence of cell initiation, hinderance to normal cell growth, and proneness to carcinogenic conditions.^[4]

Montreal Definition and Classification Agreement defined gastroesophageal reflux disease (GERD) as “a condition characterized by reflex of the contents in stomach causing symptomatic problems and/or complications.”^[5]

Extraneousophageal discomforts arising from GERD are associated with respiratory, esophageal, and digestive systems including laryngeal (reflux laryngitis, hoarseness,

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chronic cough in some cases, vocal cord ulcers and granulomas), pharyngeal (mucositis), respiratory (asthma, bronchitis, chronic cough, and aspiration pneumonia), sinus (sinusitis), middle ear (otitis media), and oral conditions (tooth erosion, sour taste, and mucositis).^[6] Chronic periodontitis may be caused by GERD since it can induce poor oral conditions through poor salivary function or microbial colonization.^[7]

Studies have shown that inflammatory chronic periodontitis is associated with pathological gastroesophageal reflux. Patients suffering from inflammatory periodontitis and GERD can be managed with treatments isolated for chronic inflammatory periodontitis.^[8,9]

GERD patients are often predisposed for chronic periodontitis; this may be due to poor salivary function. The salivary coats on internal anatomical surfaces along with mucin-rich secretions provide a diffusion barrier that protects against chemical, mechanical, thermal, and microbial damages. Saliva is the endogenous antiacidic agent protecting the organs against symptomatic gastroesophageal reflux, and reduced secretion of saliva can prove inefficient in neutralizing the acids being formed.^[10] The exposure of esophageal mucosal to gastroesophageal reflux reduces the pH and triggers esophageal mechani/chemoreceptors, resulting in activated afferent fibers. Parasympathetic (nerve VII or IX) fibers stimulate active secretion of water and electrolytes including buffers in the glands producing saliva. Saliva secretion depends on the volume of gastroesophageal refluxate, whereas the quantity of saliva in GERD patients is independent of the volume of refluxate. Therefore, GERD patients usually have affected esophagosilivary reflex caused by hyposalivation, even in the case of high volume of refluxate. This reduced salivation in GERD patients causes their internal oral surfaces to be prone to acidic as well as proteolytic contents in the epidigestive system, ultimately resulting in chronic periodontitis.^[11]

Hyposalivation could be linked to intraoral proliferation of bacteria in people affected with GERD. Different types of antimicrobial proteins (AMPs) in human saliva and gingival fluid protect the epithelial cells from various invasive microbes and ensure oral homeostasis of pathogenic and commensal bacteria.^[12] One such AMP is chemokine ligand 28 (CCL28) that is effective in destroying anaerobic pathogens, such as *Porphyromonas gingivalis* and *Aggregatibacter Actinomycetemcomitans*, responsible for periodontal problems. Reduced salivary secretion also reduces the volume of CCL28, leading to weak oral self-defense.^[13] Thus, in GERD patients, hyposalivation should have a strong linkage to the cause of chronic periodontitis, allowing the spread of intraoral bacteria. This being the background, the present study is aimed at investigating the possible linkage between GERD and functional dyspepsia while analyzing risk factors in chronic periodontitis patients.

Materials and Methods

Ethical consideration and study design

This cross-sectional observational study has been approved by the Ethical Committee of Mamata Dental College and Hospital, Khammam, Telangana, India. The participants who have volunteered were enlightened with detailed information on the purpose of the proposed research, and their written/signed consent were obtained. Institutional ethical committee approved the present study on December 5, 2016.

Study population and inclusion criteria

The control group consisted of 20 individuals who are systemically healthy, but diagnosed with chronic periodontitis. Test group, similarly, contained twenty individuals suffering from GERD and functional dyspepsia. Both the groups included those patients who were referred to the outpatient department of periodontics, Mamata Dental College and Hospital, Khammam during the period from December 2016 to January 2018.

Exclusion criteria

Patients diagnosed with gingivitis, acute periodontitis, and other systemic diseases like acquired immunodeficiency syndrome, diabetes mellitus, carrying or lactating, smokers and those who were under treatment for orthodontics or even submitted to periodontal therapy in the previous six months of the study have been excluded.

Case definitions

The catalyzing factors to GERD include gaining weight, intake of fatty and nonfibrous foods, caffeine-containing gaseous drinks, and use of alcohol, tobacco, and drugs. Drugs that alleviate sphincter pressure at esophagus are antihistamines, anticholinergics, antidepressants classified as tricyclic, CBB, progesterone, and nitrate-based applications. The most common symptom for GERD is heartburn along with regurgitation of acidic contents upward to mouth along the esophagus. Other symptoms include epigastric pain, chest pain, hoarseness, throat irritation, and sometime cough.^[5]

The symptoms like postprandial fullness, feeling of early satiation, epigastric problems including burning, if diagnosed, could be attributed to functional dyspepsia, but without any structural evidence of the disease.^[14] No additional tests were performed

Chronic periodontitis' diagnosis is corroborated with clinical findings of inflammation on gingiva, clinical attachment loss ≥ 2 mm interdentially and ≥ 3 mm buccally, and the size of probing pocket depths >3 mm at ≥ 2 teeth per quadrant.^[15]

Salivary flow volume (Milliliter/minute)

Saxon test was conducted twice for every participant where the average quantity of saliva excretion was considered as

the salivary flow. For this test, the participants were made to bite and clamp-down upon a folded, weighed gauze piece for two minutes, after which the gauze inserted and the lab dish that contained the rest of intra-oral saliva were weighed. Standard conditions were maintained while collecting saliva for each volunteer.^[12]

Swallowing function (Number of swallows/second)

The Repetitive Saliva Swallowing Test (RSST) was used to evaluate the swallowing function so as to understand the saliva-swallowing potential. The frequency of swallowing for a 30 × period and “time-to-onset of the first swallowing” were clinically recorded. RSST was carried out keeping the conditions for each and every participant same as that of the volume of salivary flow.^[12]

Oral examinations for teeth - dental caries

Various indexes were used in the present study, such as dental caries, remaining tooth count, decayed tooth count (D), missing teeth (M), filled (treated, F) teeth, total number of DMF (decayed, missing and filled), and DMF indexes. Calculation of DMF index was done on the basis of “total number of decayed, treated, and missing teeth/residual teeth × 100 (%)”.^[16]

Oral examinations for soft tissue gingivitis

The severity of gingivitis was assessed and studied to establish its link to GERD with respect to optical findings. Papillary, marginal, and attached (PMA- Massler M, 1967) indices^[17] were used for the evaluation of gingiva inflammation. The simplified oral hygiene index (OHI-S- Greene JC & Vermillion JR, 1964) was used for oral hygiene.^[18] The tests identified PMA index scores as 0: zero inflammation, and 1: for inflammation of papillary, marginal, and attached gingiva to all teeth.

OHI-S outcomes included two subset of debris scores and calculus scores, each having proneness range of scores from zero to three.

Statistical analysis

Data were analyzed using SPSS version 22 (IBM Inc. Chicago, USA). Descriptive statistics, such as mean and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables were

determined. The data were found to be normally distributed. Tests performed were descriptive for scale data. T test was performed for intergroup comparison and Pearson correlation test was conducted for evaluating correlation between various parameters.

Results

This study was carried out on 40 patients with chronic periodontitis with age group range 40–60 years. The test group included 20 patients, 18 male and 2 female, were diagnosed with gastroesophageal reflux disease (GERD) and chronic periodontitis. The control group comprised of 20 systematically healthy people (17 male, 3 female) suffering from chronic periodontitis.

Intergroup comparison of various parameters like salivary flow rate, RSST, PMA, OHI S and DMFT(%). The mean salivary flow rates in test and control groups were 4.90 ± 0.66 and 6.45 ± 0.06 , respectively. The mean RSST values were 0.21 ± 0.02 and 0.23 ± 0.04 in test and control groups, respectively. In addition, PMA scores of the test and control groups were 4.07 ± 0.57 and 3.77 ± 0.73 , respectively. The OHI S scores of both the groups were 3.26 ± 0.55 and 2.19 ± 0.58 , respectively. The DMFT(%) of the test group and control group were 57.44 ± 11.73 and 49.22 ± 9.38 , respectively. Inferences drawn from the above results revealed a statistically significant difference between swallow function as evaluated by RSST and also in DMFT (%), where $P < 0.05$. Highly statistically significant differences with a $P < 0.001$ were seen between the two groups with respect to salivary flow rate, PMA, and OHI S [Table 1].

Pearson correlation between various clinical parameters. It can be inferred that salivary flow rate has r value (0.641) with OHI S Score, which indicates a statistically significant moderate negative correlation with OHI S. In other words, if the OHI S score increases, there is a decrease in salivary flow rate. It was also observed that DMFT has r value (0.598) with OHI S, which indicates a statistically significant moderate positive correlation with OHI S. In other words, if OHI S increases, there is a definite increase in DMFT [Table 2].

Comparison between probing depth and CAL. The mean probing depth and CAL in the test group were 6.52 ± 1.35 and 3.62 ± 0.74 , respectively. In comparison, the mean

Table 1: Intergroup comparison of various parameters like salivary flow rate, RSST, PMA Index, OHI-S Index and DMFT index [independent t-test]

Parameter	n	Test		Control		Mean Difference	P
		Mean	Std. Deviation	Mean	Std. Deviation		
Salivary flow rate	20	4.90	0.66	6.45	0.66	-1.5550	<0.001**
RSST	20	0.21	0.02	0.23	0.04	-.02300	0.026*
PMA Index	20	4.07	0.57	3.26	0.55	0.8100	<0.001**
OHI-S	20	3.77	0.73	2.19	0.58	1.5750	<0.001**
DMFT (%)	20	57.44	11.73	49.22	9.38	8.21500	0.019*

*Significant ($P < 0.05$), **Highly significant ($P < 0.001$)

Table 2: Correlation between various parameters like salivary flow rate, RSST swallow function test, PMA index, OHI-S index, DMFT index, PD, and CAL (Pearson Correlation)

Parameter	Statistics	Correlations						
		Salivary Flow Rate	RSST swallow function test	PMA Index	OHI-S	DMFT (%)	PD	CAL
Salivary flow rate	Pearson correlation	1	0.311	-0.269	-0.641**	-0.801**	-0.502**	-0.487**
	P		0.051	0.094	0.000	0.000	0.001	0.001
	n	40	40	40	40	40	40	40
RSST swallow function test	Pearson correlation	0.311	1	-0.399*	-0.530**	-0.203	-0.547**	-0.628**
	P	0.051		0.011	0.000	0.210	0.000	0.000
	n	40	40	40	40	40	40	40
PMA index	Pearson correlation	-0.269	-0.399*	1	0.629**	0.305	0.548**	0.486**
	P	0.094	0.011		0.000	0.056	0.000	0.001
	n	40	40	40	40	40	40	40
OHI-S	Pearson correlation	-0.641**	-0.530**	0.629**	1	0.598**	0.784**	0.789**
	P	0.000	0.000	0.000		0.000	0.000	0.000
	n	40	40	40	40	40	40	40
DMFT(%)	Pearson correlation	-0.801**	-0.203	0.305	0.598**	1	0.660**	0.541**
	P	0.000	0.210	0.056	0.000		0.000	0.000
	n	40	40	40	40	40	40	40
PD	Pearson correlation	-0.502**	-0.547**	0.548**	0.784**	0.660**	1	0.747**
	P	0.001	0.000	0.000	0.000	0.000		0.000
	n	40	40	40	40	40	40	40
CAL	Pearson correlation	-0.487**	-0.628**	0.486**	0.789**	0.541**	0.747**	1
	P	0.001	0.000	0.001	0.000	0.000	0.000	
	n	40	40	40	40	40	40	40

**Correlation is significant at the 0.01 level (2-tailed). *.Correlation is significant at the 0.05 level (2-tailed)

Table 3: Comparison of PD and CAL in the both Test group and Control group

Parameter	Group	n	Mean	Std. Deviation	Std. Error Mean	Mean Difference	P
PD	Test	20	6.525	1.3521	0.3023	2.575	<0.001**
	Control	20	3.950	0.7592	0.1698		
CAL	Test	20	3.625	0.7412	0.1657	1.75	<0.001**
	Control	20	1.875	0.5350	0.1196		

**-Highly Significant (P<0.001)

probing depth and CAL in control group were 3.95 ± 0.75 and 1.87 ± 0.83 , respectively. A statistically significant difference was observed in probing depth and CAL levels between both groups with higher levels in the test group [Table 3].

Discussion

This study attempts to establish reduced salivary flow volume in GERD patients as the cause for oral dryness. All patients with GERD covered in this study showed symptoms of typical reflux. This draws verifiable correlations between reflux of acid as a symptom of typical GERD and dryness in the oral cavity and atypically symptomatic dental erosions. Reduced salivary flow can induce gingivitis in GERD patients, and thus, it acts as an aggravating factor. In GERD patients, swallowing function was observed to have been substantially reduced.^[11]

Gastric contents like acid, bile-salt, pepsin, as well as trypsin with a pH value of 1–1.5 may impact the

esophagus and oral cavity in GERD patients. This can lead to numerous typical dental erosions including lesions, reflecting extraesophageal problems to soft tissues in the oral cavity with failure in suitably adapting themselves affected by the oral buffer mechanism.^[13]

Similar to periodontal disease, dental caries is one of the major oral disorders. Therefore, the risks of dental caries were also evaluated. The DMF indices were significantly higher in GERD patients than in systemically healthy controls. Certain oral bacteria, especially major pathogens of periodontal diseases, may play a more direct role through local inflammatory responses and carcinogenic transformations.

In major pathogens of periodontics, certain types of bacteria can directly influence the localized inflammatory sensitiveness and reactions and may result in carcinogenic occurrences. An example typical in periodontitis is *A. actinomycetemcomitans*, a Gram

negative bacteria growing in oral cavities of almost more than one-third of the total population, besides *A. actinomycetemcomitans* when separated from many similar lesions in different parts of human body, regarded as a systemic pathogen.^[19] Increased production of carcinogenic nitrosamines is another plausible mechanism which may explain the observed relationships between periodontal diseases and gastric cancer. This relationship could be promoted by tobacco use and certain dietary factors, wherein endogenous nitrosamines were formed by nitrate-reducing bacteria.^[20] In spite of being cleared from gastric mucosa, triple or quadruple therapies fail to defend *H. pylori* infection on the dental plaque. This raises the possibilities that dental plaque could potentially be a source of reinfections in gastric-mucosa.^[21,22]

Oral cavity functions like a vault of Pylori and often as a pathway of transmission. According to Gebara *et al.*, periodontics manifest high incidences (43%) of *H. pylori* in the dental plaque of patients.^[23] Similarly, Umeda *et al.* established that 41.2% of people diagnosed with periodontics had *H. pylori* in their stomach and duodenum, in a depth more than 4 mm.^[24] Dye *et al.* conducted a survey of 4,504 periodontitis patients with a pocket depth of 5 mm or even more to conclude that those pockets were impacted with odds of *H. pylori* seropositivity.^[25] Annand *et al.*, Chitsazi *et al.* and Kamat *et al.* also reported that periodontal diseases and *H. pylori* infection were not much related.^[26] Interestingly, frequently occurring chronic gastritis or GERD may aggravate to atrophic gastritis, intestinal metaplasia, dysplasia, and sometimes into a gastric adenocarcinoma.^[27,28]

Shakerei and team studied 309 GERD patients and observed a strong relationship between tooth loss and low DMFT score, leading to the risk of gastric cancer. Moreover, a visible linkage was observed only in respect to gastric cardia cancer, for those who used to brush teeth less than daily brushing. Removal of *H. pylori* from plaques and oral cavity should play a clear and significant role in the comprehensive management of *H. pylori*-associated GI problems.^[29] Eradication of *H. pylori* from dental plaques and oral cavity should be an important part of the comprehensive management of *H. pylori*-associated GI diseases.^[30,31]

Thus, spreading of awareness about oral health and hygiene should be a part of community health programs so as to reduce possible outbreak of systemic health concerns.^[32]

The limitations of the study include its small sample size that limited the power to assess associations between GERD and chronic periodontitis. The cross-sectional study precludes any definitive statement regarding a causal association. The possibility of potential selection bias cannot be overruled completely, even though the participants were unaware of their health status at the time of recruitment of oral health examinations.

Conclusion

Though chronic periodontitis is associated with deterioration of health status and subsequently increased cost for medical care among the elderly, the association between GERD and chronic periodontitis has less often been reported. The observation made in the present study that GERD is an independent risk factor for chronic periodontitis gives us a heads-up on the need to inform gastroenterologists about considering the incidence of chronic periodontitis while providing care for subjects with GERD. Furthermore, subjects with unexplained chronic periodontitis must be evaluated for the presence of GERD. It is suggested that large epidemiological studies should be conducted to confirm the association between GERD and chronic periodontitis.

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Nil.

Conflicts of interest

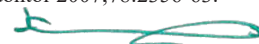
There are no conflicts of interest.

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Assessment of knowledge, attitude, and practice related to ergonomics among the students of three different dental schools in India: An original research

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Abstract:

BACKGROUND AND OBJECTIVE: Dental students and practitioners are frequently prone to work-related musculoskeletal disorders (WMSDs) due to the unawareness of ergonomic principles when practicing dentistry. The aim of this study was to assess the dental student knowledge, attitude, and practice toward ergonomics in three different dental schools.

METHODOLOGY: A cross-sectional study was conducted among 1166 dental students from three different dental schools of Andhra Pradesh, India, to assess knowledge, attitude, and practice regarding ergonomics. All the participants were provided with prestructured questionnaire form comprising 13 questions to know their knowledge, attitude, and practice related to ergonomics before and after providing guidelines on ergonomic principles.

RESULTS: The majority of the dental students appreciate that the most affected regions due to work-related disorders were back (475 before and 559 after instructions), neck (354 and 420), hand and wrist (205 and 117), and shoulder and elbow (132 and 70), respectively. Knowledge and practice scores of dental students (mean and standard deviation) regarding WMSDs before and after applying ergonomic principles were 3.92 ± 2.44 , 1.29 ± 1.67 and 5.81 ± 0.87 , 3.03 ± 0.60 , respectively.

CONCLUSION: The present study provides an insight into ergonomics for dental students during routine dental procedures. The knowledge, attitude, and practice related to ergonomics were satisfactorily increased among the participants. Thus, ergonomic education of the dental health-care personnel must be focused in all the educational institutions and at continuing dental health programs by delivering ergonomic principles both theoretically and practically and should be a part of the curriculum.

Keywords:

Attitude, dental students, ergonomics, knowledge, musculoskeletal disorders

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Introduction

Ergonomics is the scientific study of people and their working environment. Ergonomics was derived from a Greek word "Ergo" means work and "Nomos" means natural laws or systems. Ergonomics consequently is the science concerned with designing products and procedures for

maximum efficiency and safety (American Dental Association 2011).^[1]

Ergonomics in dentistry is defined as reduction in cognitive and physical stress, preventing occupational diseases, thereby improving efficiency, with better quality and greater comfort for both the practitioners and patients.^[1,2]

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Musculoskeletal disorder is the term that refers to the conditions that involve the nerves, tendons, muscles, and supporting structures of the body. When a specific job plays the main causative factor, then the term becomes work-related musculoskeletal disorders (WMSDs).^[2,3]

Dental professionals should have unique skills to perform different dental operative procedures. During these procedures, movements are repetitive and are restricted to the mouth. Working posture, long working hours, and the use of different types of instruments also play a factor to be considered among dentists while working. All the above factors consequently result in WMSDs among dentists.^[4,5]

Most of the clinicians and students often complain of uneasiness, discomfort, and lack of strength to work for long hours. Back pain, hand and wrist pain, and neck pain are the common problems associated with dental clinicians. The symptoms are definitely due to the lack of awareness in applying ergonomic principles while working.^[6,7]

In order to avoid these problems, one needs to gain awareness regarding ergonomic principles at institutional levels and also at continuous dental health programs. The maximum efficacy and safety will be achieved by providing education on ergonomics which then show a positive impact on long-term run of the profession.^[7,8]

Thus, the study aimed to assess the dental student knowledge, attitude, and practice toward ergonomics in three different dental schools, before and after providing instructions related to ergonomic principles.

Methodology

This comparative study was conducted between January 2017 and March 2018 in three dental institutions of Andhra Pradesh, India. Permissions were obtained from all the three institutional review boards before the start of the study.

All the 3rd and 4th year BDS students, interns, and 1st, 2nd and 3rd year postgraduates present during the course time of the study in the respective institutions, taken admission into a BDS and MDS course in the academic year 2013–2014 and completed at least 12 months of clinical exposure during the start of the study were included in the study. Students who were not willing to provide consent and preclinical students were excluded. A total of 1166 dental students were selected using convenience sampling, of which 835 were female and 331 were male, aged between 19 and 30 years.

The proforma was adopted from previously published studies^[5-8] and modified by taking care that it covered the basic principles in dental ergonomics. The proforma comprised of two sections. The first section collected to demographic details of the participants and the second part comprised 13 questions, of which seven were related to knowledge, three related to attitude, and three relevant to practice toward ergonomics. A pilot study was conducted among 90 students (30 from each dental school) to assess the feasibility and validity of the questionnaire. The observed Cronbach's alpha was 0.75, which was acceptable.

The questionnaire was distributed among all the participants and data were collected. After collection of the baseline data, all the participants were provided with guidelines on ergonomics and the respective faculty in three dental schools had taken necessary steps to provide knowledge on how to apply ergonomic principles while treating dental patients.

A washout period of 14 days was given following the intervention and data was collected using the same proforma to assess and compare their knowledge, attitude and practice toward ergonomics in dental institutions [Chart 1]. The data collected were entered in MicroSoft Excel spreadsheet and analyzed using the IBM SPSS Statistics for Windows, version XX (IBM Corp., Armonk, N.Y., USA) program and compared with Chi-square test ($P < 0.05$). The results were processed by age, gender, work experience, and their knowledge, attitude, and practice related to ergonomics.

Results

The results of the study demonstrate that knowledge and practice scores of dental students (mean and standard deviation) regarding WMSDs before applying ergonomic principles were 3.92 ± 2.44 and 1.29 ± 1.67 , respectively. Whereas, the knowledge (5.81 ± 0.87) and practice scores (3.03 ± 0.60) increased after the instructions [Table 1].

Table 1: Comparing the knowledge and practice scores regarding work-related musculoskeletal disorders before and after the instructions

Time	n	Mean	SD	P
Knowledge				
Before	1166	3.92	2.44	0.000
After	1166	5.81	0.87	
Practice				
Before	1166	1.29	1.67	0.000
After	1166	3.03	0.60	

SD=Standard deviation

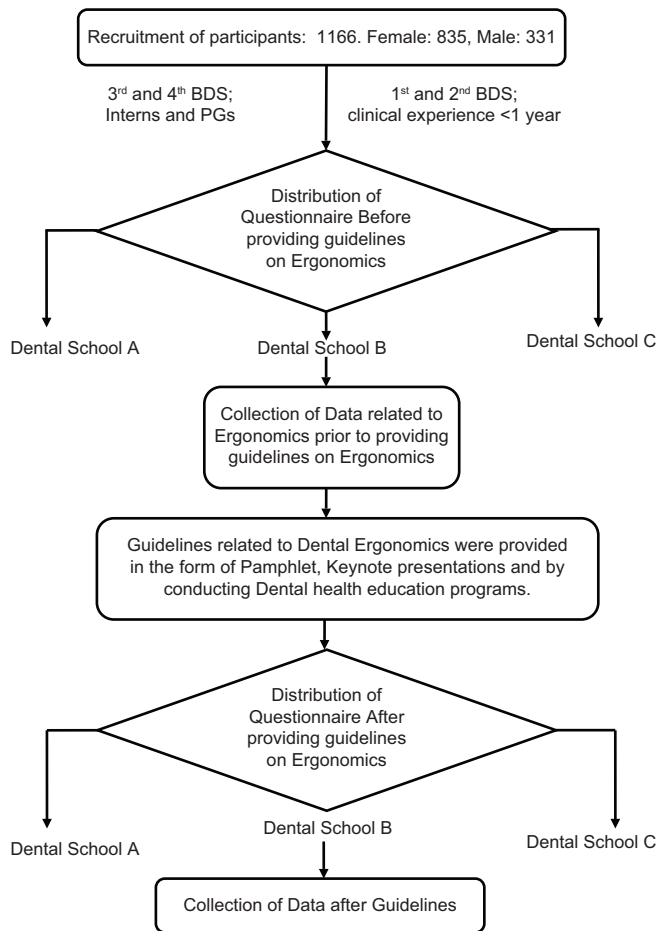


Chart 1: Flowchart sequence of methodology followed in three dental schools

The majority of the dental students appreciate that the most affected regions due to work-related disorders were back (475 before and 559 after instructions), neck (354 and 420), hand and wrist (205 and 117), and shoulder and elbow (132 and 70), respectively. Most of the dental students accepted that WMSDs are the common reason for early retirement to practice [Table 2].

There was a positive correlation (+0.737) between knowledge and practice scores among the study participants, which were found to be statistically significant. For every 1 unit increase in knowledge score, there is a 0.737 unit increase in practice score and vice versa [Table 3].

Intragroup comparison showed that there was a significant difference in both knowledge and practice scores before and after instructions across all the year dental students. Inter-group comparison showed that PGs had high knowledge and practice scores compared to interns, final years, and 3rd years. However, there was an increase in knowledge and practice scores among 3rd years, final years, and interns following the instructions [Table 4].

Table 2: Comparing the attitude regarding work-related musculoskeletal disorders before and after instructions

	Time		Total	P
	Before	After		
Most affected regions due to work-related disorders				
Back	475	559	1034	0.000
Neck	354	420	774	
Hand and wrist	205	117	322	
Shoulder and elbow	132	70	202	
Accept work-related disorders are the common reason for early retirement				
Yes	858	1100	1958	0.000
No	308	66	374	

Table 3: Correlation between knowledge and practice scores among the study participants

Parameters	Knowledge	Practice
Knowledge		
Pearson correlation	1	0.737**
P-value		0.000
n	2332	2332

**Highly significant (P<0.01)

After the instructions at institutional level, 92.7% of the study participants responded that ergonomics should be a part of curriculum.

Discussion

Adopting healthy life-style is an important aspect, and it comes only through education. The dental profession is the one where you see many work-related disorders affecting many of the clinicians. These disorders affect different regions of the body, such as lower back, neck, hand, fingers, wrist, arms, elbows, and shoulders, and this results in the leading cause for their absence from professional work. Disorders affecting muscles, tendons, ligaments, and bone are mainly due to the higher exertion of mechanical force during dental operating procedures.^[9,10]

In dental profession, these problems are due to the repetitive movements within the small place and also long-standing exertion of forces during dental procedures will result in work-related problems. The number of exposures and the total time of exposures are important factors in WMSDs Short-term and long-term loadings due to the dental surgical procedures will result in acute and chronic disorders, respectively, thus affecting the overall occupational life. To prevent the unnecessary fatigue or to diminish the exertion forces used during dental work, it is wise to know about dental ergonomic principles.^[11,12]

All the three dental schools had given the instructions to the students after obtaining the attitude, knowledge, and practice related to ergonomics. Guidelines or principles

Table 4: Intra and inter-year comparison of knowledge and practice scores among the study participants

Years	Knowledge	Knowledge after instructions	Practice before instructions	Practice after instructions	P
Third					
n	344 ^{a,A}	344 ^a	344 ^{b,B}	344 ^{b,B}	0.000
Mean	3.4128	5.8634	0.7064	2.9651	
SD	2.28915	0.72558	1.35025	0.46317	
Final					
n	311 ^{a,A}	311 ^a	311 ^{b,B}	311 ^{b,B}	0.000
Mean	3.0096	5.8296	1.0997	3.0096	
SD	2.72087	0.83852	1.70191	0.59831	
Interns					
n	280 ^{a,A}	280 ^a	280 ^{b,B}	280 ^{b,B}	0.000
Mean	4.7214	5.8250	1.4464	3.0821	
SD	2.00918	0.79068	1.74462	0.63107	
PGs					
n	231 ^{a,A}	231 ^a	231 ^{b,B}	231 ^{b,B}	0.000
Mean	4.9610	5.6883	2.2294	3.1342	
SD	2.02662	1.18240	1.57541	0.73081	
P	0.000	0.131	0.000	0.0000	

Knowledge=^{a,A}Refers intergroup and intragroup significance, ^AIntragroup significance, Practice=^{b,B}Refers intergroup and intragroup significance. SD=Standard deviation

of ergonomics were mainly based on the following factors: operator chair position instructions, patient chair position guidelines, selection of working instruments with modified design, training of the dental personnel, and using magnification systems.^[5,13]

In this study, the knowledge and practice scores of dental students were increased after applying ergonomic-related instructions than before in all the three different colleges of all the year students.

In this study, there was a difference in the opinion among the participants before and after the instructions. Furthermore, 244 participants have changed their opinion regarding WMSDs after applying ergonomic principles. Inter-group comparison showed that PGs had high knowledge and practice scores compared to interns, final years, and 3rd years. Intra-group comparison showed that there was a significant difference in both knowledge and practice scores before and after instructions across all the years. However, there was an increase in knowledge and practice scores among 3rd years, final years, and interns following the instructions.

The main objective of ergonomics in dental profession is to prevent the symptoms of work-related musculoskeletal disorders. Education related to ergonomic principles at institutional level and in continuing dental programs plays a vital role in improving the quality of dental professional life.^[14,15]

This study will help the dental students and professionals to recognize the risks for musculoskeletal disorders at the earliest and modify their work design and environment.

Limitations of the study

Although we have assessed the knowledge, attitude and perception, we did not assess the implementation of the gained knowledge of the students on clinical practice.

Advantages by knowing ergonomic principles and future perspectives.

Although the ergonomic principles will help in reducing the risk of developing WMSDs, still many of the dental professionals facing the problems related to ergonomics. In order to overcome these problems, the following are the futuristic changes that need to explore in detail to know the advantages in dental ergonomics.

1. Use of zero concept or proprioceptive derivation concept: Much research work is yet to come on this concept where the dental chair is modified and flat-bed or PD support is used
2. Selection of ergonomically friendly equipment: Dental professionals should get to know which instruments the best suit for their physical capabilities in order to reduce fatigue during the usage of instruments
3. Continuing dental education programs: There is a lack of dental education regarding ergonomics and WMSDs before the clinical exposure of dental students in almost all the institutions.

Conclusion

WMSDs can be prevented by modifying the work environment, including the design of working tools and equipment, and by organizing training programs in educational institutions and by conducting dental health programs. The successful application of principles of dental ergonomics will not only increase the productivity

but also decrease the unwanted injuries or illness among dental profession.

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Conflicts of interest

There are no conflicts of interest.

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Scanning Electron Microscopic Evaluation of Erosive Potential of Pediatric Liquid Medicaments on Primary Teeth

Abstract

Introduction: Pediatric liquid medicaments (PLMs) are the most accepted form of medication prescribed for children to treat different infirmities, however, their harmful effects on a child's dental health are unaware for most of us. The present *in vitro* study was aimed to evaluate the erosive potential of two commonly used PLM'S on primary teeth and the remineralizing potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste on these erosive lesions. **Materials and Methods:** Forty deciduous teeth were randomly assigned to five groups (n=8) in which group I samples were treated with Meftagesic-P, group II with Kofarest-PD, group III with Meftagesic P+CPP-ACP paste, group IV with Kofarest-PD+CPP-ACP paste, and group V samples were immersed in artificial saliva (control). Photomicrographs were taken at 2000× and 5000× magnification using a scanning electron microscope (SEM). **Results:** SEM photomicrographs elicited erosive changes in the enamel surface of all the samples in both groups I and II, however, the erosive changes in group II samples were more pronounced. The enamel surface of group III and IV samples that were treated with CPP-ACP after exposure to medicaments showed remineralization of eroded areas. **Conclusion:** Both the PLMs used in the study showed an erosive effect on the primary enamel surface. The severity of erosion increased with the exposure time. However, the application of CPP-ACP paste following the exposure to PLMs showed noticeable remineralization.

Keywords: Casein phosphopeptide amorphous calcium phosphate, dental erosion, pediatric liquid medicaments, remineralization, scanning electron microscope

Introduction-

Diseases can be devastating for anyone, but it seems particularly unfair because of their belligerent nature when they affect children. Poor dietary, hygiene practices and feebly developed immunological defenses increase the predisposition of children to diseases. To tackle such conditions, medicines in liquid form are widely used to facilitate easy administration. Apart from having main active ingredients medications are usually colored, flavored, and sweetened with various excipients such as acids and sugars to improve their palatability and shelf life. Long-term or frequent usage of these medications with low pH values will have a high erosive and cariogenic effect on primary teeth.^[1] Over the past decade, there is a decline in the caries prevalence

worldwide, however, it has been accompanied by a remarkable increase in the incidence of noncarious lesions, such as dental erosion, that lead to an irreversible loss of tooth structure.

It is currently recognized as a challenging clinical condition affecting the younger age group, as deciduous teeth are more susceptible to erosion compared to their permanent successors because of the softer, thinner, and less mineralized enamel.^[2] In the event of illness consumption of medicines at bedtime, reduced salivary flow, content, and prolonged salivary clearance time have also been linked to its increased prevalence. Erosion in children may lead to various clinical problems such as dental hypersensitivity, altered occlusion, eating difficulties, poor aesthetics, pulp exposure, and abscesses. Therefore, it is

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important to prevent and control the progression of these erosive lesions that can be achieved by removing the cause and the factors that enhance it or making the tooth more resistant to acid attack.^[5]

Over the years with clinically proven research, fluoride has been documented to promote remineralization, however, the distribution of fluoride is not the same throughout the world, there are some areas with endemic fluoride which may increase the risk of toxicity.^[4] To overcome these drawbacks newer remineralizing agents were developed that are derived from a major protein found in milk called casein. The casein phosphopeptide (CPP) binds to amorphous calcium phosphate (ACP) in metastable solution preventing the dissolution of calcium and phosphate ions. The ACP-CPP also acts as a reservoir of bio-available calcium and phosphate, and maintains the solution supersaturated, thus facilitating remineralization.^[5]

Many studies have emphasized the erosive potential of Pediatric liquid medicaments (PLMs) on primary teeth but the surface changes after application of remineralizing agents have not yet been evaluated to date. Thus, considering these facts, the present *in vitro* study was aimed to analyze the surface changes of the enamel caused by the consumption of PLM's and subsequent remineralization of erosive lesions following the application of CPP-ACP paste under SEM.

Materials and Methods

Ethical approval for this study (protocol no: 30/IEC/SIBAR/2015) was provided by the Ethical Committee of SIBAR Institute of dental sciences, Guntur. On 8 November 2015. The sample included sound human caries-free primary teeth (either extracted for orthodontic reasons, over-retained, or exfoliated teeth). Carious, hypoplastic, discolored teeth, and teeth with cracked areas and white spots were excluded from the study. The selected teeth were washed, cleaned of debris, and were stored in thymol solution until further use. The sample size was determined using G* power 3.1.9.2. The power of the study was 80%, the effect size was 1.518, Type I error was 0.05, and the sample size derived was 40. The teeth were randomly divided into five groups with eight teeth in each using the computer randomization method. Group I samples were treated with Meftagesic-P (Medication I); Group II with Kofarest-PD (Medication II); Group III: Medication I+CPP-ACP paste (GC Tooth Mousse, GC India Dental Private Limited, Europe); Group IV: Medication II+CPP-ACP paste, and Group V samples are kept in artificial saliva [Table 1]. The viscosity of PLMs and the artificial saliva was measured in centipoises (cP) using a calibrated digital rotational viscometer (ViscoStar Plus, Funjilab, Spain).

Viscosity of Medication I, II, and artificial saliva were 401.54 ± 0.60 cP, 508.41 ± 7.10 cP, and 15.5 ± 2.8 cP, respectively. The total sugar content of Medication I was

73% wt/wt and Medication II was 77% wt/wt, which was determined by the volumetric method.

The pH of the medicaments used in the study was determined using a pH meter that was calibrated with standard solutions between each measurement for accuracy. The values obtained for medication I, II, and artificial saliva were 6.31, 5.66, and 7.2, respectively.

Immersion cycling protocol proposed by Lussi and Amechi *et al.*^[2] was adopted to simulate the usual number of intakes by the patients. Group I and II samples were immersed in 5 mL undiluted syrups in separate air-tight containers and agitated using a magnetic stirrer bath (300 rpm) for a period of 2 minutes thrice daily for a test period of 28 days. Later the samples were washed using distilled water and placed in 10 mL of artificial saliva at 37°C until the next immersion cycle. Fresh medicament was used for each immersion.

After treating the groups III and IV samples with their respective medicaments, they were washed with distilled water and stored in artificial saliva for 30 minutes. Later CPP-ACP paste was applied on the test site of the samples for 3 minutes using gloved finger as per the manufacturer's instructions. Further these samples were washed with distilled water and stored in artificial saliva. This procedure is repeated thrice daily for the whole test period.

Group V (Control) samples were preserved in artificial saliva for the whole test period and the solution was changed daily. The specimens were analyzed for surface changes under SEM at the end of 7, 14, 21, and 28 days. The mesio-buccal surface of the test site of each tooth was scanned and photomicrographs were taken at 2000× and 5000× magnifications, respectively.

Photomicrographs in all the groups were examined for the surface erosion and remineralizing changes in the enamel. The entire procedure was performed by a single operator. However, to avoid bias, a second operator who was unaware of the prior results evaluated the samples randomly. An interexaminer reliability statistic of 0.93 was achieved indicating excellent agreement (intraclass correlation coefficient = 0.93), hence the prior observations were only considered.

Results

Qualitative analysis of the SEM photomicrographs [Figure 1] showed erosive changes in the enamel surface of all the samples in both groups I and II. The intensity of surface changes increased with the exposure time. The erosive changes produced in group II samples were more pronounced than those of group I samples.

After 28 days, the SEM images of group II samples clearly displayed structural loss with hardly identifiable enamel

Table 1: Table showing the composition, pH, viscosity, and uses of medicaments and CPP-ACP

Serial number	Product	Type of use	Composition	pH	Viscosity
1	Meftagesic-P	Pyrexia, headache, cold, ear pain, cough, asthma.	Mefenamic Acid (50mg/5ml), Paracetamol (125mg/5ml), Other additives	6.31	401.54 ± 0.60 cP
2	Kofarest-PD	Cold, cough with mucus, asthma.	Ambroxol hydrochloride 15 mg, guaiphenesin 50 mg +terbutaline sulphate 1.25 mg	5.66	508.41 ± 7.10 cP
3	CPP-ACP	Remineralization of incipient carious lesions and white spot lesions.	Pure water, glycerol, CPP-ACP, d-sorbitol, xylitol, CMC-Na, propylene glycol, H ₂ O, SiO ₂ , TiO ₂ , ZnO ₂ , H ₃ PO ₄ , MgO ₂ , guar gum, sodium saccharin, ethyl <i>p</i> -hydroxybenzoate, butyl <i>p</i> -hydroxybenzoate, and propyl <i>p</i> -hydroxybenzoate	5.0 to 9.0	–

CPP-ACP, casein phosphopeptide amorphous calcium phosphate.

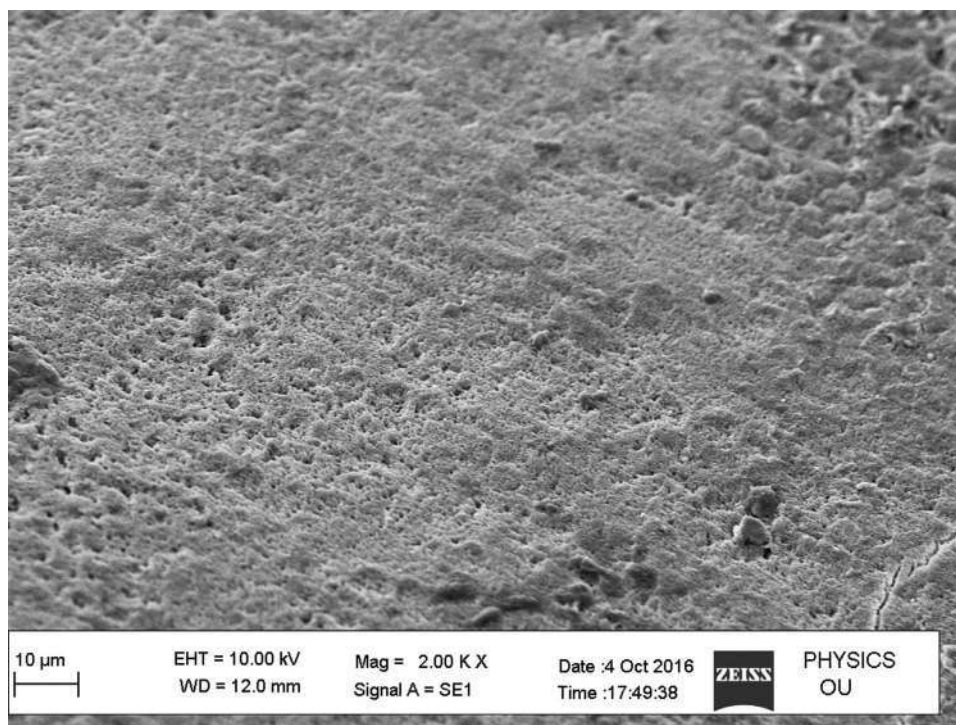


Figure 1: Evident irregularities on the enamel surface with prominent enamel rods with focal areas of distorted enamel rods after 28 days (group II).

prisms [Figure 1]. Group I specimens exhibited a noticeable structural loss. The surface was irregular with small enamel depressions, with areas of enamel prisms and interprismatic substance [Figure 2]. No microstructure alterations were observed in group V specimens [Figure 3]. The photomicrographs of groups III and IV where the teeth samples were treated with CPP-ACP paste after exposing to medicaments revealed deposition of hyperdense amorphous substance in the prismatic areas of enamel surface indicating the significant amount of remineralization [Figures 4 and 5] when compared to samples in groups I and II, and also the amount of remineralization increased with timeperiod.

When endogenous pH, viscosity, and total sugar contents of both the medicaments were compared. Medication II showed low pH (5.66), viscosity (508.41±7.10), and increased total sugar content (77%) than medication I (6.31; 401.54 ± 0.60; 73%).

Discussion

Medications are becoming a part of the daily routine for children because of their increased susceptibility to diseases compared to adults.^[6] But children differ from adults in many aspects of drug pharmacokinetics, pharmacodynamics, potential routes of administration, medicine-related

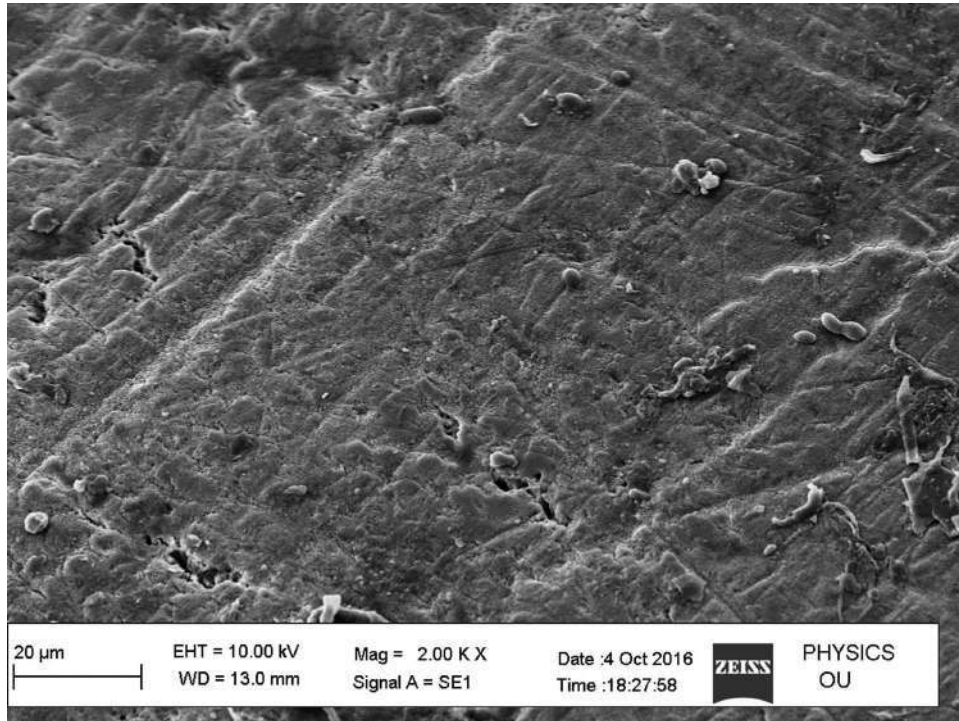


Figure 2: Irregular surface with areas of enamel prisms and inter prismatic substance were noticed after 28 days (group I).

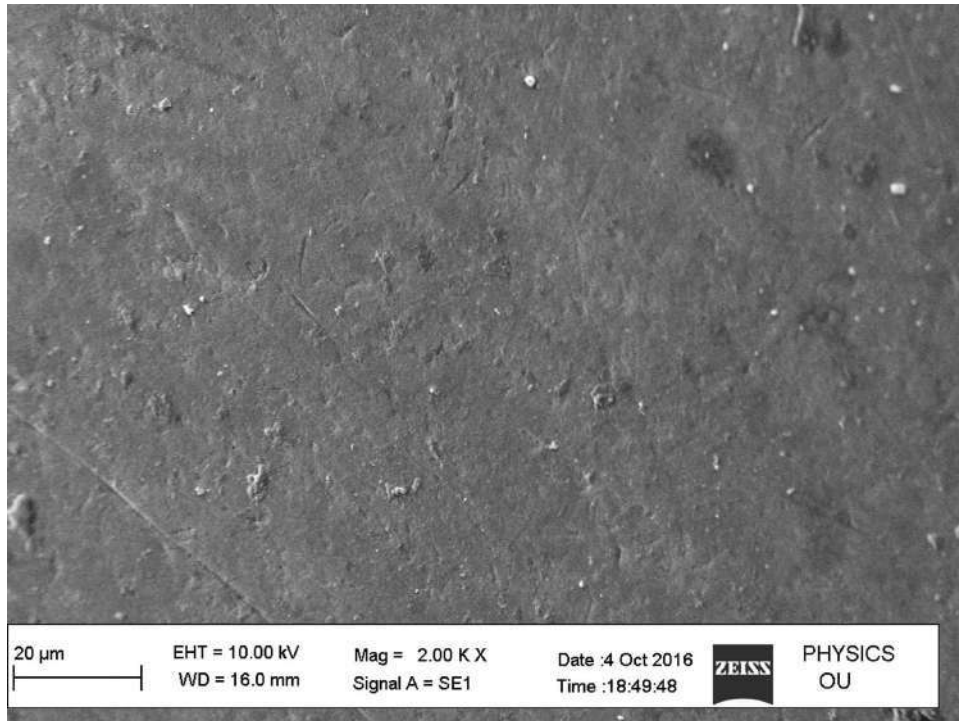


Figure 3: Smooth enamel surface with small elevations were observed after 28 days (group V).

toxicity, and taste preferences. Among the routes of drug administration, the oral route is the most preferred one in children. As solid forms of the drugs are associated with the risk of choking, PLMs were preferred in younger children because of their ease and simple mode of administration.

In order to improve the patient's acceptance and palatability, various ingredients are added to the PLMs along with their main active ingredients. The frequent usage of PLMs that are acidified with acids such as citric and malic acids and sweetened with sucrose, fructose, or a combination of both

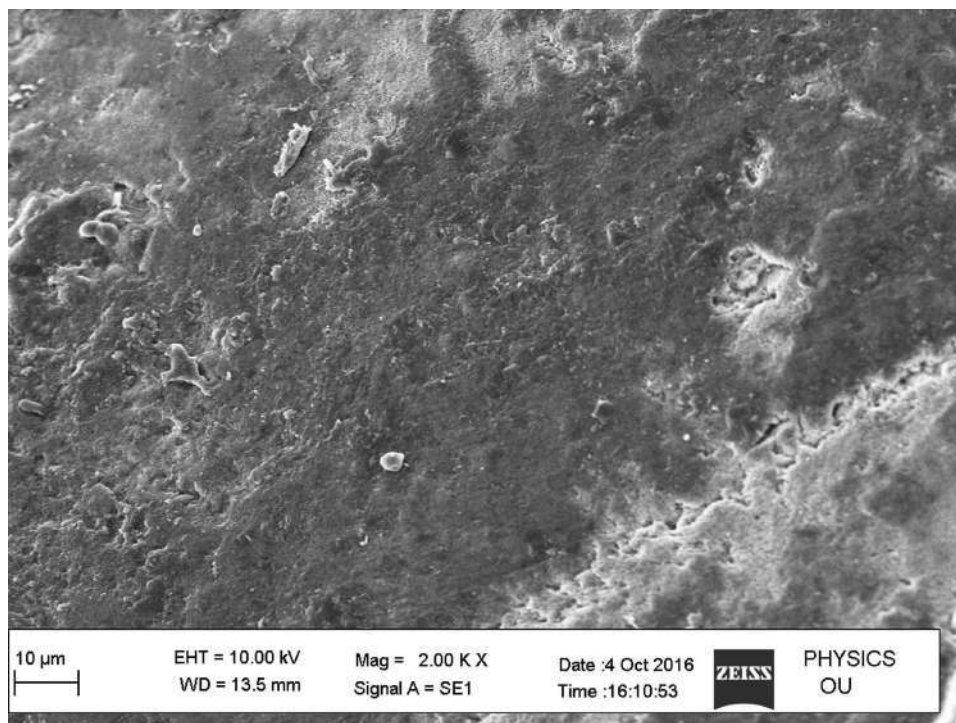


Figure 4: After 28 days enamel surface showed hyperdense areas with round- to ovoid-shaped enamel rods (group III).

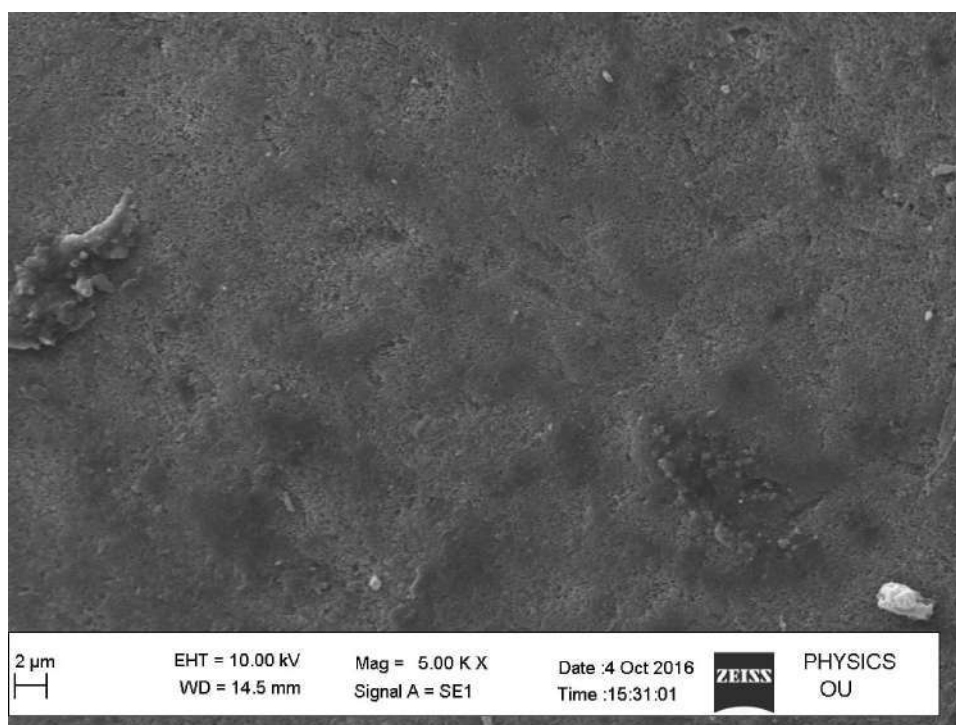


Figure 5: Surface illustrates smooth hyperdense areas covering the enamel rods after 28 days (group IV).

has been linked to causing dental erosion, caries, and a drop in the plaque pH.^[7,8]

The deleterious effect of PLM's was first studied by Imfeld way back in 1953. But Maguire *et al.*^[9] in their study reported that over half of the PLMs surveyed had pH values significantly below the critical pH and have high erosive

potential. Studies done by Greenwood *et al.*^[10] and Mackie and Hobson^[11] have confirmed that these preparations were cariogenic and acidogenic in nature.

Development of erosion is a complex process and occurs either by hydrogen ion attack or by the action of chelating anions that combine with carbonate, phosphate, or both, and

detach the mineral ions from the surface. The result of exposure to erosive agents decreases enamel microhardness, which upon exposure to mechanical forces, makes the surface more susceptible to disruption.^[12]

The biological factors that play a crucial role during an erosive challenge involve mainly the salivary protective mechanisms such as dilution and clearance of an erosive agent from the mouth, neutralization and buffering of acids, and slowing down the rate of enamel dissolution.^[13] Numerous studies that evaluated the relationship between various salivary parameters and the risk of dental erosion have supported the protective value of saliva, with the strongest association of decreased salivary flow rate and low buffering capacity.^[14]

Dental erosion is more prevalent in children due to reduced salivary flow and content that prolong the salivary clearance rate, thus making the nocturnal exposure to erosive agents more destructive. Moreover, studies have reported that 75.7% of parents would use a fever reducer or cough suppressants to relieve their discomfort while the child is partially asleep, which further increases its deleterious effect.^[15]

An interesting correlation between erosion and caries arises from the postulation that the acidic oral environment is likely to encourage the growth of acidophilic *Streptococcus mutans*, thus increasing the individual's susceptibility to caries. Supporting studies by Kazoullis *et al.*^[16] showed elevated counts of *S. mutans* and early childhood caries in primary dentition in children with severe erosion. Apart from encouraging the growth of cariogenic bacteria these PLMs contribute to caries indirectly by reducing the salivary secretion and also cause deleterious effects on the restorations.^[17]

As it may not be practical to eliminate the cause of tooth wear, it is desirable to develop effective preventive strategies like the usage of topical fluoride, which is considered as a gold standard agent for remineralization. However, to overcome certain drawbacks^[18] of fluorides, Longbottom *et al.*^[19] have proposed an ideal remineralizing agent that releases calcium and phosphate ions (CPP-ACP) into the oral environment. This CPP-ACP (GC Tooth Mousse) paste, unlike remineralizing the carious lesions, repairs the eroded tooth structure by depositing the mineral ions into the porous zone rather than crystal regrowth.^[20]

Hence, the present *in vitro* study was conducted to analyze the surface changes of the enamel caused by frequent consumption of PLMs and subsequent remineralization of surface erosive lesions following the application of CPP-ACP paste under SEM.

The commonly prescribed PLMs for the management of conditions such as pyrexia, headache, cold, ear pain, cough, asthma, and acute sore throat by the pediatricians were selected (Meftagesic-P; Kofarest-PD). Both the selected medicaments displayed the ingredients, but none contained any information regarding the pH. Therefore, the pH values

were measured using pH electrode meter (Meftagesic-P 6.31; Kofarest-PD 5.66), which are acidic. The findings of the present study were in accordance with the study conducted by Neves *et al.*^[21] in which all the 23 tested pediatric medications showed an acidic pH.

PLMs are usually viscous syrups that penetrate in fissures and proximal areas of teeth that are inaccessible to the toothbrush. Regular and long-term use of these medications with prolonged oral clearance may increase the risk of dental erosion and caries as they contain sugars and acids.^[22] Viscosity values of PLMs used in this study were 401.54 ± 0.60 cP (Meftagesic-P) and 508.41 ± 7.10 cP (Kofarest-PD), respectively.

Sugars added to these PLMs are metabolized by bacteria to acidic end-products and lower the plaque pH that causes ionic dissolution of the hydroxyapatite crystals, leading to enamel and dentin demineralization. The total sugar content of medicaments I and II used in this study is 73% and 77%, wt/wt, respectively.

Enamel erosion can be measured by various methods such as loss of enamel weight, SEM, light microscope, microradiograph, image analysis, electron probe analysis, and profilometry. However, in the present study, SEM was used as it is a simple, rapid, and cost-effective method to observe the topographical changes of the enamel.^[23]

The results of the present study revealed that both groups I and II showed erosive changes on the enamel surface, whereas the control group (group V) showed no significant surface variations and areas of remineralization on the eroded enamel surface were noticed in group III and IV samples. The surface changes of the enamel in group II samples were more pronounced than group I, which could be due to high viscosity, low pH, and the chelating properties of ambroxol hydrochloride and salbutamol present in Kofarest-PD. Similar results were noticed in a study by Kulkarni *et al.*^[24] in which antitussive drug (Ambrolite-D) showed the highest erosive potential compared to other drugs.

The surface changes were noted from 7th to 28th day in both the groups. In group I samples, the enamel surface illustrated minimum irregularities on the seventh day, whereas irregular enamel surface with areas of enamel prism and areas of inter prismatic substance was observed at 28 days interval. In the case of group II samples on the seventh day, prominent irregular structures with few enamel rods were noticed but at the end of 28 days, irregular surfaces with prominent enamel rods and distorted enamel sheaths were evident. The experimental period of 28 days was chosen to simulate the changes that would happen with long-term usage of these PLMs. Similar results were noticed in studies conducted by Tupalli^[25] and Mittal *et al.*^[26]

The photomicrographs of the samples in groups III and IV revealed the deposition of granular structures on the enamel surface that is a sign of remineralization and it was more

pronounced in group IV. Similar findings were observed in studies by Ranjitkar^[27] and Gaber *et al.*^[28]

The results of this *in vitro* study may not be completely applicable for clinical situations because of over/underestimation of the values, however, they give a brief overview of the possible consequences. As the presence of pellicle in *in vivo* conditions will protect the teeth from acidic challenges, even it has been suggested that the amount and quality of saliva, in particular its buffering capacity, were important in the occurrence of dental erosion.^[28] From the standpoint of both hard tissue quality and salivary conditions, deciduous teeth seem to be at greater risk from erosive challenges than permanent teeth.

Conclusion

Thus, based on the results obtained from the present study, it can be concluded that both the PLMs were erosive on the primary enamel surface. The antitussive drug used in group II showed more surface changes. The severity of the erosive changes increased with exposure time. Application of remineralizing agents such as CPP-ACP paste following the exposure to PLMs had considerably reduced their erosive effect.

Thus, it is important to educate the professionals and parents about the possible ill effects of long-term usage of PLMs and the importance of using remineralizing agents.

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Conflicts of interest

There are no conflicts of interest.

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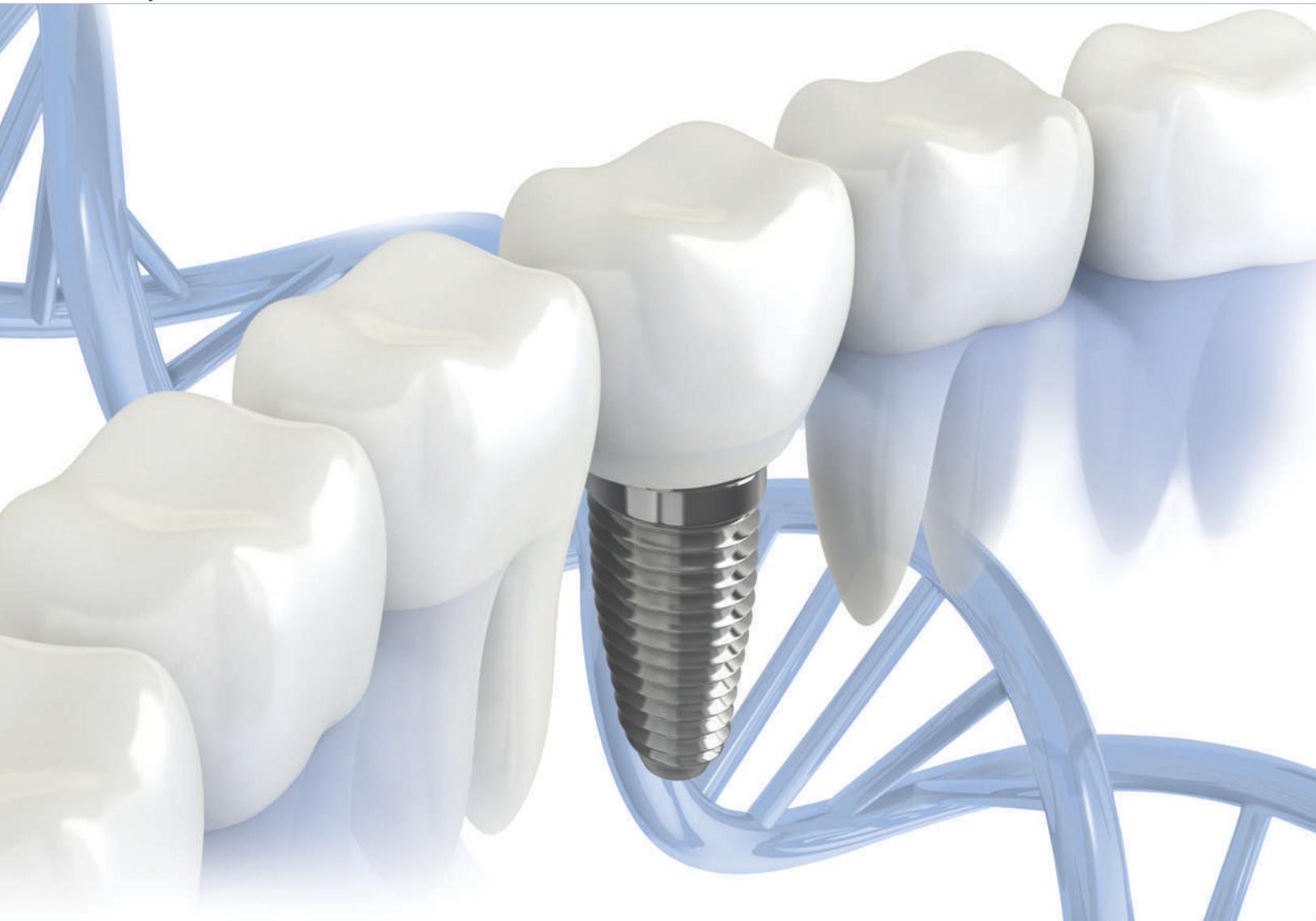
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An *In Vitro* Evaluation of Biodegradability of Stainless Steel Crowns at Various Salivary pH

Abstract

Aim: To evaluate the biodegradability of preformed stainless steel crowns at varying salivary pH and the cytotoxic effect of leached out elements on fibroblasts. **Methodology:** A total of 243 stainless steel crowns were selected and were divided into 3 groups (I, II, III) based on Ph of immersion media. The pH of samples in group I, II, III were 4.3, 5.5 and 6.3 with 81 crowns in each group. Each group has 9 samples with 8 crowns in each sample. All samples were immersed in polyethylene bottles containing 10ml of artificial saliva and incubated at 37°C for 4 weeks. All the samples were analyzed on 1,7,14 and 21 days by atomic absorption spectrophotometer for the quantitative assessment of Ni, Cr and Fe. Fibroblast tissue culture was used to assess the cytotoxicity of the samples. **Statistical Analysis:** Analysis of variance. **Results:** Maximum release of Ni, Cr, Fe ions were observed at pH 4.3 followed by pH 5.5 and least release of ions were observed at pH 6.3 from SS crowns. The cytotoxic results showed that the least cell viability of cells was seen at pH 4.3. **Conclusion:** With decrease in pH, there is an increase in ion release from stainless steel crowns and the mean release of nickel, chromium and iron were very much below the average dietary intake. But the allergic manifestations of ions like nickel can't be ruled out.

Keywords: Atomic absorption spectrophotometer; biodegradability, chromium, iron, nickel, stainless steel crowns

Introduction

Prefabricated stainless steel crowns (SSCs) are one of the most common dental devices used to restore primary molars which are severely damaged or endodontically treated and as an abutment tooth for a space maintainer. The use of preformed crowns for primary tooth restoration reduces treatment time and number of visits to clinics.^[1] The chemical composition is 65–73% iron, 17–20% chromium, 8–13% nickel and less than 2% manganese, silicon and carbon.^[2] The frequent use of preformed crowns has led to concerns that heavy metals in the crowns could be released into the oral cavity and accumulate in the body.^[1]

Oral release of dental metals is caused by mechanical stimulation due to abrasion and by chemical and thermal stimulation from eating and drinking.^[1] Dental metals were found to be cytotoxic to DNA and cultured cells, although the amounts of such metals released into the oral cavity were not harmful to human health.^[3] However, systemic accumulation of heavy metals could trigger allergies.^[4]

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The major corrosion products of stainless steel are iron (Fe), chromium (Cr), and nickel (Ni). Although all three elements potentially have adverse effects, Ni and Cr have received the most attention because of their reported potential for producing allergic, toxic, or carcinogenic reactions. Approximately 10% of the general population exhibit hypersensitivity to Ni, with females being reported to be 10 times more sensitive than males presumably because of sensitization from nickel-containing jewelry.^[5] In pediatric patients, the prevalence of sensitization is around 15–16%.^[6]

It has been well established that these metals possess the propensity to produce hypersensitivity, dermatitis, asthma, and ulcers of the oral mucous membrane. In addition, a significant carcinogenic and mutagenic potential have been demonstrated for the compounds of these metals. Metals leaching out of crowns can cause toxicity reactions if they exceed the maximum recommended daily intake levels.^[7]

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Hence, the purpose of the present study was to assess the leach out of Ni, Cr, and Fe at varying salivary pH on 1, 7, 14, and 21 days and to assess the cytotoxicity of these leached out elements on cultured fibroblasts (L929 cells).

Methodology

A total of 216 stainless steel crowns were selected and divided into three groups (I, II, III) based on pH of immersion media with 72 crowns in each group. The pH of samples in group I, II, and III were 4.3, 5.5, and 6.3, respectively. Each group has 9 samples with 8 crowns in each sample. All the samples were immersed in polyethylene bottles containing 10 ml of artificial saliva and incubated at 37°C for 4 weeks.

Artificial saliva was prepared by dissolving 0.8 g of NaCl (Sodium chloride), 2.4 g of KCl (Potassium chloride), 1.5 g NaH₂PO₄ 2H₂O (Sodium dihydrogen phosphate dihydrate), 0.1 g Na₂S, 9H₂O (Sodium sulphide), 2 g CO (NH₂)₂ (Urea) in 2000 ml of distilled deionized water.^[8] The pH of the artificial saliva was measured by pH meter, with the addition of an acid/and base. The pH was adjusted to obtain the different pH ranges designated for the study.

The samples were placed in the solution on day 0. After day 1 and every 7 days, they were taken out from the solution and placed in another container with fresh artificial saliva in order to avoid saturation of solution with released ions. Following immersion the samples were shaken gently to ensure bathing of all crowns in saliva and to obtain a uniform solution.

The amounts of released elements were measured on days 1, 7, 14, and 21 by Atomic absorption spectrophotometer (AA- 6300, Shimadzu, Kyoto, Japan).

Cell culture and MTT assay

The fibroblast (L929) were plated separately using 96-well plates with the concentration of 1×10^4 cells/well in Dulbecco's Modified Eagle's medium (DMEM) with $1 \times$ Antibiotic-Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in a CO₂ incubator at 37°C with 5% CO₂. The cells were washed with 200 µL of $1 \times$ Phosphate-buffered saline (PBS), then the cells were treated with various test concentration of IB, IIB, IIIB samples on the 1st and 7th day in serum-free media and incubated for 24 hrs. The medium was aspirated from cells at the end of the treatment period. 0.5 mg/mL MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) prepared in $1 \times$ PBS was added and incubated at 37°C for 4 hrs using CO₂ incubator. After the incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals were dissolved with 100 µL of Dimethyl sulfoxide (DMSO) and thoroughly mixed. The development of color intensity was evaluated at 570 nm. The formazan dye turns to purple-blue color. The absorbance was measured at 570 nm using a microplate reader.

Results

The results depicted that maximum release of Ni was observed from group I samples on all the experimental day's. The values were 0.272 ppm, 0.330 ppm, 0.218 ppm, 0.185 ppm on 1,7,14 and 21 days, respectively. By comparing the release of Ni on all days at three designated pH, the maximum leach out was seen on the 7th day with 0.330 ppm, 0.264 ppm, 0.243 ppm and there was a gradual declination to 21st day with values being 0.185 ppm, 0.114 ppm, 0.100 ppm at 4.3, 5.5, and 6.3 pH, respectively [Graph 1].

The maximum release of Fe was observed at pH 4.3 with 0.673 ppm, 0.650 ppm, 0.563 ppm, 0.516 ppm on 1, 7, 14, and 21 days, respectively. By comparing the release of Fe on all days at 3 selected pH, the maximum leach out was seen on 1st day with values 0.673 ppm, 0.509 ppm, 0.425 ppm and there was a gradual declination to 21st day with 0.516 ppm, 0.414 ppm, 0.375 ppm at 4.3, 5.5, and 6.3 pH, respectively [Graph 2].

In case of Cr, maximum release was observed at pH 4.3 with 0.476 ppm and 0.173 ppm on 1st and 7th day, respectively. There was a maximum leach out of Cr on 1st day with 0.476 ppm, 0.43 ppm and 0.003 ppm at 4.3, 5.5, and 6.3 pH, respectively. By the 7th day the leach out had declined to 0.173 ppm at 4.3 pH -0.13 ppm at 5.5 and -0.0259 ppm at 6.3 pH, respectively [Graph 3].

Evaluation of mean cell viability of cultured fibroblasts on day 1 at 4.3, 5.5 and 6.3 pH is 80.8, 84.5, 88.7, respectively and on day 7 is 66.6, 94.2, 109.9, respectively. The values do not represent any significant alteration in the viability of cells; however, least cell viability was observed at pH 4.3 on both 1st and 7th days [Table 1].

Few numbers of fibroblasts were seen on day 7 at pH 4.3 using Phase contrast microscopy magnification 100x with reflected light fluorescence system at different pH on 1st and 7th day [Figure 1].

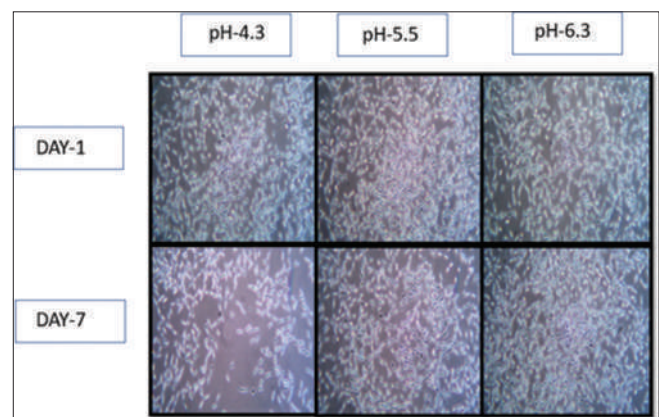
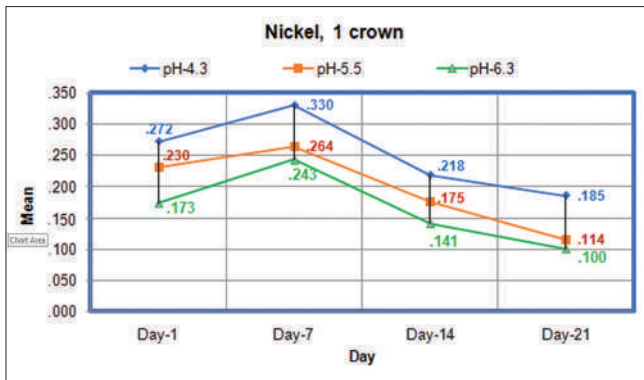
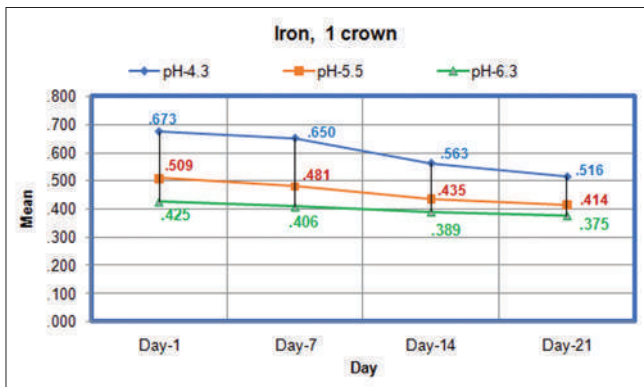


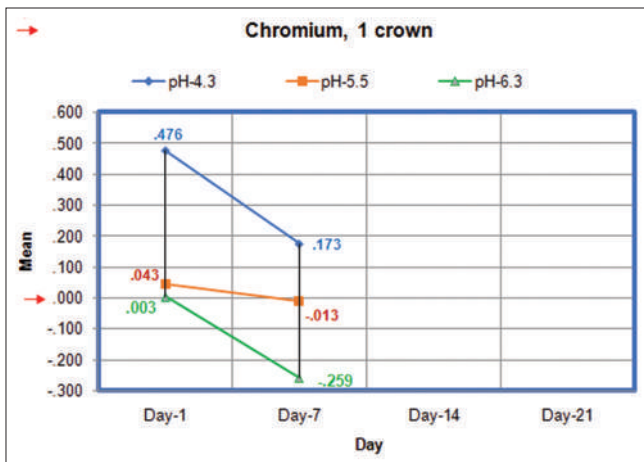
Figure 1: Phase contrast microscopy magnification 100 × with reflected light fluorescence system of fibroblasts at different pH on 1st and 7th day



Graph 1: Comparison of the mean release of Ni (ppm) in groups I, II, III on 1, 7, 14, and 21 days



Graph 2: Comparison of the mean release of Fe in groups I, II, III on 1, 7, 14, and 21 days



Graph 3: Comparison of the mean release of Cr in groups I, II, III on 1st and 7th days

Discussion

In pediatric dentistry, the stainless steel crowns are widely used which are made of a base metal alloy mainly containing nickel, chromium and iron as main constituents.^[9] The oral environment is particularly an ideal climate for the biodegradation because of its microbiologic and enzymatic phenomena.^[8] The general mechanism for the corrosion and subsequent release of metal ions from stainless steel involves loss of the passivating layer

of chromium oxide and chromium hydroxide that forms on the surface upon contact with oxygen.^[10]

Nickel is a strong sensitizer and one of the most common cause for contact allergies and claimed to have carcinogenic and mutagenic effects. The ability of nickel to induce allergic reaction has been contributed to the high haptenic capacity of released metal. Allergic response to nickel-containing alloys is mainly type 4 hypersensitivity reaction. It has been suggested that long-term exposure to nickel-containing dental materials may adversely affect both human monocytes and oral mucosal cells. In addition to nickel, chromium and cobalt ions also cause hypersensitivity and dermatitis. These metals can also induce cytotoxicity and genotoxicity. WHO (1988) and WHO (1991) stated that 0.2 mg/kg body weight of nickel and 50 mg/kg body weight of chromium can cause systemic manifestations.^[8]

Hence, the purpose of the present study was to assess the leach out of Ni, Cr, and Fe which are the main components of preformed stainless steel crowns used in restoring the primary teeth at varying salivary pH and to assess whether the corrosion products are within the acceptable limits.

In the oral cavity, the temperature and pH variations caused by diet, decomposition of foods, cell debris, oral microflora and their by-products are the important factors to be considered while evaluating the clinical behavior of orthodontic components that remain in the oral cavity for months or years.^[11] So in the present study three pH variants - 4.3 was considered to simulate a condition that can occur when people feed on acidic foods or drinks; 5.5 is the critical pH and 6.3 is the normal pH of human saliva. In each sample eight crowns were taken, considering the fact that a maximum of eight crowns can only be placed excluding anterior teeth.

Corrosion is measured in a number of ways such as electro-chemical tests that measure elemental release indirectly through the flow of the released electrons current or by tests that measure of the elements directly by spectroscopic methods. Perhaps the most relevant measure of corrosion from the standpoint of biocompatibility is identifying and quantifying the elements that are released. Thus, atomic absorption spectrophotometer was used in the study to measure the leach out of the elements.^[12]

In the present study, maximum release of Ni, Cr, Fe ions was observed at pH 4.3 followed by pH 5.5 and the least release of ions were observed at pH 6.3. This observation illustrate that as the pH of saliva increases, the leach out of ions from the stainless steel crowns decreases. Similar findings were observed in the studies conducted by Menek *et al.* (2012)^[13] and Tiwari *et al.* (2016).^[2] Huang *et al.* (2004)^[5] observed elevated levels of nickel released from stainless steel brackets at lower pH. The reason attributed to this could be that acidic conditions provide a reducing environment in which the stainless steel oxide film required for corrosion resistance is less stable.

Table 1: Mean cell viability of L929 cells at 4.3,5.5,6.3 pH on 1st and 7th day

Cell viability	pH						Control	
	4.3		5.5		6.3		Mean	SD
	Mean	SD	Mean	SD	Mean	SD		
Day 1	80.8833	4.5495	84.5867	16.5273	88.7700	15.6070	100.0	0.0
Day 7	66.6667	4.3590	94.2667	14.8178	109.9167	15.0120	100.0	0.0
	P=0.08		P=0.52		P=0.2			

When the concentrations of nickel were measured at the various time intervals, peak level was noticed on day 7 and the concentration levels showed a progressive decline on day 14 and 21. Park and Shearer (1983)^[14] and Menne *et al.* (1987)^[15] also found that the corrosion of the appliances reached a plateau after 6 days and did not increase appreciably thereafter. Two explanations for this behavior can be contemplated. First, the nickel present on the surface of the stainless steel may quickly corrode during the first 7 days of the experiment. Subsequently, the rate of release falls as the surface nickel is depleted. Second, the corrosive products formed on the surface after 7 days slows down the corrosion of nickel.^[8] When the overall findings including those of chromium levels are taken into consideration, the first hypothesis is the more appropriate.

The release of Cr and Fe ions in the present study are same as the study conducted by Matos de Souza (2008)^[16] where they assessed the *in vivo* release of Ni, Cr, Fe ions into saliva by three commercial metallic brackets and the results showed that both Cr and Fe levels were maximum on the 1st day and declined gradually. In contrast to these results Barret *et al.* (1993)^[17] demonstrated increased release of Cr during the first two weeks and levelled off in the subsequent weeks in *In Vitro* conditions. This difference in the leach out of ions could be due to methodological differences such as storage medium, sample size and the study variables.

The amount of Ni, Cr, and Fe released in all the test solutions were below the critical level so its systemic toxic effects are so improbable. However, even this low amount of Ni has the ability to induce allergic reactions. For the occurrence of allergic reaction in the mucosa the antigen should be 5-12 times stronger than what is required to create an allergic reaction on the skin. This amount of Ni can be enough to induce an allergic reaction due to high haptenic capacity of the released metal.^[18]

When a patient is suspected of allergy, a thorough history taking and clinical examination should be done. Prick test and scratch test are used to confirm immediate hypersensitivity while patch test confirms delayed hypersensitivity and MELISA (memory lymphocyte immuno-stimulation assay test) is used to measure the sensitization induced by metals.^[19]

To evaluate the cytotoxicity of the leached out elements, cultured fibroblasts (L929 cells) were used. MTT, an enzyme-based method which relies on a reductive

coloring reagent and dehydrogenase in a viable cell was used to determine the cell viability with a colorimetric method.^[14] The MTT assay determines the functional state of mitochondria that indicates cell viability. A mitochondrial dehydrogenase enzyme in living cells reduces the yellow tetrazolium salt MTT to blue MTT formazan, which is precipitated in uninjured cells. The MTT assay is frequently used to evaluate the biocompatibility of dental materials.^[15]

The mean cell viability of L929 cells on day 1 at 4.3, 5.5, and 6.3 pH were 80.8, 84.5, 88.7, and on day 7 it was 66.6, 94.2, 109.9, respectively. This represents that there is no significant alteration in the cell viability however the least cell viability was seen at pH 4.3 on both 1st and 7th days which could be due to the maximum release of ions.

The results of this *In Vitro* study are limited and extrapolation to the clinical situation is difficult because the methodologies used are unable to reproduce precisely the highly complex and dynamic oral environment. The interaction between the characteristics of the human saliva, alterations of pH due to food variety, bacterial colonization and its by-products make the oral cavity an extremely favorable environment to corrosive process and is difficult to reproduce in laboratory conditions. Moreover, an important factor in metal corrosion is the flow rate of saliva. Most of the studies in the literature used static conditions, but more metal release could be observed in real life because of the composition and fluidity of the saliva and also because oxide layers are removed by tooth brushing.

Conclusion

- Initial leach out of the tested elements was noticed at different salivary pH.
- The mean release of Ni, Cr and Fe were very much below the average dietary intake (200–300 µg/day; 50–200 µg/day and 18 mg, respectively) and are not capable of causing any toxic effects.
- pH of saliva and exposure period is indirectly proportionate to the leachability of the ions.

Even though the ion leach out is negligible, there is possibility of allergic reactions in few cases so it is upto the clinician to explore alternative viable options.

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Conflicts of interest

There are no conflicts of interest.

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Accidental displacement of primary anterior teeth following extraction of neonatal teeth

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ABSTRACT

Eruption of the first tooth at 6 months of age is a significant stage in a child's life. However, the presence of a tooth in the oral cavity of a newborn can lead to a lot of delusions. Natal and neonatal teeth are of utmost importance not only to a dentist but also for a pediatrician due to parental anxiety, folklore superstitions, and numerous complications associated with it. The present case report describes a 1.5 cm × 1.5 cm, slow-growing, soft-tissue gingival mass which developed following the extraction of a tooth-like structure in a 4-month-old male patient. Histological examination revealed that it contained a tooth-like hard tissue intermingled with bone and fibrous tissue. Based on clinical and histological findings, the present case was diagnosed as gingival hyperplasia with displaced tooth buds of 71 and 81, which might be due to chronic irritation or traumatic extraction of the neonatal teeth. No abnormal recurrence of the lesion was detected during the follow-up period. However, postoperative clinical and radiographic photographs further reconfirmed the absence of tooth in relation to 71 and 81.

KEYWORDS: Gingival hyperplasia, neonatal teeth, odontoblasts

Introduction

Development of a child from conception through the first few years of life is characterized by many changes, which make the parents more anxious; one among these is the appearance of tooth/tooth-like structures at birth.^[1] The eruption of the first primary tooth takes place around 6 months of age, which is the first milestone both in terms of functional and psychological changes in a child's life. Occasionally, children are born with tooth-like structures that erupt even before the eruption of the first deciduous teeth. These precociously and prematurely erupted teeth are called natal and neonatal teeth, respectively, which must be differentiated from the true deciduous teeth.

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^[2] Even though the etiology is not clear, a number of factors are attributed to its incidence, which include the superficial position of the tooth germ, infection or malnutrition, febrile state, hormonal stimulation, hereditary transmission of a dominant autosomal gene, osteoblastic activity, and hypovitaminosis. Some investigators suggested that the presence of natal/neonatal teeth is associated with various syndromes or systemic conditions.^[3]

Case Report

A 4-month-old male infant was brought to the outpatient department by his parents, with the chief

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complaint of a bulbous soft-tissue mass in the lower front tooth region, which is causing difficulty while feeding. History revealed that the child was born healthy, at full term, and through normal delivery. The parents noticed tooth-like structures in the lower front teeth region at birth, for which they have consulted a general dental practitioner, which were extracted uneventfully when the child was 10 days old. Eventually, after a few months, a small, slow-growing mass appeared at the previous site of extraction. On intraoral examination, an exophytic growth measuring approximately 0.5 cm × 1.5 cm was noticed extending from the corner of the mouth on either side with a pink, smooth, shiny surface [Figure 1]. On palpation, the growth was nontender and firm inconsistency. To ascertain the presence of any hard-tissue inclusions, an intraoral periapical radiograph was taken in the same region, which revealed the presence of radiopaque tooth-like structures within the mass, and sockets in relation to 71 and 81 were empty. However, the adjacent developing tooth buds in relation to 72 and 82 regions were normal [Figure 2]. Based on the clinical and radiographic findings, it was provisionally diagnosed as gingival hyperplasia with displaced developing tooth buds. The suspicion of eruption cyst, Epstein pearls, Bohn's nodules, and natal teeth were ruled out as they are of developmental origin. Pulp polyp is omitted as it is associated with a carious tooth.^[4,5] However, the possibility of trauma or local irritation was considered. As the present case needs to be differentiated from other similar conditions through histological examination, the whole soft-tissue mass was excised using a low-grade, soft-tissue diode laser with gallium arsenide medium 810 nm at 2-3 W after obtaining the informed consent from the parents. Postsurgical healing was uneventful, and the patient was recalled after 3 months for reevaluation [Figure 3].

Histopathological picture revealed a hyperparakeratinized stratified surface epithelium with fibrous connective tissue stroma that contained numerous immature collagen fibers that were loosely



Figure 1: Preoperative photograph showing the soft-tissue mass

arranged with well-formed blood vessels. The elongated cells with an oval-shaped nucleus were suggestive of odontoblasts and the uniform eosinophilic structure above these cells was the predentin [Figure 4]. Absence of marked proliferation of endothelial cells, multinucleated giant cells, and osteoid material, ruled out the diagnosis of pyogenic granuloma, peripheral giant cell granuloma, and peripheral ossifying fibroma. Thus, a final diagnosis of reactive fibrous hyperplasia with displaced deciduous tooth buds in relation to 71 and 81, was reconfirmed in the immediate postoperative radiograph and clinical photograph after 10-month follow-up [Figures 5 and 6].

Discussion

The presence of natal or neonatal teeth may be a source of suspicion while deciding on the treatment plan, whether to maintain these teeth in the oral cavity or not. Factors such as degree of mobility, difficulty during suckling, interference with breastfeeding, the possibility of traumatic injury to the tongue and oral tissues, and whether the tooth is part of the normal dentition or is a supernumerary need to be evaluated.^[6,7] If the tooth is diagnosed as one from the normal series, maintenance of the same is important unless it causes injury to the individual. However, if these teeth are to be extracted, precaution should be taken to prevent hemorrhage, assessing the need for administration of Vitamin K before extraction, avoiding unnecessary injury to the gingiva, and creating alertness regarding the risk of aspiration during removal.^[8] In most of the cases, the extraction site heals uneventfully, but in rare cases, a proliferative growth may occur at the site of extraction, due to inflammation or procedural errors.^[9]

In the present case, the gingival overgrowth following the extraction of neonatal teeth could be due to excessive pressure to control postoperative bleeding, local curettage to remove the remnants of dental lamina, or chronic low-grade irritation/microtrauma. One rare finding that was noticed in this case was



Figure 2: Radiograph showing the displacement of tooth buds of 71 and 81 out of sockets


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Figure 3: Postoperative healing after 2 weeks

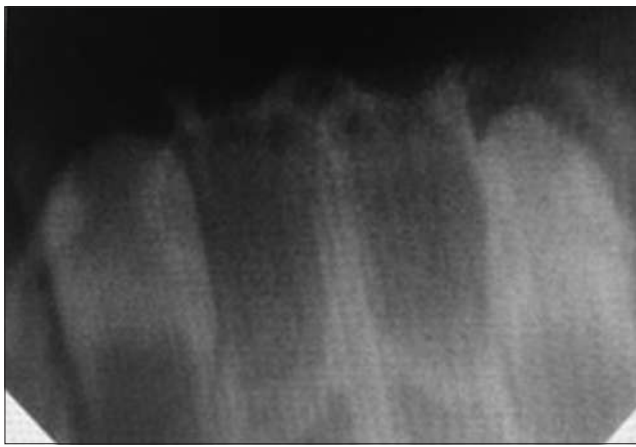


Figure 5: Postoperative radiograph

the dislodgement of the primary tooth buds from the socket into the gingival overgrowth. This could be due to the pressure exerted by proliferating collagen fibers of dental follicle that displace the developing tooth buds out of its sockets.

Conventionally, these sort of lesions are excised surgically. However, in this case, soft-tissue laser was used to excise the lesion to have an accurate, bloodless field with minimal trauma and faster healing. Even a study by Mustafa and Kawas^[10] noticed that there is no effect on the developing enamel, cementum, periodontal ligament, and eruption process following the usage of soft-tissue lasers. Moreover, the usage of lasers reduces the operator's chairside time and postoperative discomfort has less or no requirement of local anesthesia.

In the present case, replacement of the missing lower anterior primary teeth with a functional space maintainer is planned after the eruption of the adjacent teeth during the follow-up visit.

Conclusion

Complications following the extraction of natal/neonatal teeth are a rare finding. However, in this case,

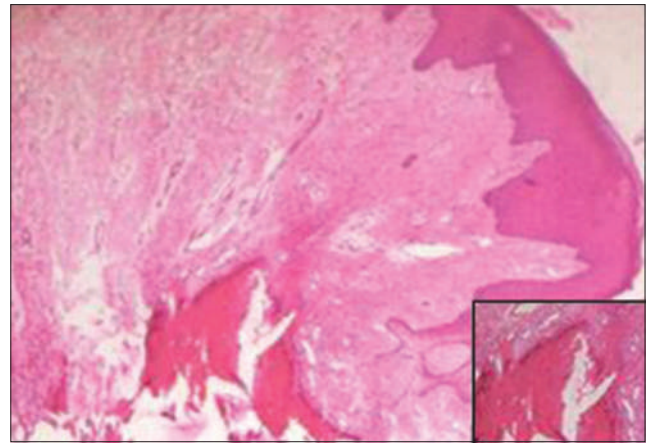


Figure 4: Photomicrograph ($\times 10$) showing the hyperplastic stratified squamous epithelium with fibrous connective tissue stroma and eosinophilic dentin-like structure



Figure 6: Ten-month follow-up postoperatively revealing the absence of 71 and 81 and eruption of 72 and 82

the presence of low-grade irritation and improper execution of the surgical procedure led to this condition. Restoring the form and function of the missing primary teeth followed by long-term follow-up till the succedaneous permanent teeth erupt into the oral cavity is mandatory. Thus, early diagnosis, prompt treatment, and gentle handling of the tissues should be the primary concern in management to prevent the occurrence of these types of anomalies.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Cephalometric Assessment of Dentoskeletal Characteristics in Children with Digit-sucking Habit

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ABSTRACT

Background: Nonnutritive sucking can turn into a continuous behavior practiced unconsciously, leading to a deleterious oral habit. Digit-sucking habits are an important etiological factor for malocclusion.

Aim: To investigate the effect of the digit-sucking habit on dentofacial structures by employing a cephalometric analysis.

Materials and methods: Selected 120 children were grouped as 60 with the digit-sucking habit and 60 without the digit-sucking habit in the age ranging between 6 and 12 years without gender discrimination. All were subjected to the standardized cephalometric technique, radiographs were traced by a single operator on a standard matte acetate tracing paper in a darkened room, and a total of 8 linear and 11 angular variables were measured for each patient in both the groups.

Statistical analysis: The unpaired Student's *t*-test was used to compare the mean difference between the two groups.

Results: The digit-sucking group showed significant difference in linear skeletal measurements such as value from anterior nasal spine (ANS) to posterior nasal spine (PNS), condylon to gnathion, nasion to ANS, sella to basion, and angular measurements such as angle between maxillary incisor to cranial plane, mandibular incisors to mandibular plane, sella nasion to point A, sella nasion to point B, and mandibular plane to cranial plane, when compared to the control group.

Conclusion: Within the confined parameters, digit sucking has led to significant variations in certain dental and skeletal cephalometric measurements.

Keywords: Digit sucking, Lateral cephalometry, Malocclusion.

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INTRODUCTION

Most of the valuable contributions to our knowledge of orofacial behavior have been made by astute clinicians who assert that the form of oral structures determines their function.¹ Nonetheless, the myofunctional hypothesis proclaims that teeth move to occupy a neutral zone and any change in muscle force equilibrium can initiate morphological variation in the normal configuration of the teeth and the supporting bone, or it can enhance an already existing inherent malocclusion due to compensatory or adaptive muscle activity and functions.²

Many activities do not normally enter consciousness; movements follow a stimulus as a patterned behavior.¹ In children, oral habits have concerned dentists and other healthcare professionals for many years because of the harmful unbalanced pressures, which may be brought to bear upon the immature, highly malleable alveolar ridges, the potential changes in the position of the teeth, and occlusion that may become abnormal if habits are continued beyond preschool age.³

One of the most common and earliest repetitive behaviors seen in the infantile period is digit sucking with prevalence between 61% and 90%. It may extend until the period of 1–4 years, 6 years, and 7–11 years with the incidence of 46, 13, and 6%, respectively.⁴ It has a well-established reputation within the orthodontic profession for causing anterior open bites, an increased overjet, high palatal vault, abnormal facial growth, posterior crossbites, crowding, increased probability of developing class II malocclusion, and further disturbs the normal development of the orofacial system causing facial deformities. The literature evidenced that these changes cause discernible disfigurements if persist beyond preschool age, ensuing

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complicated surgical orthognathic procedures, multibanded mechanotherapy at a later age.⁵

Hence, one of the most valuable services that can be rendered as part of an interceptive orthodontic procedure is intervening oral habits during the primary and mixed dentition stages, which can enable the greatest possible control over growth changes and occlusal development. A pedodontist is at an advantage that he/she can see the child during the period that the habit is developing and gets the opportunity to examine the child before the detrimental effects of the habit manifest.

As the emerging field of myofunctional therapy spreads its wings, it is important to revisit the foundation that this field is built upon. With the advent of unique hybrid developments such as myobrace and preorthodontic trainer systems, this field has enjoyed great popularity and proven to elicit impressive results for habit correction and proper retraining of the oral musculature. It would

be beneficial if we can exactly know where the skeletal changes have occurred secondary to the habit for proposing more accurate decisions on the time and type of intervention.

Thus, the present cephalometric study was aimed to evaluate the effect of the digit-sucking habit on the dentofacial skeleton by assessing the linear and angular measurements of various dental and skeletal changes.

MATERIALS AND METHODS

Ethical clearance from the institutional ethical committee and an informed written consent from parents/guardians of the selected children were obtained prior to the conduct of this cephalometric study.

Selection of the Cases

Healthy patients in the age range of 6–12 years with and without the history of digit sucking were included in the study. Patients with history of orthodontic treatment, birth injuries, oral and nasal surgeries, patients who have bone deformities in the craniofacial region, muscular dystrophies, cleft palate, hereditary syndromes like craniofacial dysostosis, etc., were excluded from the study. Selected patients with history of the digit-sucking habit were grouped as the study group and patients without the habit were included in the control group.

Study Design

It was a cross-sectional study, where the sample size was obtained using the formula:

$$n = \frac{(Z\alpha^2)(pq)}{d^2} = 120$$

METHODS

Following the inclusion and exclusion criteria, the subjects were selected from the outpatients attending the Department of Pedodontics between January 2017 and August 2018. Total 120 subjects were selected and are equally placed in both the groups (60 in each).

Radiographic Technique

All radiation protective measures were considered for all the subjects to minimize radiation exposure. The subjects were asked to look straight in a long mirror, which was placed in front of them. Films were obtained from all the subjects by a cephalostat with the Frankfort horizontal plane parallel to the floor and teeth in centric occlusion with relaxed lips Fuji X-ray film (8" × 10") with speed E were exposed at 80 Kvp: 40 mA for 2 seconds from a fixed distance of 60 inches. To obtain a standardized cephalogram, all the radiographs were orientated similarly for all the patients with same amount of magnification.

Further, the radiographs were traced by a single operator on a standard matte acetate tracing paper in a darkened room, with the area of the light box surrounding the cephalogram shielded for optimum landmark identification. Linear and angular measurements that were recorded to assess the dental and skeletal variations have been shown in the Figures 1 to 3.

STATISTICAL ANALYSIS

The quantitative analysis of results was performed using the means. The unpaired Student sample's *t*-test was used to compare the mean between digit and nondigit suckers.

RESULTS

In the present study, the deviations in the dentofacial growth were determined by comparing the lateral cephalogram of digit-sucking with nondigit-sucking children.

On comparing the mean values of skeletal linear variables between the groups, statistical significance was seen for ANS-PNS ($p < 0.001$), CO-GN ($p = 0.008$), N-ANS ($p = 0.007$), and S-BA ($p = 0.005$). The significant higher mean values for the variables were noticed with ANS-PNS, CO-GN, and S-BA. Whereas N-ANS elicited reduced mean value in the study group than in controls (Table 1).

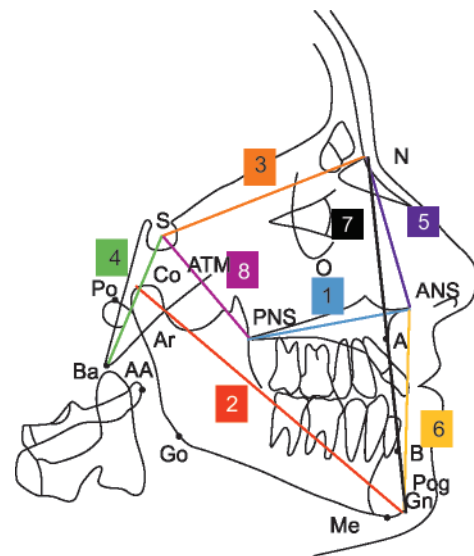
Statistical significance was observed for MNI-MNP ($p < 0.001$) and MXI-SNL ($p < 0.001$) when mean dental angular variables were compared (Table 2).

Whereas Table 3 depicts the comparison of mean skeletal angular variables between the groups. It was evident that the mean values for SNA ($p < 0.001$), SNB ($p < 0.001$), and MNP-SNL ($P = 0.045$) were significantly higher among the study group as compared to controls.

DISCUSSION

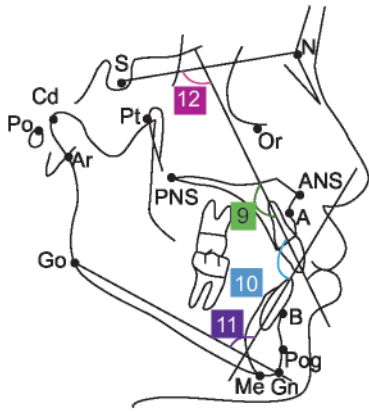
In the present study, cephalometric linear and angular variables were measured to assess the effects of digit sucking on the maxilla, mandible, and cranial base morphology by measuring incisor angulation, maxillary mandibular lengths, cranial base, and vertical height measurements to evaluate the prognathism, sagittal, and transverse variations in the dentofacial skeleton.

Some significant differences were observed, which can reasonably be attributed to the digit sucking. In the present study, a total of four skeletal linear variables ANS-PNS, CO-GN, S-BA, and N-ANS showed the significant difference between study and control groups. The mean values of ANS-PNS, CO-GN, and S-BA increased significantly in the digit-sucking group (48.77 mm, 43.86 mm, and



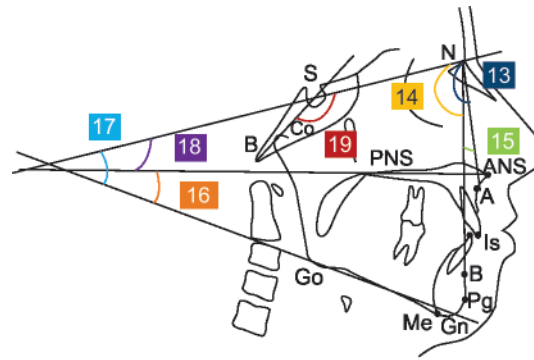
1. Maxillary length (ANS-PNS)
2. Mandibular length (CO-GN)
3. Anterior cranial base length (N-S)
4. Posterior cranial base length (S-BA)
5. Upper anterior facial height (N-ANS)
6. Lower anterior facial height (ANS-GN)
7. Total anterior facial height (N-GN)
8. Posterior facial height (PNS-S)

Fig. 1: Linear variables



- 09. Angle between maxillary incisors ANS to PNS (MXI-MXP)
- 10. Inter incisal angle (MXI-MNI)
- 11. Angle between mandibular incisors and gonion to gnathion (MNI-MNP)
- 12. Angle between maxillary incisors and nasion to sella (MXI-SNL)

Fig. 2: Dental angular measurements



- 13. Angle between sella nasion to point A (SNA)
- 14. Angle between sella nasion to point B (SNB)
- 15. Angle between point A-nasion-point B (ANB)
- 16. Angle between gonion to gnathion and ANS to PNS (MNP-MXP)
- 17. Angle between gonion to gnathion and nasion to selle (MNP-SNL)
- 18. Angle between ANS to PNS and nasion to selle (MXP-SNL)
- 19. Cranial base angle: Angle between basion selle to nasion (B-S-N)

Fig. 3: Skeletal angular measurements

Table 1: Comparing the mean values of linear variables between the study group and the control group

Linear variables	Study group mean	Control group mean	Difference between means	t value	p value
ANS-PNS	48.77	44.97	3.800	5.253	0.000*
CO-GN	108.63	103.47	5.167	2.691	0.008*
N-ANS	47.53	50.93	-3.400	-2.760	0.007*
ANS-GN	60.17	59.60	0.567	0.375	0.709
N-GN	107.47	103.67	3.800	1.536	0.127
S-PNS	44.30	44.87	-0.567	-0.388	0.698
N-S	70.27	69.30	0.967	0.979	0.329
S-BA	43.86	41.20	2.664	2.881	0.005*

*p value < 0.05 indicates statistically significant. Measurements are expressed in millimeter

Table 2: Comparing the mean values of dental angular variables between the study group and the control group

Dental angular variables	Study group mean	Control group mean	Difference between means	t value	p value
MXI-MXP	116.20	118.23	-2.033	-0.704	0.483
MNI-MNP	99.17	105.33	-6.167	-4.122	0.000*
MXI-MNI	110.80	110.23	0.567	0.305	0.761
MXI-SNL	109.47	95.40	14.067	4.362	0.000*

*p value < 0.05 indicates statistically significant. Measurements are expressed in millimeter

Table 3: Comparing the mean values of skeletal angular variables between the study group and the control group

Skeletal angular variables	Study group mean	Control group mean	Difference between means	t value	p value
SNA	80.50	77.37	3.133	4.755	0.000*
SNB	76.00	73.03	2.967	4.018	0.000*
ANB	4.63	4.97	-0.333	-0.804	0.423
BA-S-N	137.53	135.87	1.667	1.412	0.161
MXP-MNP	24.83	25.40	-0.567	-0.449	0.654
MXP-SNL	19.50	20.57	-1.067	1.219	0.225
MNP-SNL	44.90	41.51	3.333	2.031	0.045*

*p value < 0.05 indicates statistically significant. Measurements are expressed in millimeter

108.63 mm) than those of nondigit suckers (44.97 mm, 41.20 mm, and 103.47 mm). This might be due to the digit interference between maxilla and mandible, generating a light long-term continuous force

on the maxilla resulting in forward positing of ANS. This habit also causes alterations in the condylar position, creating a gap between the head of the condyle and glenoid fossae, thus stimulating the

condylar cartilage, which leads to increased measurement from condylo to gnathion. Posterior cranial base length is measured from sella to basion; this has been increased because the altered position of condyle secondarily would have affected the position of basion due to bone remodeling. These results were in agreement with the study done by Taft and Hempstead.⁶

The statistical decrease in the mean of upper anterior facial height (N-ANS) has been noticed in digit suckers (47.53 mm) than in nondigit suckers (50.93 mm). This might be due to the resistance offered by the digit to the downward growth of the anterior maxilla tilted the maxillary plane anteriorly upward and posteriorly downward resulting in reduced upper anterior facial height. But these findings are in contrary to the study done by Moore and Mc Donald where they have reported overall rotation of the maxilla without significant reduction in upper anterior facial height.⁷

On intergroup comparison, insignificant differences were noticed in linear skeletal variables when measured from sella to nasion, ANS to gnathion, and nasion to gnathion. These findings are in accordance with the study done by Moore and Mc Donald.⁷

The angle between mandibular incisors and mandibular plane (MNI-MNP) reduced statistically in the study group (99.17°) when compared to the control group (105.33°), depicting accentuated retroclination of mandibular incisors. This might be due to increased perioral muscular activity during digit sucking and this finding was in accordance with the study conducted by Subtelny,⁸ Backlund,⁹ and Gardiner.¹⁰

Maxillary incisors were significantly proclined relative to the cranial base (MXI-SNL) in the study group (109.47°) when compared to the control group (95.40°); this might be due to the lever effect of the digit creating an anteriorly directed force on the maxillary alveolar process and incisors further resulting in more anteriorly positioned point "A." Similar findings were reported by Moore and Mc Donald⁷ and Willmot.¹¹

Generally, increased proclination is characterized by the increased angle between the maxillary plane and maxillary incisors; though the proclination is prominent in most of the digit suckers, the study results did not show a significant increase in this angle. The bidirectional changes such as the increase in the length of maxillary plane and incisors proclination have maintained the angle without much deviation. Hence, the angle between the maxillary plane and maxillary incisors (MXI-MXP) might have not been altered significantly in the study group. The angle between maxillary and mandibular incisors (MXI-MNI) also did not show a significant difference in the study group; this might be due to the increased mandibular length, which led to the bodily movement of mandibular incisors that have maintained the angle.

The mean value of SNA angle in the present study was increased significantly in study group 80.50° controls 77.37°, indicating maxillary protrusion relative to the cranial base. The reason attributed could be the forces generated during the habit encourage forward growth of the maxilla resulting in more anteriorly positioned point "A." These results are in accordance with the study done by Moore and Mc Donald.⁷

The mean value of SNB angle was significantly increased in the study group (76.00°) than controls (73.00°), indicating mandibular protrusion relative to the cranial base. The reason for this could be that prolonged periods of altered position of the condyle and subsequent bone formation secondary to the habit could have placed the point B in forward position. However, these findings were in contrary to the study results by Moore and Mc Donald.⁷

The angle between gonion to gnathion and nasion to sella (MNP-SNL) suggests growth patterns in individuals; the mean values of this angle were 44.90° and 41.51° in study subjects and controls, respectively, indicating a vertical growing facial pattern in digit suckers.

An interesting facet of this study is that along with maxillary changes, mandibular and cranial changes are also evidently emphasizing the deleterious effects of the habit on the growing craniofacial skeleton and it also reflects that growth of the face will differ in both speed and velocity between children with or without the sucking habit, making them more vulnerable to permanent skeletal deviation at a faster rate. To know exactly the habit-related changes, variables such as the position of the digit and the action of the tongue during the sucking process, intensity, duration, and frequency of the habit need to be considered. However, because of the limited samples in the study group, it was not possible to break down into subgroups depending on these variables. Thus opening the paths for further research.

CONCLUSION

From the observations of this study, it can be concluded that the changes from the normalcy either dental or skeletal have been limited to maxilla/mandible. The sites far from the oral cavity such as cranial base have not shown significant changes.

Thus evaluation of dental and skeletal changes in a patient with the digit-sucking habit will help to discern the importance of early identification with a reliable diagnostic aid and intercepting them at an early age, thereby ensuring a functional environment adequate for physiological growth that helps in establishing occlusal harmony and dentofacial esthetics.

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Impact of Covid-19 Pandemic on Orthodontic Practice in India: A Qualitative Study

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ABSTRACT

Background: The purpose of our research was to assess the impact of COVID- 19 pandemic on orthodontic practices according to perceptions of practicing orthodontists.

Materials and Methods: A questionnaire was circulated amongst 16 orthodontists where the questions were based upon the measures that orthodontists are taking to curb the spread of COVID-19 in clinical settings as well as easing patient anxiety and in turn increasing their compliance to the treatments.

Results: We observed most of the orthodontists (89%) are facing problems while running their dental clinics in this pandemic outbreak. Around 65% orthodontists felt that existing patient compliance to the treatment appointments has decreased, which has led to increased incidence of relapse in the on-going treatments.

Conclusion: Given the high transmissibility of COVID-19, controlling aerosol and human-to-human contact while limiting treatment to emergency patients only is advised during the outbreak. It is the responsibility of the orthodontic team to ensure safety and stop cross contamination within the clinical facility.

Keywords: COVID-19, patient anxiety, aerosol spread, orthodontics.

INTRODUCTION

The COVID-19 is a viral infection caused by the novel coronavirus; interpersonal transmission occurs mainly via respiratory droplets and contact transmission, in addition to these characteristics, asymptomatic subjects and patients in the incubation period are also carriers of the novel coronavirus.¹ A recent study showed that coronavirus (previously known as SARS-CoV-2) aerosol transmission is plausible, since the virus can remain viable and infectious in aerosols for hours and on surfaces up to days.² Besides that, aerosols from infected people may pose an inhalation threat

even at considerable distances and in enclosed spaces, particularly if there is poor ventilation.³ In many countries, recommendations of the national councils of dentistry are to interrupt elective dental treatments and only emergency or urgency cares are allowed. In other places, social distancing is recommended but dental offices are still able to remain open, with usual dental care, providing the necessary biosafety measures, according to the recommendations of the national dental associations. However, many patients are not aware whether or not to attend their appointments with

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the orthodontist.⁴ Orthodontists may see dozens of patients in a single day. This fact makes strict infection control measures with the highly transmissible SARS-CoV-2 an area of concern. Children comprise the vast majority of orthodontic patients. Studies have reported asymptomatic children infected with COVID-19. The incubation period of this disease is 14 days up to 24 days. The virus is still highly contagious during this latency period. This finding rings the alarm bell of a potential hazard: treating asymptomatic patients and spreading infection within the orthodontic clinic. Furthermore, aerosol generation—a routine occurrence in the orthodontic clinic—is a confirmed route of infection transmission. Thus, to face this highly contagious infection, it is important to re-evaluate infection control measures within the orthodontic practice.⁵ Although most archwires are packaged and sealed individually, some orthodontists have recycled and reused wires.⁶ This is a huge risk of cross contamination if wires are not thoroughly sterilized. In addition, reusing tried-in orthodontic bands, orthodontic brackets, elastomeric chains, tungsten carbide debonding burs, miniscrews, orthodontic markers, and photographic retractors without proper sterilization and disinfection are tremendous potential hazards.⁷ Orthodontic instruments that come in direct contact with patients' saliva and blood, including band seaters, band removers, scalers, and ligature directors are considered contamination dangers as well.⁸ Improper handling and disinfection of such instruments and supplies would compromise infection control measures within the orthodontic practice. Using a high-speed handpiece or ultrasonic scaler during dental cleaning at bonding, bracket repositioning, and debonding visits produces aerosol and splatter in the operatory vicinity. This aerosol could be contaminated with patient's blood, saliva, or high concentrations of infectious microbes exceeding those produced by coughing or sneezing.⁹ Moreover, aerosol containing microbes was found to reach as far as 2 m from the patient's mouth, with the highest concentrations reported the furthest away from the patient.¹⁰ This finding means microbes could contaminate surfaces throughout the operatory. A study performed using a fluorescent dye with a high-speed handpiece found that the fluorescence dye reached more than 2 ft from the dental chair. The dye was even found in

the noses of the operator and assistant, having penetrated their facial protective gear.¹¹ Another study reported traces of aerosol were found on operators' scrub jacket sleeves and chest area.¹² The aerosol could contaminate the dental unit waterline, resulting in the spread of infection.¹³ Aerosols containing germs of 0.5-10 mm or less can remain airborne longer, increasing the risk of being inhaled and entering deeper areas of the lung, posing a potential infectious hazard.¹⁴ This collectively presents an alarming threat with the highly contagious COVID-19.

Aim of the study

The purpose of our research was to assess the impact of COVID-19 pandemic on orthodontic practices according to perceptions of practicing orthodontists. We intend to analyze the various precautionary measures needed for appointments and patient anxiety related to continuing orthodontic treatments from orthodontist's perspective.

MATERIALS AND METHODS

A questionnaire containing 8 questions; (Table 1) was circulated amongst 16 orthodontists; who had resumed their clinical practice after lockdown due to COVID-19 pandemic. Survey questionnaire was emailed to the participants. The questions were in English language and in an open-ended format. The responses from the participants were recorded in an MS excel spreadsheet and were subjected to descriptive statistical measures using latest SPSS. The survey questions were based upon the measures that orthodontists are taking to curb the spread of COVID-19 in clinical settings as well as easing patient anxiety and in turn increasing their compliance to the treatments. Another important factor that was considered the problems orthodontists were facing in planning their treatment strategies.

RESULTS

In our study, we observed most of the orthodontists (89%) are facing problems while running their dental clinics in this pandemic outbreak. Around 65% orthodontists felt that existing patient compliance to the treatment appointments has decreased, which has led to increased incidence of relapse in the on-going treatments. 51% of the

survey participants are only carrying emergency procedures like- management of protruding arch wires, loose brackets and bands, increased gingival swelling etc. however, only 34% are trying their hands-on virtual consultations with the help of telephonic calls, video calls as well as internet chats.(Table 2) Due to the pandemic and less incoming patients in the clinics has led to increased financial constraints on the orthodontists (56%). 92% of all the survey participants are trying to reassure the patients, of proper sanitation measures, screening in their clinics; so that patient can adhere to their orthodontic appointments.

Table 1: Questionnaire utilized in the study.

S. No.	Questions
1	Are you facing troubles due to COVID-19 situation in your dental practice?
2	Has the patient compliance to the appointments and treatments changed?
3	Has the new patient output decreased in your clinical settings?
4	Are you aware of the precautionary measures used in orthodontic clinical setting to avoid COVID-19?
5	Due to pandemic situation, how has the financial situation changed for you?
6	How are you trying to increase patient compliance in this pandemic period?
7	Are you trying virtual consultations?
8	Are you carrying out only emergency treatment in clinical settings?

Table 2: Data recorded in the present study.

Question No.	Responses	Reasons & Remarks
1	89%	Faced troubles due to COVID-19
2	65%	Patient compliance decreased
3	82%	Decrease in new patient output
4	78%	Are familiar with precautionary measures
5	56%	Have experienced increased financial constraint
6	92%	Tried to increase the patient compliance by reassuring, including cutting off the costs of the treatment
7	34%	Are trying virtual consultations
8	51%	Are only carrying emergency orthodontic procedures

DISCUSSION

In general, orthodontic emergencies can arise from the following scenarios: Loose intraoral fixed appliances, either fully retrievable by the patient or parent or partially loose requiring orthodontic intervention; fixed intra-oral appliance impinging on the palate or gingival tissue; broken, ill-fitting, or missing removable appliances, aligners or retainers; a missing or broken bracket; pcky wire; and broken or loose-ended fixed retainer. In addition, there are many scenarios for which an orthodontist may not be able to leave a patient unattended for a period greater than 10-12 weeks. Examples include patients with a reverse-curve NiTi wire or patients having treatment to retrieve an impacted tooth.¹⁵ Upon returning to clinic, dental professionals worldwide, including orthodontic faculty and residents, face the risk of a decreased patient population as a result of the economic tribulations, this pandemic has caused. Over 16 million individuals and counting have filed for unemployment due to the pandemic.¹⁶ The decline in employment, and the prevailing fear of acquiring SARS-CoV-2 may translate to a decrease in patients willing to receive elective dental treatment. The recommended precautions given by the Indian Orthodontic Society (IOS) are similar to the guidelines of other countries. The IOS stressed the assessment of chair operatories, making sure that they are a minimum of 6 feet away. They also recommend that if an employee or patient reports a fever, they should not come in for at least 24 h after being fever free with no fever-reducing medications.¹⁷ This varies from the US recommendation of 3 days by the ADA. There should also be a daily screening log for employees to log temperature and symptoms.¹⁸ The American Association of Orthodontists also listed recommendations. Emergency situations must first be assessed by phone or video calls to triage patients.¹⁹ Orthodontic practices typically have a pool of pediatric patients; considering that the pediatric population can be asymptomatic carriers of the virus, a key factor determining the ability to reopen should be the availability of personal protective equipment and access to acquiring more. It is recommended to order PPE for a 2-week period or longer as delivery times have been extended. Upon reopening, clinics should restart equipment, perform spore test sterilizer, test ultrasonic and

instrument washer, shock waterlines, and train employees on proper PPE guidelines.²⁰ In our study, we noticed that orthodontists are worried related to less patient compliance to the treatment appointments as well as extra sanitation measures which are needed to combat the spread of COVID-19 spread. Less compliance has led to relapse of treatments of their patients, which is concerning. Some of them are also trying virtual consultations, however it is still in initial stages and it will be difficult to match up with the less turn up of patients to the dental clinics.

CONCLUSION

SARS-CoV-2 is the first highly contagious pandemic infection of this millennium. Although cross-contamination within any dental setting has not been reported, dentists in all disciplines, including orthodontists, need to be constantly aware of the emerging infectious threats and informed of updates in infection control guidelines.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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Evaluation of stability of three different mini-implants, based on thread shape factor and numerical analysis of stress around mini-implants with different insertion angle, with relation to *en-masse* retraction force



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Objectives: Assess the stability of three different mini-implants, based on thread shape factor (TSF), and evaluate stresses at the mini-implant site and surrounding cortical bone on application of retraction force, at two different insertion angles. **Methods:** Mini-implants of three different diameters (M1 - Orthoimplant, 1.8mm), (M2 - Tomas, 1.6mm) and (M3 - Vector TAS, 1.4mm) and length of 8mm were used. Using scanning electronic microscopy, the mean thread depth, pitch and relationship between the two (TSF) were calculated. The mini-implants were loaded into a synthetic bone block and the pull-out strength was tested. One way ANOVA and Tukey *post-hoc* tests were used to compare the pull-out strength of mini-implants. P values < 0.05 were considered statistically significant. Finite element models (FEM) were constructed with insertion angulation at 90° and 60°, with retraction force of 150g. The results were analyzed using ANSYS software. **Results:** Statistically significant difference was found among all the three mini-implants for thread depth and pitch (<0.001). Statistically significant higher pull-out force value was seen for Orthoimplant. The stress distribution level in mini-implant and surrounding bone was observed to be smaller for Orthoimplant. **Conclusion:** Orthoimplant mini-implants have more favorable geometric characteristics among the three types, and less stress with 90° angulation.

Keywords: Thread shape factor. Primary stability. FEM.

Objetivos: Avaliar a estabilidade de três diferentes tipos de mini-implantes, com base no fator formato da rosca (*thread shape factor*, TSF), e avaliar a tensão no local de inserção e no osso cortical ao redor dos mini-implantes inseridos com dois ângulos diferentes, durante a aplicação de força para retração. **Métodos:** Foram usados três diferentes diâmetros de mini-implantes, sendo eles 1,8mm (M1, ORTHO Implant), 1,6mm (M2, Tomas) e 1,4mm (M3, Vector TAS), todos com comprimento de 8mm. Por meio da microscopia eletrônica de varredura, foram calculados a profundidade da rosca, o passo da rosca (distância entre os filetes da rosca) e a relação entre os dois (TSF). Para realização do teste de tração (*pull-out*), os mini-implantes foram inseridos em um bloco de osso sintético. Os testes ANOVA de uma via e *post-hoc* de Tukey foram usados para comparar as forças de resistência à tração dos mini-implantes, considerando-se estatisticamente significativos valores de $p < 0,05$. Modelos de elementos finitos (MEF) foram gerados com ângulos de inserção dos mini-implantes a 90° e 60°, com força de retração em 150g. Os resultados foram analisados usando-se o *software* ANSYS. **Resultados:** Diferenças estatisticamente significativas foram encontradas entre os três mini-implantes quanto à profundidade da rosca e o passo da rosca ($p < 0,001$). O ORTHO Implant apresentou a maior força de resistência à tração, com significância estatística. O nível de distribuição das tensões no mini-implante e no osso circundante também foi menor para o ORTHO Implant. **Resultados:** Entre os diferentes tipos de mini-implantes analisados, o ORTHO Implant apresentou as características geométricas mais favoráveis e a menor tensão com o ângulo de inserção de 90°.

Palavras-chave: Procedimentos de ancoragem ortodôntica. Implantes de carga imediata. Análise de Elementos Finitos.

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INTRODUCTION

The need for orthodontic treatment modalities that maximize anchorage control and minimize patient compliance has led to the development of mini-implant-assisted orthodontics.¹ Temporary anchorage devices (TADs) in the form of mini-implants are used as a skeletal anchorage and their utilization has become a reliable and acceptable method.²

Primary stability of mini-implant is due to the mechanical interlock between the bone and mini-implant, and it depends on many factors, including bone quality, mini-implant site and insertion angle, and design of mini-implants, such as diameter, thread form, pitch, thread size, mini-implant material,³⁻⁵ and the recently introduced thread shape factor (TSF)². TSF is calculated as the geometrical relationship between the mean thread depth and the pitch (D/P) and is expressed as a percentage.²

Bone remodeling processes at the bone/screw interface are correlated with the structural response of the bony tissue to the TADs and then to the stress/strain field, developing within themselves and the surrounding bone.⁶ Studies of stress allow optimization of the shape and geometric parameters. A key to the success or failure of mini-implant is the manner in which stresses are transferred to the surrounding bone.⁷

The proper insertion angle is important for cortical anchorage, patient safety (root damage), and biomechanical control. It also provides increased surface contact area between the mini-implant and the bone.⁸

Measurement of the stresses *in vivo* is virtually impossible. The finite element method (FEM) is thus a valid technique used to analyze structural stress.⁹ In order to understand better how a viscous-elastic material, such as the bone (cortical and cancellous layer), reacts to the insertion of rigid material like titanium, and which kind of stress can be generated by a specific thread design, FEM analysis can be utilized to serve this purpose.²

However, the literature lacks information on the combination of ideal geometric design characteristics, i.e., TSF and optimal insertion angle during *en-masse* retraction. To address that, this study was conducted to evaluate the effect of TSF of 3 different mini-implants, and their various insertion angle combinations, on the pull-out strength and stresses at the mini-implant site and surrounding bone during *en-masse* retraction, using a FEM study.

MATERIAL AND METHODS

Detailed geometry of all three mini-implants was studied through scanning electron microscope (SEM), to measure the TSF.

Pull-out test was carried out to determine the primary stability.

FEM was done to evaluate stress distribution at the mini-implant site and in the surrounding cortical bone, with the application of retraction force at two different insertion angles (60° and 90°).

Material

The three mini-implants used in the study were as follows:

- 1) ORTHOImplant (3M Unitek, Monrovia, CA, USA): 1.8-mm diameter and 8-mm length (M1).
- 2) TOMAS (Dentaurum): 1.6-mm diameter and 8-mm length (M2).
- 3) VECTOR TAS (Ormco): 1.4-mm diameter and 8-mm length (M3).

According to the manufacturer's description, these mini-implants are available in the above mentioned diameter with three different lengths. All mini-implants are made of Ti-6Al-4V alloy.

For the pull-out test, double layer artificial bone block (Sawbones, Pacific Research Laboratories Inc, Vashon, Washington;) was used. The block is composed by a polyurethane foam, measuring 120 x 170 x 41 mm thick, having a 1.1-mm top layer with a 40-pcf density, and a 39-mm base layer with a 10-pcf density (Table 1).¹⁰

Methods

Scanning electron microscopy

Each mini-implant was examined using a scanning electron microscope (Tescan Vega3, Czech Republic), operating at 30.00 kV, which was performed at Sanray laboratories Pvt Ltd (Hyderabad, India). Images of each mini-implant were captured with VEGA 3.0 software, and obtained at 27× and 33× magnifications (Figs 1 and 2). The pitch and thread depth was measured using team measurement tool of Biovis Materials VA4.59 software.

Pull-out test

Pull-out strength tests were performed at the same laboratory on bone blocks constructed featuring a superficial layer with biomechanical characteristics (elas-



Table 1 - Material properties of artificial bone materials (poisson ratio = 0.3).

Density pcf (g/cc)	Compressive		Strength and modulus (MPa)		Shear	
	Strength	Modulus	Tensile Strength	Tensile Modulus	Strength	Modulus
10 (0.16)	22	58	2.1	86	1.6	19
40 (0.64)	31	759	19	1000	11	130

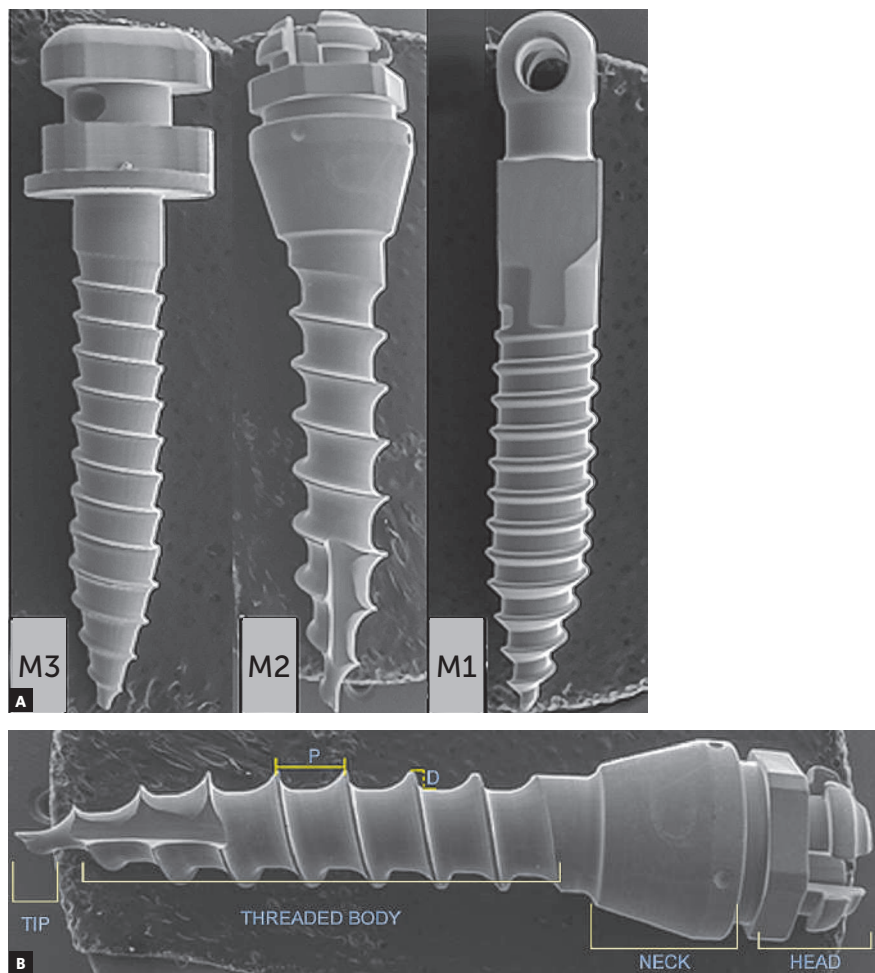


Figure 1 - A) M1, M2, M3: SEM images of mini-implants at magnification x27. B) Illustrating parts of mini-implant (D is thread depth and P is pitch).

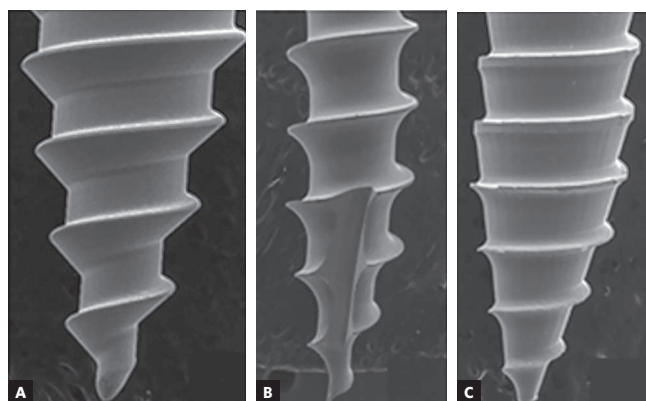


Figure 2: SEM images of mini-implants at magnification x 33.


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ticity, hardness and density) similar to the cortical bone and a deeper layer with characteristics mimicking the trabecular bone. Computed tomography study by Migliorati et al.¹³ reported a mean cortical thickness of 1.10mm on the buccal side of the maxilla. So a bone block with 1.1-mm cortical thickness was used in the present study.

This bone block was divided into small blocks measuring 1.5cm x 2.5cm, so that it could fit accurately in between the metal plates of the testing machine. The geometric center was marked on each bone block and the mini-implants were inserted at these points, to a thread depth of 6mm. The pull-out test was carried out by a universal testing machine Shimadzu AGS-X featuring 5 kN load. The mini-implant was loaded with a traction speed of 2mm/minute and the pull-out strength was measured as the peak force recorded by the built-in machine software (Trapezium v. 1.4.5). The method was repeated for each mini-implant.

Finite element method

For creating a finite element model, a 3D CAD model was constructed from a CT scan of the craniofacial complex of a 15-years-old female patient. CT scan images of the maxillary bone were taken by Siemens Somatom Definition 64 (120kVp; 290mAs) in axial direction. Sequential CT images were taken at 0.5 mm intervals to reproduce finer and detailed aspects of the geometry. A total of 625 images were stacked over one another and converted to a finite element meshed model by the software MIMIC (version 18.0). Tetrahedron elements were used to mesh the skull and teeth. Archwire, brackets, crimpable hooks and NiTi closed coil spring were modeled by the software ANSYS Design Modeler (version 19; ANSYS Inc., Integrated Design Analysis Consultants,

INDIA Pvt Ltd) with beam elements. The total number of elements in the geometry was 864,650 and the total number of nodes created was 247,119 (Fig 3). Nodes and elements defined for each model of mini-implants (M1, M2 and M3) for 90° and 60° angulations, respectively, is presented in Table 3.

Only one side of the maxilla was generated, as results on the other side are expected to be the same. To simulate the extraction space, maxillary first premolar was removed from the model. Geometric model of brackets, mini-implants, archwire with crimpable hook and nickel-titanium closed coil spring were constructed using reverse engineering technique. Brackets models were constructed using stainless steel MBT prescription of slot size 0.022x0.028-in. Stainless steel archwire of 0.019x0.025-in dimension and a nickel-titanium closed coil spring was fabricated and attached from crimpable hook to the mini-implant head, generating retraction force of 150g (Fig 3). The mini-implants were inserted at angulations of 60° and 90° to the buccal surface of alveolus. Three FEM models were generated, in which all the parameters were kept the same, except the insertion angulation of the mini-implant. Material properties assigned to the FEM were tabulated in Table 2.⁹

Statistical analysis

Descriptive statistics included calculation of mean and standard deviation for TSF and pull-out tests of three different mini-implants. Shapiro-Wilk's normality test was used to verify the equality of variance. One-way ANOVA and Tukey *post-hoc* tests were used to compare the TSF and pull-out strength of the mini-implants, within as well as between the groups. The level of significance was $p < 0.05$. Data were analyzed using SPSS software v. 23.0.



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Table 2 - Material property data representation (10).

Material	Elastic modulus E (GPa)	Poisson's ratio
Tooth	20.7	0.30
Compact bone	14.7	0.30
Cancellous bone	1.5	0.30
Titanium mini-implant	114	0.34
Bracket and wire	179	0.30
Nickel-titanium	36	0.33

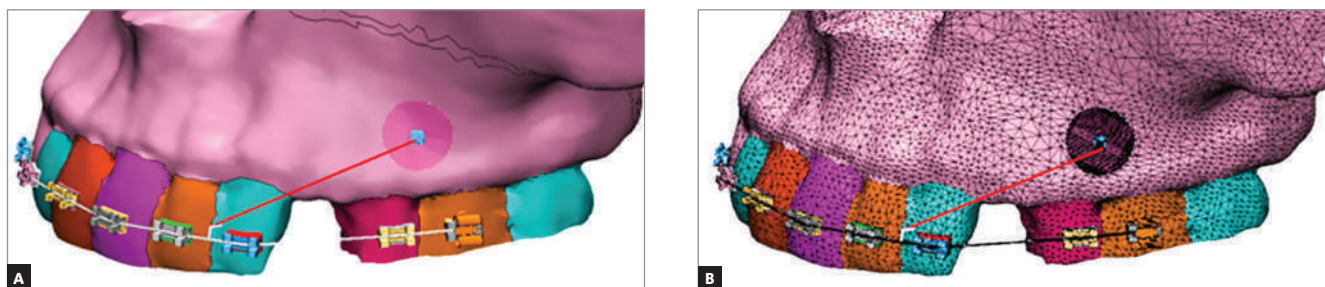


Figure 3: Three-dimensional geometric model of half maxilla with brackets, mini-implant, archwire with crimpable hook and NiTi closed coil spring.

Table 3 - Nodes and elements defined for each model.

Mini-implants	Nodes	Angulation		Nodes	Elements
		90°	60°		
M1	357,580	Elements		357,737	1,529,610
M2	357,822	1,531,187		357,714	1,531,233
M3	357,103	1,527,129		357,214	1,527,246

RESULTS

SEM and pull-out test

Since the Shapiro-Wilk's normality test confirmed the equality of variance, one-way ANOVA was used for the between-group comparisons. The mean thread depth, pitch and TSF of M1 was found to be 0.088mm, 0.426mm and 20.667%, respectively; for M2, it was 0.217mm, 0.849mm and 25.483%, respectively; and for M3, it was 0.097mm, 0.507mm and 19.100% respectively. ANOVA showed statistically significant difference for thread depth and pitch for all the three mini-implants, and statistically insignificant for TSF (Table 4). *Post-hoc* Tukey test showed: statistically significant difference for thread depth between M1 and M2, and M2 and M3; not significant difference between M1 and M3; and statistically significant difference between all the groups for the pitch of the mini-implants (Table 5).

The mean values of M1, M2 and M3 for the pull-out test were 0.181kN; 0.142kN and 0.138kN, respectively. Differences were statistically significant (Table 4).

Finite element method

The results showed changes in terms of von Mises stress and principal stresses. The magnitude of stresses developed in reaction to applied retraction force is men-

tioned in Table 6 and the pattern of stress distribution is described below.

Mini-implant

For M1 at 90° insertion angle, maximum stress was observed on the head of the mini-implant at the point of attachment with the retraction spring and at the junction of the head and transmucosal collar (neck). The stresses gradually decreased from first thread until fourth thread. Minimum levels of stress remained constant throughout the length of the mini-implant (Fig 4, M1).

At 60° insertion angle, a small portion of maximum stress was observed at the junction of the head and neck. The stresses gradually decreased from first thread until fourth thread. Minimum levels of stress remained constant throughout the length of the mini-implant. The maximum von Mises stresses at 90° and 60° insertion angle were 23.72 MPa and 29.01 MPa, respectively (Fig 5, M1).

For M2 at 90° insertion angle, maximum stress was observed in the first and second threads. Stresses decreased towards the neck and below the third thread. The stresses gradually decreased from fourth and fifth thread. The stresses remained minimal from fifth thread to the tip of the mini-implant (Fig 4, M2).

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Table 4 - Comparisons of mean depth, pitch, TSF and peak load among all three groups, by analysis of variance.

Parameter	Orthoimplant (M1)		Tomas (M2)		Vector TAS (M3)		ANOVA p value
	Mean	SD	Mean	SD	Mean	SD	
Depth (mm)	0.088	0.019	0.217	0.046	0.097	0.027	<0.001*
Pitch (mm)	0.088	0.049	0.849	0.024	0.507	0.010	
TSF (%)	20.667	4.894	25.483	4.967	19.100	5.277	0.107
Peak load (kN)	0.181	0.018	0.142	0.030	0.138	0.025	0.017*

* p < 0.05.

Table 5 - Multiple comparisons between groups by Tukey *post-hoc* test.

Parameter	M1-M2		M1-M3		M2-M3	
	Mean difference	p value	Mean difference	p value	Mean difference	p value
Depth (mm)	-0.130	<0.001*	-0.010	0.871	0.120	<0.001*
Pitch (mm)	-0.423	<0.001*	-0.080	0.002*	0.343	<0.001*
TSF (%)	-4.817	0.255	1.567	0.854	6.383	0.105
Peak load (kN)	0.039	0.040*	0.043	0.024*	0.004	0.962

* p < 0.05.

Table 6 - Magnitude of stresses developed under same load and different mini-implant angulations.

Mini-implant	Mini-implant				Cortical bone			
	90 degree		60 degree		90 degree		60 degree	
	Maximum (MPa)	Minimum (MPa)	Maximum (MPa)	Minimum (MPa)	Maximum (MPa)	Minimum (MPa)	Maximum (MPa)	Minimum (MPa)
M1	23.72	0.1056	28.01	0.062	2.4184	0.078	2.9524	0.077
M2	80.03	0.1382	107.06	0.014	6.7626	0.134	5.4152	0.115
M3	17.01	0.0625	14.89	0.008	3.8516	0.081	3.6095	0.071

At 60° insertion angle, maximum stresses were observed at the larger portion of first and second threads, and a smaller portion of third thread and neck of the mini-implant. Stresses were decreased to a small portion of fourth thread, and the stresses reached minimum levels and remained constant at the head and a larger portion of neck and from fifth thread to the tip of the mini-implant. The maximum von Mises stresses at 90° and 60° insertion angle were 80.03 MPa and 107.06 MPa, respectively (Fig 5, M2).

For M3 at 90° insertion angle, maximum stress was observed at the first and second threads and a small portion of the head, at the point of attachment of retraction spring. The stresses gradually reduced at the neck and a small portion of third and fourth threads, and from there the stresses reached minimum level and remained constant throughout the length of the mini-implant (Fig 4, M3).

At 60° insertion angle, maximum stresses were observed at the junction of head and neck. The stresses reduced from the threaded body and at the small portion

of third thread. The stresses reached a minimum level and remained constant throughout the length of the mini-implant. The maximum von Mises stresses at 90° and 60° insertion angle were 17.01 MPa and 14.89 MPa, respectively (Fig 5, M3).

Cortical bone

For M1 at the 90° and 60° insertion angle, the pattern of stress distribution was the same, where maximum stresses were observed at the mesial, distal and apical to the mini-implant. Stresses uniformly decreased in the form of concentric circles as it is moved away from the mini-implant and reached closer to the upper small portion of the lower crest of the cortical bone (Figs 6 and 7, M1).

For M2 and M3 at the 90° insertion angle, the pattern of stress distribution was similar to what was observed for the M1. At 60° insertion angle, the stresses at M2 and M3 were close to M1, with a main difference that the stresses reached the broader area of lower crest of the cortical bone (Figs 6 and 7, M2-M3).



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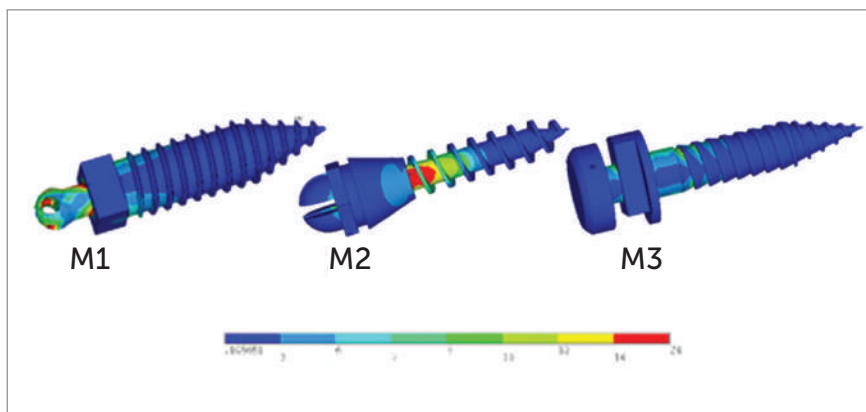


Figure 4 - M1, M2, M3: Pattern of stress distribution along mini-implant length at 90° insertion angulation.

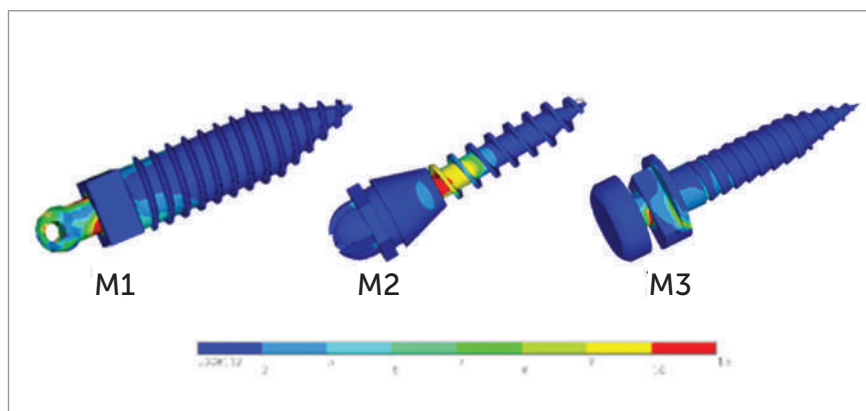


Figure 5 - M1, M2, M3: Pattern of stress distribution along mini-implant length at 60° insertion angulation.

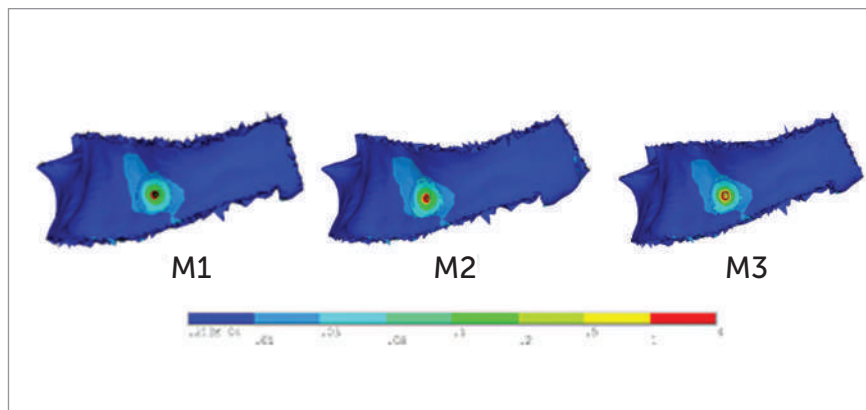


Figure 6 - M1, M2, M3: Pattern of stress distribution in cortical bone at 90° insertion angulation.

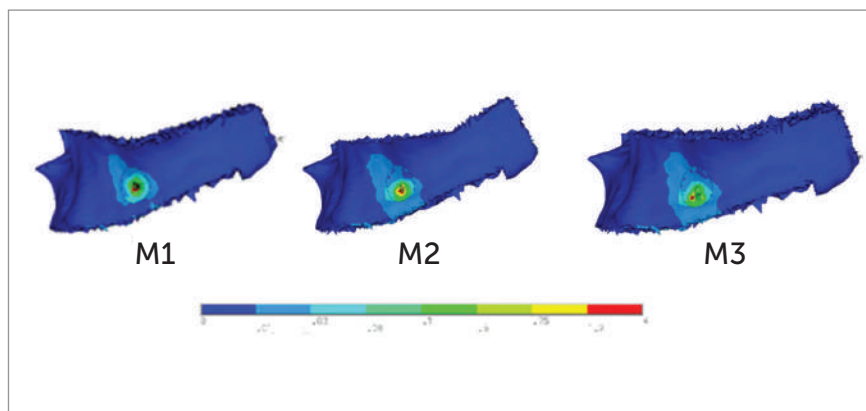



Figure 7 - M1, M2, M3: Pattern of stress distribution in cortical bone at 60° insertion angulation.


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DISCUSSION

SEM and pull-out test

The fundamental parameter for primary retention of TADs is the pull-out strength, which is linked to bone related factors¹¹⁻¹² and mini-implant design factors like diameter, pitch, thread depth and TSF.^{2,13} The TSF and relative pull-out strength values in the current study were: 20.6%, 0.181kN for M1; 25.4%, 0.142 kN for M2; and 19.1 %; 0.138kN for M3.

The results of this study showed no correlation between TSF and pull-out strength. Previous literature has shown contradicting conclusions. Radwan et al⁵ concluded that decreased TSF led to increased pull-out forces and thus, to higher primary stability; however, Migliorati et al^{2,13} reported that a larger TSF provided higher primary stability.

The results in the current study showed a non-significant difference in TSF and significant difference in pull-out force between the three groups. M1 had the highest value of pull-out force, followed by M2 and, finally, M3 (Table 4). The results of the current study indicate that different geometric design parameters like pitch, thread depth and diameter of mini-implant, apart from TSF, influenced the mechanical stability of the mini-implant.

The results of the current study showed no definitive correlation between the pitch and pull-out values, as shown in Table 4. Brinley et al¹⁴ reported that a decrease in pitch led to increase in pull-out force and therefore higher primary stability. In contrast, Migliorati et al² reported that there was a positive correlation between pitch and pull-out force when mini-implants of less than 1mm pitch were inserted in cortical thickness of 2.2mm. The reason for increased primary stability in that study could be due to more thread engagement in cortex in mini-implants with <1mm pitch when cortical bone was 1.0 - 2.0mm in width.

The current study showed no definitive correlation between the thread depth and pull-out values, as shown in Table 4. Chang et al⁴ concluded that pull-out resistance decreased abruptly as the thread depth increased from 0.32 to 0.40mm. In the present study, the thread depth is within 0.32mm for all the three mini-implants. Mini-implants used in this study have three different diameters (1.8mm, M1; 1.6mm, M2; and 1.4mm, M3). The results of this study showed a definitive correlation between diameter and pull-out force (Table 4).

The greater the diameter of the mini-implant, the greater the bone compression is, leading to an increased primary stability.¹⁵ Results of this study were in agreement with results reported in previous studies^{16,17,18} Walter et al¹⁸ stated that mini-implants with <1.2 mm in diameter should be avoided to prevent failure. Studies on fracture resistance have related the relationship between diameter and strength; they considered that a 0.1 mm increase in core diameter should give greater fracture resistance.¹⁹ OrthoImplant implants can safely resist the high levels of orthodontic forces used for *en-masse* teeth retraction and molar uprighting.

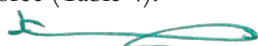
FEM study

The insertion angle of mini-implant varies most often according to clinical preference. Therefore, it is necessary to compare the efficacy in terms of stress induced in the metal and bone among mini-implants of various design and insertion angle with orthodontic loading.²⁰

Stress analysis on the mini-implant and cortical bone

In the present study, it was observed that for a given load, i.e. 150g, the stress values on mini-implant and in surrounding bone were higher for M2 with 60° and 90° insertion angle, followed by M1 and M3, respectively (Table 6). M2 mini-implant, which has a greater thread depth and smaller taper design, showed higher stresses when compared to the other two mini-implants. The results of the present study are in agreement with Chang et al,⁴ who concluded that mini-implant with greater thread depth, smaller taper and short taper length generated higher stresses on the bone and thread elements in lateral loading condition.

The stress levels in the mini-implant increased with reduction in the insertion angle for M2 and M1. The results are in agreement with studies by Woodall et al,²¹ and Lee et al,²² who concluded that placing mini-implant at 90° insertion angle increases the biomechanical stability of mini-implant. The authors also stated that oblique/acute angulations potentially creates longer lever arms, making the threads not completely engaged into the bone, creating increased stress and displacement around the mini-implant, negatively contributing to the primary stability.^{15,23} In the present study, the stress levels in M3 increased with an increase in the insertion angle, and the reason could be the reduced diameter of the mini-implant.



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For M2 mini-implant, high stresses were distributed on the uppermost threads at the neck of the mini-implant near the margin of bone, with both insertion angulations. High stresses were observed on the head of mini-implant at the point of attachment of the retraction spring, with respect to M1 and M3 with both insertion angulations. This pattern of stress distribution on mini-implant (M1 and M3) was in agreement with studies conducted by Ammar et al²⁴ and Gracco et al.⁶ Benedict et al²⁵ and Ammar et al²⁴ in their studies suggested that 2–3mm of the implant's endo-osseous length is most critical in terms of stress response under tangential loading, and the results of the present study were in agreement with that. Mini-implants manufacturers should expect more failures at top three threads.

However, the stress values in the current study were below the yield stress of titanium (692Mpa),²⁶ thus indicating that all mini-screws have sufficient strength to resist forces during orthodontic loading.

Highest amount of principal stress in the bone were seen with the M2 mini-implant and the least amount of principal stress were seen for the M1 type. The results were in agreement with previous studies^{27–28} that concluded that mini-implants with smaller pitch showed less stress within the bone. In the present study, M1 and M3 had smaller pitch when compared to M2, so smaller amount of stresses was observed with M1 and M3 mini-implants.

The maximum principal stress in bone for both insertion angles indicated that the stress decreased from 60° to 90° for M1, but this decrease in stress distribution was observed to be marginal. These findings were in agreement with previous studies,^{8,29} which reported that when the mini-implant insertion angle was increased from 60° to 90°, the stress in the surrounding bone decreased. However, for M2 and M3 there was a marginal increase in stress distribution from 60° to 90° insertion angle. The maximum stress value of 6.7626MPa was

seen with 150-g load and at 90° insertion angulation. As this value is way smaller compared to the 133MPa yield stress of cortical bone, it can be inferred that no significant adverse changes will be seen in cortical bone.

CONCLUSION

» Within the limitation of this study involving the finite element analyses and mechanical testing of different mini-implants, the result demonstrated that Ortho-implant type with a larger diameter, smaller pitch and shorter taper length have better primary stability, and also have low stresses within the mini-implants and surrounding bone amongst the three groups.

» The favorable insertion angulation found was 90°, as it provides better primary stability and low stresses in the mini-implant and surrounding bone under orthodontic loading.

» Further research is required for optimization of thread-parameters and its validation on living bone tissue.

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Conception or design of the study: SS, RR, AKT. Data acquisition, analysis or interpretation: SS, RR, AKT, AH, SLB, AF. Writing the article: SS, RR. Critical revision of the article: SS, RR, AKT, AH, SLB, AF. Final approval of the article: SS, RR, AKT, AH, SLB, AF. Obtained funding: RR. Overall responsibility: SS.



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Evaluation of Three Commercially Available Materials in Reducing the White Spot Lesions During Fixed Orthodontic Treatment: A Prospective Randomized Controlled Trial

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Abstract

Objectives: Treating white spot lesions (WSL) to create a sound and esthetically pleasing enamel surface is a question yet to be fully answered. The objective of this randomized controlled trial was to measure and compare the degree of regression of WSL during orthodontic treatment achieved by using three commercially available materials.

Methods: A single-blinded randomized prospective clinical trial, comprising 80 patients categorized into four groups (one control group and three experimental groups, with 20 subjects per group) using block randomization, was conducted. Group A (control group): Colgate strong toothpaste; and experimental groups were Group B: GC tooth mousse, Group C: Phos-Flur mouthwash and Group D: SHY-NM. Subjects were instructed to use the designated dentifrice/mouthwash and photographs were taken at baseline, third and sixth months, and white spot lesions were reassessed in the maxillomandibular anterior teeth.

Results: All the three groups had shown an improvement in WSL. But Group B has shown the greatest difference in mean values of decalcification index (DI) scores.

Conclusion: All three commercially available products showed a regression of WSL over a 6-month duration. GC tooth mousse proved to be the most effective means of treating WSL over other regimens.

Keywords

White spot lesions, dentifrices, orthodontic therapy, remineralization

Introduction

White spot lesion (WSL) is the earliest macroscopic evidence of enamel caries and is commonly seen during orthodontic therapy.¹ Fejerskov et al.² defined WSLs as ‘the first sign of a carious lesion on the enamel that can be detected with naked eye’.

After four weeks of orthodontic bonding, WSLs become noticeable and are commonly seen on tooth surface around the bracket, especially in the gingival areas.³ Patients treated orthodontically showed broadly about 2% to 96% of white spot lesions. This large range is thought to be mainly due to the difficulty in standardizing clinical examinations.⁴

Clinically visible white spot lesions were seen in patients under orthodontic treatment 1 month following the start of the treatment in studies done by O’Reilly et al.⁵ and Ogaard et al.⁶. This is a very small estimated time interval between two successive orthodontic treatment sessions. WSLs that are crescent in shape are commonly seen near the bracket base;

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they are also seen under loose bands or as linear defects near the margin of the band.

Gorelick et al.⁷ investigated the prevalence of carious white spots after removing bonded appliances and reported that about 50% of the patients reported with white spot lesions at least on a single tooth. In the same study, incidence of white spot lesions in the maxillary anterior segment was observed to be 15.3%, and the maxillary lateral incisor was the tooth with the highest percentage of WSL incidence (23%).

Remineralization of teeth can be brought about by various resolution procedures and products, such as saliva, mouth rinses and toothpaste, with various components such as increased fluoride concentrations, casein phosphopeptide-amorphous calcium phosphate (CPPACP) and calcium sodium phosphosilicate glass (CSPG). Microabrasion and composite restorations are aggressive techniques used to treat white spot lesions.^{8,9}

A decrease in the size of white spot lesion was noted over time mainly due to two mechanisms:

1. remineralization by calcium, phosphate and fluoride present in the saliva;^{10,11} and
2. removal of the dissolved enamel surface exposing the underlying enamel crystals which are tightly packed and thus reflect light properly.¹⁰

Fluoride acts as a remineralizing agent by depositing fluoroapatite in place of dissolved hydroxyapatite.¹² High fluoride concentration results in remineralization of WSL via hypermineralization, which usually gives unsightly tooth.¹³⁻¹⁵ The unsightly tooth appearance is because of surface remineralization which inhibits the movement of ions into the subsurface of the lesion, thereby affecting light reflection touching the tooth surface.^{16,17}

Materials that carry calcium and phosphate ions to enamel have been recommended to assist in remineralization with a more esthetically pleasing result. CPPACP is one such material¹²⁻¹⁸ that acts by binding itself to the tooth surface and the plaque coating it. This bond helps maintain a high concentration gradient of soluble calcium and phosphate ions, which in turn results in the stabilization of amorphous calcium phosphate, both of which play an important role in remineralization.^{12,19,20} These calcium and phosphate ions get deposited in the crystal voids present in the crystal structure of the enamel and promote crystal growth; this has a positive impact on the reflective properties of the enamel.¹⁵ CSPG is marketed as a treatment for dentin sensitivity.¹⁶ Its success and mechanism of action as a sensitivity-reduction agent promoted interest in it being used as a remineralization agent.²¹ This product is believed to maintain high concentrations of calcium and phosphate on the enamel surface. NovaMin is composed of inorganic calcium phosphate minerals and silica.²⁰ The method of action is that the release of sodium ions through interaction with oral fluids results in an increase in pH, which in turn releases calcium and phosphate to form a layer of calcium phosphate.

This layer then crystallizes, producing a crystalline hydroxycarbonate apatite layer filling the crystal voids in the enamel.²² Therefore, the aim of this *in vivo* study was to compare the regression of WSL treated with three commercially available products: GC tooth mousse (CPPACP), Colgate Phos-Flur mouthwash (fluoride and amulated phosphorus) and SHY-NM (calcium sodium phosphosilicate glass).

Methodology

The *in vivo* study was carried out in the Department of Orthodontics and Dentofacial Orthopaedics. The study protocol was reviewed and approved by the institutional ethical committee (No. IERB/2015-16/01). Patients undergoing orthodontic treatment using fixed appliances in the postgraduate orthodontic clinic were included for the study.

Inclusion Criteria

Included in the study were patients:

- aged between 12 and 25 years;
- with fair oral hygiene;
- with satisfactory general health;
- with a decalcification index (DI) score of 2 (visible white spot lesions), with at least 6 months of orthodontic treatment remaining; and
- with maxillary and mandibular anterior teeth bonded with fixed orthodontic appliance;

Exclusion Criteria

The exclusion criteria for the study were:

- enamel hypoplasia
- amelogenesis imperfecta
- fluorosis
- active periodontal disease
- active dental caries

Sample Size

The mean changes in decalcification index and standard deviation estimates used for sample size calculations were from a previous *in vivo* study.¹ Twenty to 25 individuals were selected in each group, with a power greater than 85% to calculate the difference of 1.0 unit change in the decalcification index (assuming a standard deviation of 1.0) and a greater than 80% power to calculate the difference of 1.25 if the standard deviation was more than anticipated (1.3).

A total of 80 (20 per group) patients were enrolled in this prospective single-blinded randomized clinical trial categorized into four groups with the help of a computer as follows:

Control group

Group A: Colgate strong toothpaste

Experimental Group

Group B: GC tooth mousse (CPPACP)

Group C: Colgate Phos-Flur mouthwash (CPF)

Group D: SHY-NM (CSPG)

Before the start of orthodontic treatment, professional dental cleaning and oral hygiene instructions were given. Frontal intra-oral photographs were taken at the baseline for assessment of decalcification index. Decalcification index was measured on the anterior teeth.

Subjects were instructed to use the designated dentifrice/mouthwash. The follow-up photographs were taken at 3 months and 6 months for assessment of WSL in the maxillary and mandibular anterior teeth. All the photographs were taken using a standardized camera (Nikon D5300, with 90–100 mm micro lens, ISO of 200, F of 18 and shutter speed of 1/125) and the photographs were taken in a well-illuminated environment.

Measurement of Decalcification

Modified Gorelick et al.⁵ DI, which is being currently used, scores each tooth as follows:

- 0: absence of a WSL
- 1: visible white spots without any surface interruption (mild decalcification)
- 2: visible white spot lesion with a roughened surface that does not require a restoration (moderate decalcification)
- 3: visible white spot lesion with surface interruptions (severe decalcification)
- 4: cavitation

For DI scoring, the photographs were taken at the beginning of the study for documentation purpose. The camera settings mentioned in the methodology were maintained throughout the study.

Statistical Analysis

Data were analyzed using SPSS version 23, and normality assumption was tested using Shapiro-wilk test. ANOVA and post-hoc Tukey tests were done for intragroup and intergroup comparisons, respectively.

Results

Mean and standard deviation (SD) of DI scores at baseline, 3 months and 6 months for the four groups are given in Table 1.

The score in Group A at baseline is 1.03 ± 0.33 , at 3 months is 1.18 ± 0.52 and at 6 months is 0.97 ± 0.49 . The score in Group B at baseline is 1.04 ± 0.39 , at 3 months is 0.78 ± 0.37 and at 6 months is 0.49 ± 0.31 . The score in Group C at baseline is 1.06 ± 0.32 , at 3 months is 0.91 ± 0.30 and at 6 months is 0.72 ± 0.27 . The score in Group D at baseline is 1.05 ± 0.38 , at 3 months is 1.01 ± 0.39 and at 6 months is 0.83 ± 0.40 .

Results of ANOVA at baseline showed statistical insignificance as $p > 0.05$, and statistically significant differences were seen at third month and sixth month as $p < 0.05$.

Post-hoc Tukey test showed a statistically significant difference between Colgate strong and GC tooth mousse at the third month and between Colgate strong toothpaste and GC tooth mouse, and GC tooth mousse and Novamin at the sixth month, while the rest of the comparisons were insignificant, as shown in Table 2.

Discussion

Many studies have been done to evaluate the ability of remineralizing agents to remineralize enamel in an artificial environment; this *in vivo* study illustrates the importance of oral environment and gives patient-centered outcomes. The products that claimed to resolve white spot lesions were selected for this study, and each of them influences the enamel surface and substrate in a different manner. Studies done by previous researchers have shown a reduction in the size of WSL with the use of the calcium and phosphorous.^{17,19,23}

The outcome of our study, which utilizes four delivering systems for calcium and phosphorous compounds, shows subsequent remineralization of white spot lesions with all the delivery systems. The remineralization of the demineralized enamel was the greatest with CPPACP, followed by Colgate Phos-Flur mouthwash, CSPS and least with a general fluoridated toothpaste.

The results of our study showed statistically significant difference in remineralization of enamel with all the four groups at the end of 3 months and 6 months.

When comparing the DI scores between demineralization products, normal fluoride toothpaste and GC tooth mousse showed a statistically significant difference at the third and sixth months, whereas GC tooth mousse and Novamin showed statistically significant difference only at the sixth month.

Previous studies also showed similar results.^{17,19,24,28} A favorable effect was observed from either supplemental fluoride or CPPACP. Du et al.²⁵ reported that a high concentration of fluoride varnish reversed WSLs at the third- and sixth-month follow-ups following de-bonding. Use of fluoridated chewing sticks have shown a greater remineralization effect on the white spot lesions in the study done by Baeshen et al.²⁶ over a period of 6 weeks.

TABLE 1: COMPARISON OF MEAN WSL SCORES AMONG ALL FOUR GROUPS AT BASELINE, 3RD MONTH AND 6TH MONTH

Comparison at	Groups	WSL scores				p value
		Mean	SD	Minimum	Maximum	
BASELINE	Colgate strong tooth paste	1.03	0.33	0.33	1.53	0.157
	G.C Tooth Mousse	1.04	0.39	0.33	1.83	
	Colgate Phos –Flur Mouthwash	1.06	0.32	0.5	1.66	
	SHY-NM	1.05	0.38	0.58	1.83	
3RD MONTH	Colgate strong tooth paste	1.18	0.52	0.25	1.48	0.019*
	G.C Tooth Mousse	0.78	0.37	0.16	1.5	
	Colgate Phos –Flur Mouthwash	0.91	0.30	0.41	1.41	
	SHY-NM	1.01	0.39	0.41	1.66	
6TH MONTH	Colgate strong tooth paste	0.97	0.49	0.16	1.83	0.001*
	G.C Tooth Mousse	0.49	0.31	0.00	1.00	
	Colgate Phos –Flur Mouthwash	0.72	0.27	0.33	1.16	
	SHY-NM	0.83	0.40	0.25	1.58	

Note: * significant at 5% level of significance ($p < 0.05$), p value is based on ANOVA test

TABLE 2: COMPARISON OF MEAN WSL SCORES BETWEEN TWO GROUPS AT BASELINE, 3RD MONTH AND 6TH MONTH

Comparison at	Comparison between	Mean Difference	SE	p value
BASELINE	Colgate strong toothpaste & G.C Tooth Mousse	-0.01	0.13	0.181
	Colgate strong toothpaste & Colgate Phos –Flur Mouthwash	-0.03	0.13	0.220
	Colgate strong toothpaste & SHY-NM	-0.02	0.13	0.460
	G.C Tooth Mousse & Colgate Phos –Flur Mouthwash	-0.01	0.13	0.999
	G.C Tooth Mousse & SHY-NM	-0.01	0.13	0.939
	Colgate Phos –Flur Mouthwash & SHY-NM	0.01	0.13	0.965
3RD MONTH	Colgate strong toothpaste & G.C Tooth Mousse	0.40	0.13	0.012*
	Colgate strong toothpaste & Colgate Phos –Flur Mouthwash	0.27	0.13	0.149
	Colgate strong toothpaste & SHY-NM	0.18	0.13	0.514
	G.C Tooth Mousse & Colgate Phos –Flur Mouthwash	-0.13	0.13	0.741
	G.C Tooth Mousse & SHY-NM	-0.22	0.13	0.294
	Colgate Phos –Flur Mouthwash & SHY-NM	-0.10	0.13	0.873
6TH MONTH	Colgate strong toothpaste & G.C Tooth Mousse	0.48	0.12	0.001*
	Colgate strong toothpaste & Colgate Phos –Flur Mouthwash	0.25	0.12	0.153
	Colgate strong toothpaste & SHY-NM	0.14	0.12	0.638
	G.C Tooth Mousse & Colgate Phos –Flur Mouthwash	-0.23	0.12	0.230
	G.C Tooth Mousse & SHY-NM	-0.34	0.12	0.027*
	Colgate Phos –Flur Mouthwash & SHY-NM	-0.11	0.12	0.780

Note: * significant at 5% level of significance ($p < 0.05$), p value is based on Post-hoc Tukey test

Anderson et al.²⁷ reported that a combination of CPPACP with 0.05% sodium mouthwash and fluoridated dentifrice demonstrated a significant improvement in WSLs. However, the authors reported that the CPPACP group was associated with a greater number of WSLs that totally disappeared

over the period of 12 months compared to 0.05% sodium fluoride mouthwash and fluoridated dentifrice combination. Robertson et al.,¹⁹ from a randomized clinical trial, reported a greater regression of white spot lesions during the initial period of orthodontic treatment with CPPACP.

However, there are studies in contrast to our results which have reported an insignificant change in the size of WSLs over time in groups using the fluoride delivery systems.^{13,28,29} Beerens et al.²⁸ concluded that the use of the CPPACP with fluoride paste supplementary to oral hygiene over the period of 4 months had no clinical advantage.

In a randomized trial²⁹ conducted, 60 adolescents were given daily application of either CPPACP (tooth mousse) or standard fluoride toothpaste to treat WSL. The study was carried out for a period of 1 month, then the results were analyzed by laser fluorescence and visual scoring (Gorelick scale) from the digital photographs. The results showed 26–58% reduction in the mean area of the lesion, but no significant differences were found between the CPPACP and the control groups. Another randomized trial¹³ compared WSLs treated with a low-fluoride mouth rinse (50 ppm) to those treated with a non-fluoride mouth rinse. At the end of 12 weeks, the lesions decreased by 40% (SD, 14.5) in the treatment group and by 51.5% (SD, 12.3) in the control group, thus indicating no significant benefits from the low-level fluoride.

The results of this current study have shown that CPPACP was superior in its ability to remineralize WSLs. The multifactorial anti-cariogenic mechanism for CPPACP has a three-fold mode of action: (a) Remineralization of enamel is brought about by preserving the supersaturated state of enamel minerals calcium and phosphate in plaque; (b) It slows down the initiation of biofilm and suppresses bacterial adherence to the tooth surface; and (c) It reduces the pH in the oral environment by acting as a buffering agent.²⁸

A limitation of our study was the lack of use of laser fluorescence and quantitative light-induced fluorescence to assess WSLs, which could have provided another superior method of assessment. However, the use of this technology in our trial was not feasible due to our budget limitations.

Conclusion

All the three commercially available products—GC tooth mousse (CPPACP), Colgate Phos-Flur mouthwash (fluoride and amulated phosphorus) and SHY-NM (calcium sodium phosphosilicate glass), showed regression of WSL over a period of 6 months. GC tooth mousse proved to be the most effective means of treating WSL over other treatment regimens.



Declaration of Conflicting Interests

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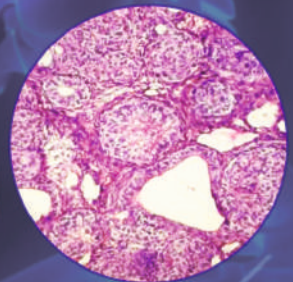
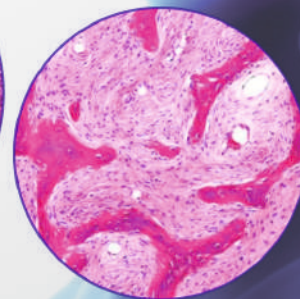
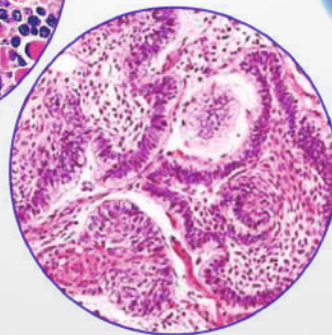
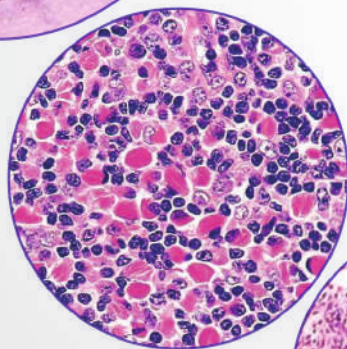
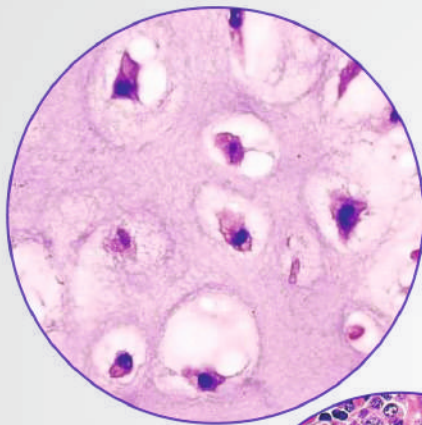
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Can coffee combat caries? An *in vitro* study

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Abstract

Background: Dental caries is a multifactorial disease caused by oral microflora, diet and oral environment. Oral microflora is crucial in initiation and progression of caries. An important strategy in the prevention of dental caries is to inhibit *Streptococcus mutans*, a caries causing bacteria. Use of natural products in the disease prevention has been increasing recently. One among such products is coffee which has anticariogenic properties against *S. mutans*. The aim of the present study is to evaluate the anticariogenic efficacy of coffee against *S. mutans*.

Materials and Methods: The study is carried out using saliva samples collected from 120 healthy individuals of 18–25 years' age group divided into two groups (cariou and noncariou). *S. mutans* is isolated from the saliva samples, cultured on mitis salivarius–bacitracin agar plates and used for checking the inhibitory activity of coffee in different formulations on these bacteria.

Results: Our study results showed that coffee, when used alone, has highest antibacterial activity against *S. mutans* and its anticaries activity reduces when it is mixed with milk and sugar ($P < 0.01$).

Conclusion: Our study proved that coffee has anticariogenic activity against *S. mutans*.

Keywords: Anticariogenic, caries, coffee, *Streptococcus mutans*

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INTRODUCTION

Dental caries is one of the most common diseases with high prevalence among human population. It is a multifactorial infectious disease in which diet, nutrition, microorganisms and host response plays a major role.^[1]

In Indian children, the prevalence of dental caries is as high as 60%–80%.^[2] It has been observed that during 1940, the prevalence of dental caries in India was 55.5%, and during 1960, it was reported to be 68%. A comprehensive national health survey conducted throughout India in 2004 has shown dental caries prevalence as follows: 51.9% in

5-year-old children, 53.8% in 12-year-old children and 63.1% in 15-year-old teenagers.^[3] There is an overall general impression that the prevalence and severity of dental caries has increased in urban and cosmopolitan population over the last couple of decades.^[4] Pain from untreated caries in children can affect school attendance, eating and speaking and, subsequently, growth and development. A practical solution to this “silent epidemic” is a preventive approach in a public health scenario. There is a tremendous need for the prevention of dental caries by population-oriented oral disease prevention programs and health promotion. In addition to these, not only preventive restorations and

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fluoride applications but also certain everyday elements of our diet can also help prevent caries.^[2]

Dental caries is the consequence of the interaction among the oral microflora, the diet, the dentition and the oral environment. Bacteria are crucial to the initiation and progression of carious lesions. *Streptococcus mutans* has been implicated as a primary causative agent of dental caries in humans.^[5]

Studies on anticariogenic effects of dietary components and natural products have increased in number only in the recent years, especially when there was a realization that foods such as milk and cheese can reduce the effects of acids and can help restore the enamel that may have been lost during eating. The caries inhibitory activity of coffee has recently come into focus when many researchers have started to investigate its bioactive components.^[6]

A cup of coffee contains 100–150 mg of caffeine. This compound was effective against Gram-positive and Gram-negative reference strains, such as *S. mutans*, the organism causing dental caries. The roasted coffee also has antiadhesive properties.^[7]

Hence, the present study is aimed to evaluate the *in vitro* antimicrobial activity of coffee-based solutions, obtained by three distinct methods, on *S. mutans*.

MATERIALS AND METHODS

The study sample included 120 individuals of age group of 18–25 years with no significant past or present medical history. The study sample consisted of two groups. Group 1 included 60 healthy individuals of 18–25 years' age group with caries and Group 2 included 60 healthy individuals of 18–25 years' age group without caries. The caries experience of the individuals was considered based on the WHO criteria. One milliliter of unstimulated saliva was obtained in sterile containers from all the individuals. Written consent for obtaining saliva sample from the individuals was obtained after giving the necessary instructions. Ethical approval was obtained from the institutional review board.

Individuals below 18 and above 25 years were excluded from the study and individuals with any significant past or present medical history were also excluded from the study.

Coffee solution was made in different formulations by boiling for 2 min, either alone or in combination with sugar and milk. One milliliter of saliva of the individuals was

diluted in 100 ml of distilled water and 1 ml of this 100 ml, which contained saliva, and distilled water was added to brain–heart infusion broth and incubated for 48 h at 37°C. One microliter of this turbid broth was plated on a culture plate containing mitis salivarius–bacitracin (MSB) agar along with sucrose, bacitracin and potassium tellurite and again was incubated anaerobically at 37°C for 48 h. Raised, convex, undulate, opaque, granular pale blue colonies were identified and counted using colony counter [Figure 1]. Confirmation for *S. mutans* was done using Gram's stain and catalase test.

Filter paper was cut into circular disks of size 5 mm in diameter and soaked in the following solutions – (1) boiled coffee with sugar and milk, (2) boiled coffee with milk and no sugar and (3) boiled coffee without sugar and milk. The soaked disks were placed on blood agar plate streaked with *S. mutans* colonies obtained from the MSB agar plate. Dilution factor of coffee was 2.2 g of coffee (BRU instant) in 5 ml of milk. The different combinations of milk and sugar with coffee were taken as this is the method by which the coffee is consumed in the world. Zone of inhibition around the filter paper disks was measured in millimeters [Figure 2]. The results were analyzed using unpaired *t*-test and ANOVA single factor.

RESULTS

When the colony count was done, the mean colony count in carious individuals was 2744 colonies/ml and that of noncarious individuals was 2595 colonies/ml. The difference in the colony count between the two groups was statistically nonsignificant ($P > 0.05$) [Table 1]. The mean values of zones of inhibition (in millimeters) observed on blood agar plate due to each solution in Group 1 were 1.49 ± 1.41 (mean \pm standard deviation) with Solution 1,



Figure 1: Photograph showing *Streptococcus mutans* colonies on mitis salivarius–bacitracin agar plate

2.37 ± 2.32 with Solution 2 and 2.99 ± 2.95 with Solution 3. The mean values of zones of inhibition (in millimeters) observed due to each solution in Group 2 were 1.5 ± 1.46 with Solution 1, 2.16 ± 1.87 with Solution 2 and 3.37 ± 3.09 with Solution 3. When the mean values of inhibition zones were compared between the two groups for each solution, there was no statistically significant difference. The difference was statistically highly significant when compared between the three different solutions individually within each group ($P < 0.01$) [Table 2].

DISCUSSION

The coffee tree belongs to the Rubiaceae family, genus *Coffea*. More than 80 coffee species were identified worldwide, of which only two are economically important. *Coffea arabica*, also known as Arabica coffee, is responsible for approximately 70% of the global coffee market, and *Coffea canephora* or Robusta coffee (commercial name of one of the main *C. canephora* cultivars) accounts for the rest.^[8] Coffee is the most commonly consumed beverage everywhere. According to the online survey by Cintin 2018, on beverages consumed regularly in India, 53.51% of respondents of India stated they drink coffee regularly.^[9] Coffee majorly contains higher amounts of antioxidant compounds and caffeine. The nonvolatile fraction of coffee is composed primarily of water, carbohydrates and fiber, proteins and free amino acids, lipids, minerals, organic acids, chlorogenic acids and trigonelline.^[8] Few studies are found in the literature that showed the antimicrobial activity of coffee-based solutions. Toda *et al.* related the effects of coffee on microbial species such as *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* and proposed that bactericidal effect of coffee might be due to tannic acid.^[10] Studies showed that caffeine and trigonelline exert a strong inhibitory performance against several microorganisms such as *S. mutans*. Few studies showed that, among all substances

studied, trigonelline was the most effective against the growth of the oral pathogens. These compounds exhibit their antimicrobial action by inactivation of cellular enzymes, which depends on the rate of penetration of the substance into the cell or caused by membrane permeability changes. Increased membrane permeability is a major factor in the mechanism of antimicrobial action, where compounds may disrupt membranes and cause a loss of cellular integrity.^[11] Coffee anticaries potential is related to its capacity of altering the biosynthesis of extracellular polysaccharides (mainly mutans).^[12] The commercial coffee powder (BRU Instant) used in the present study is a combination of 70% coffee and 30% chicory. Few studies demonstrated that chicory also exert antibacterial activity against *S. mutans*.^[11] Some studies done to assess the dietary habits (which included coffee) in relation to caries experience stated that there was less caries experience in individuals who consumed coffee in breakfast meal regularly.^[13] All these findings showed the anticariogenic effect of coffee. This study proved the antibacterial efficacy of coffee on *S. mutans* by the large significant zone of inhibition. There was a difference observed in the colony counts between Group 1 and Group 2 individuals, but it was not statistically significant. Solution 3, i.e., coffee without milk and sugar, showed greater mean values of zone of inhibition followed by Solution 2 (coffee with milk but no sugar) and Solution 1 (coffee with milk and sugar). This might be because of the

Table 1: Colony count difference between the groups

Group	Colony count (colonies/ml)	t-statistic	P	Inference
I (cariou)	2744	0.897	0.371	NS
II (noncariou)	2595			

NS: Not significant

Table 2: The difference in mean values of zones of inhibition between the groups

Group	Inhibition (mm), mean±SD			ANOVA (F-statistic)	P	Inference
	Solution-I	Solution-II	Solution-III			
Cariou	1.49±1.41	2.37±2.32	2.99±2.95	6.33	0.0022	HS
Noncariou	1.5±1.46	2.16±1.87	3.37±3.09	10.57	0.001	HS
Cariou (vs.) noncariou, P	0.94 (NS)	0.58 (NS)	0.48 (NS)			

SD: Standard deviation, NS: Not significant, HS: Highly significant



Figure 2: Photograph showing zones of inhibition on blood agar plate with different formulations of coffee

sugar in Solution 1 which favors the growth of *S. mutans*. Our study results show that black coffee shows highest antibacterial activity against *S. mutans* and its anticaries activity reduces when it is mixed with milk and sugar.

CONCLUSION

Dental caries is still considered one of the widespread problems of oral health and there is a need for new strategies to prevent the occurrence of this process. Although caries is a multifactorial disease, mutans streptococci are the main etiologic agents of caries. Our study proved that coffee has antibacterial effect against *S. mutans*. Although there are adverse effects with high amount of coffee consumption, it has more useful effects when consumed in limits. Hence, with further research, anticariogenic applications can be developed by incorporating coffee in tooth pastes, mouth washes and chewing gums. As coffee is one of the most consumed beverages in India, its taste and odor are largely appreciated. Coffee can be used at reasonable cost and help in developing new avenues of caries preventive and protective research as oral health is essential to general health and quality of life.

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Conflicts of interest

There are no conflicts of interest.

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SPECIAL ARTICLE COVID-19

Proposal of Research Model for the Detection of COVID-19 among Asymptomatic Carriers

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Abstract

Keywords

- ▶ COVID-19
- ▶ asymptomatic
- ▶ PCR
- ▶ diagnosis
- ▶ epidemiology
- ▶ saliva

Coronavirus disease 2019 (COVID-19) is a pandemic and rapidly progressing infectious disease that represents a global health care emergency due to severe acute respiratory syndrome. Rapid tests detect antigen or antibody presence, which is useful for patient care and for assisting in disease surveillance and epidemiological research. The complicating aspect of the COVID-19 spread may be due to asymptomatic individuals. Unfortunately, asymptomatic individuals are not detected in the early stages of infection, which could help to prevent community spread. The present paper shares an opinion through the proposal of a research model for detecting COVID-19 among asymptomatic carriers.

Introduction

The pandemic of coronavirus disease 2019 (COVID-19) is a global health emergency concern due to severe acute respiratory syndrome (SARS) caused by the novel coronavirus (nCoV). Severe acute respiratory syndrome-related nCoV is most probably originated from Chinese horseshoe bats (*Rhinolophus sinicus*), and pangolins are most likely the intermediate host. The spread of the COVID-19 infection initially originated from animal to human transmission, which was followed by sustained human to human spread.¹ The average estimated incubation period of COVID-19 is approximately 5 to 6 days; however, evidence reported that symptoms may appear around 14 days. Hence, medical and epidemiological observation recommend 14 days of quarantine for the identification of exposed individuals.² The most common early symptoms of COVID-19 are dry cough and fever, and some patients reported breathing difficulty and fatigue. The diagnosis of COVID-19 is established based on clinical, epidemiological, and laboratory reports. The

epidemiological information, such as history of travel, import-related or resident of affected region is also important for tracing the route of transmission. In routine practice, specimen for laboratory diagnosis is obtained from the mucosal secretion of respiratory tract through nasal swabs. The laboratory diagnosis is done by rapid test, which either detects the presence of viral proteins expressed by COVID-19 or antibodies in the blood of individuals with COVID-19 infection. However, the World Health Organization (WHO) does not recommend the use of antigen- or antibody-detecting rapid diagnostic tests for patient care but encourages its use for epidemiological and surveillance research. The WHO stated that in-vitro diagnostic tests such as real-time polymerase chain reaction (RT-PCR) and cobas SARS-CoV2 (Roche Diagnostics Corporation, Indianapolis, IN, USA) are quality-assured tests for the detection of COVID-19. However, it is important to mention that a negative RT-PCR test result from a suspected patient does not exclude infection, and the patient should be cautiously watched for any relevant clinical symptoms of COVID-19.³

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Evidence for Transmission of nCoV from Asymptomatic Individuals

A rapid and accurate detection of COVID-19 is crucial in controlling the outbreak in the community, and a model for the identification of asymptomatic COVID-19 patients is essential because these patients increase the COVID-19 burden. Studies identified that angiotensin-converting enzyme 2 (ACE2) is the functional receptor of COVID-19 infection and plays a significant role in SARS lung pathogenesis. The expression of ACE2 was identified in the respiratory tract through immunohistochemical analysis. Similarly, the expression of ACE2 was detected in the immunohistochemical method in minor salivary glands and, more specifically, in epithelial cells lining salivary gland ducts. A study mentioned that the epithelial cells lining of the salivary gland ducts are early targets of the nCoV, and ductal cells get infected eventually producing infected saliva.⁴ Thus, it can be mentioned that salivary glands appear to be potential targets for the nCoV in addition to the respiratory tract. This also indicates that early detection of the nCoV may be possible before lung lesions appear. Hence, the salivary gland can serve as a major source of virus in the saliva, which may be a source of transmission to others by asymptomatic individuals. To et al stated that detection of nCoV from salivary samples may reach 91.7%, and salivary samples can also cultivate live nCoV, thus providing an evidence that transmission of nCoV from asymptomatic individuals may be originating from the infected saliva.^{5,6}

Proposal of Research Model for the Detection of nCoV in Asymptomatic Carriers

Nasal swab tests are frequently employed in the testing for detection of nCoV. Although nasal swab remains the gold

standard specimen of choice for most patients with respiratory infections, salivary specimens are reported for assisting in detection of nCoV infection.⁷ Eight published papers of salivary-related research in nCoV infection were observed in the PubMed database. (<https://pubmed.ncbi.nlm.nih.gov/?term=Saliva%20and%20nCoV&pos=6>) Five of them were original research studies, and the findings of those papers were outlined in ► **Table 1**.^{4,5,8-10} The proposal of salivary testing is not to replace nasal swab test, but to identify the validity of salivary testing among asymptomatic patients. Salivary samples are collected by instructing patients to expectorate saliva (0.5–1 ml) into a sterile container. Two ml of viral transport media (VTM) should be added immediately to the salivary sample and submitted for molecular analysis.

Although the studies listed in ► **Table 1** outline the consistency of salivary detection of COVID-19, none of the existing studies employed both nasal swab and salivary methods of analysis on either symptomatic/asymptomatic COVID-19 patients. The present paper shares an opinion through a research model for identifying and understanding asymptomatic carriers through a comparative analysis. It would be easier to understand the efficacy of salivary detection through molecular diagnosis when both nasal and salivary samples are employed on symptomatic and/or asymptomatic patients. (► **Figure 1**) The data obtained from such research will allow us to compare the results between nasal and salivary samples and assist in arriving a conclusion on efficacy of salivary approach. The goal of this research model is to identify the asymptomatic patients through salivary-based molecular diagnosis. Reliable salivary-based detection of asymptomatic carriers will provide a guidance in quarantining with the aim to prevent community spread through asymptomatic carriers.

Considering the available evidence that states COVID-19 may be transmitted by asymptomatic individuals who may

Table 1 Studies that employed salivary samples for molecular detection of novel corona virus

No.	Study hypothesis	Study findings/ conclusion	Author and year of publication
1	Salivary testing from suspected COVID-19 patients based on clinical and epidemiological criteria.	nCoV was detected in the self-collected saliva of 91.7% of patients.	To et al 2020 ⁵
2	Ferret model of SARS-COV-2 study employed to understand the infection and transmission that recapitulates aspects of human disease.	SARS COV-2 infected ferrets showed the presence of the virus in nasal washes and saliva up to 8 days postinfection.	Kim et al 2020 ⁸
3	Analysis of COVID-19 from salivary samples of patients with known clinical and laboratory data. Samples were collected from patients with severe to very severe COVID-19 symptoms.	All the samples tested positive for nCoV. Interestingly, two patients showed positive salivary results while nasal swabs were negative.	Azzi et al 2020 ⁹
4	Prediction of intrinsic disorder in MERS-CoV/HCoV-EMC supports a high oral-fecal transmission.	Oral-saliva and oral-urine routes are also a possibility for viral transmission.	Goh et al 2013 ¹⁰
5	Viral replication in the upper respiratory tract may contribute to the rapid viral shedding into saliva droplets.	Demonstrated for the first time in the literature that the ACE2 epithelial cells of the salivary gland ducts are early targets of SARS-CoV infection. The findings provide evidence that salivary gland epithelial cells can be infected in vivo soon after infection, thus providing source of virus in saliva, particularly in early infection.	Liu et al 2011 ⁴

Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; HCoV-EMC, Human Corona Virus-Erasmus Medical Center; MERS-CoV, Middle East respiratory syndrome-coronavirus, nCoV, novel coronavirus; SARS-CoV, severe acute respiratory syndrome-coronavirus.

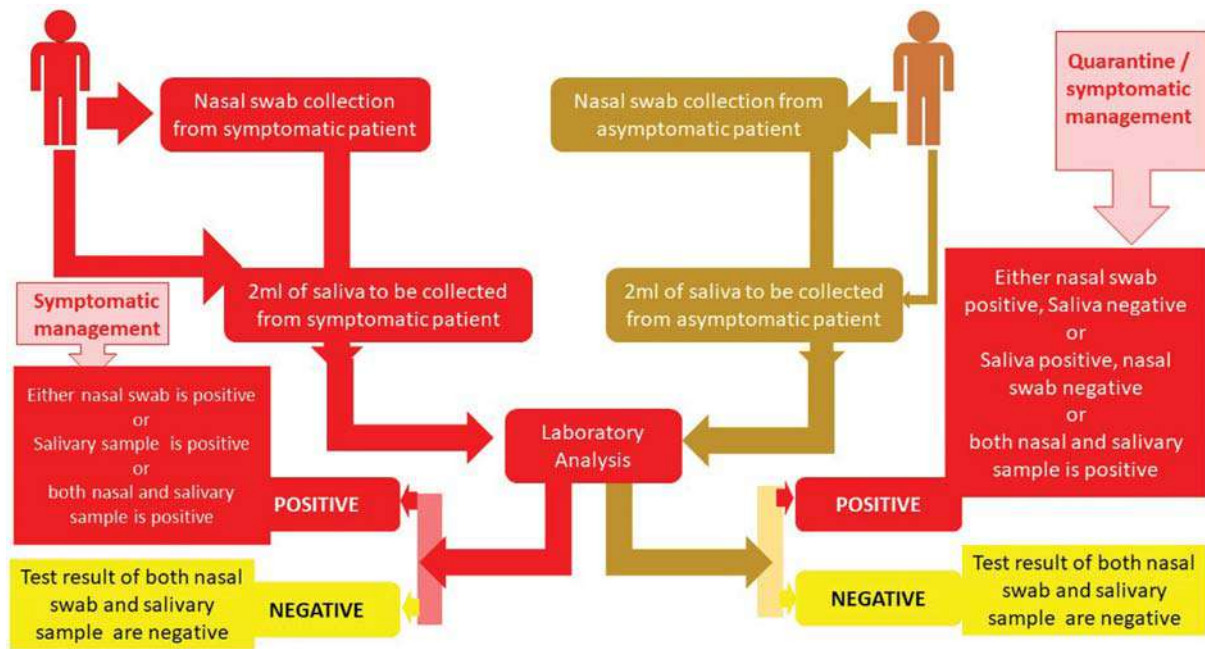


Fig. 1 Research model for comparative analysis of nasal and salivary specimens for molecular detection of nCoV in both symptomatic and asymptomatic individuals.

have an infected saliva. It is well understood that conducting a research study to employ both nasal and salivary samples on patients with suspected COVID-19 infection is resource dependent, for instance, on funding, time, and availability of adequate patients and laboratory support. The countries that could afford the cost for processing laboratory specimens should be encouraged in conducting the research to validate the salivary analysis among asymptomatic individuals. Thus, the proposed research model is aimed at identifying asymptomatic carriers and, by extension, to create a scope for policy-making decisions on preventing community spread through asymptomatic carriers.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Benign and Malignant Lesions of Jaw

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KEYWORDS

- Potentially malignant disorders • Benign • Malignant • Reactive • Oral lesions
- Child abuse

KEY POINTS

- Swelling of oral cavity may arise from surface epithelium or alternatively arise from pathology of underlying connective tissue structures.
- Most oral neoplasms are benign. Fibroma is the most common soft tissue tumor of oral cavity.
- Leukoplakia is the most common precancerous condition of oral cavity. Leukoplakia is strongly associated with smoking.
- Cancer of the head and neck is the fifth most common type of cancer in the world. Oral squamous cell carcinoma is the most common type of oral cancer.
- Benign/malignant lesion of jaw associated with child abuse is discussed.

INTRODUCTION

Benign, malignant, and reactive lesions of oral cavity present clinically as swelling/growth or an ulcerated swelling. Academically swelling is termed as tumors and growth of tumor is called as tumefaction, and hence many of the lesions described in this article are suffixed with “oma” (*fibroma*), which represents tumor.¹ Oral tissues are vulnerable for benign or malignant growth due to factors such as trauma, infection (bacterial/viral/fungal), local irritation, smoking, alcohol misuse, or genetic damage. Tumors of oral cavity constitute to a small number of cases identified in a clinical practice and therefore challenges dentist in their office for diagnosis and/or management. Hence dentists are required to be more academically familiar, and continuously educating them on these conditions, especially on common type of oral conditions of this category, will enable dentists either to institute appropriate management or refer their patient to oral disease expert and oral surgeon at a right time. Although

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clinical and/or radiological details provide a clue in diagnosis, biopsy is required for arriving to a final definitive diagnosis, because some of the swellings may be associated with dysplastic features that may require an intensive surgical management.

This article discusses potentially malignant disorders and reactive lesions, benign, malignant conditions of oral cavity. In addition, oral lesions of abovesaid category that are associated with child abuse are also discussed. For the convenience of the reader, commonly encountered oral conditions on this category are listed in **Table 1**. The oral conditions discussed will provide information on diagnosis, investigation, and outline on management.

DIAGNOSTIC APPROACH FOR SWELLING

The tumors can originate from epithelial or mesenchymal tissue and may be the result of inflammatory, neoplastic, developmental, or systemic diseases. The differential diagnosis on swelling from oral tissue must be formulated on parameters of surface appearance of swelling, location, consistency, and the presence or absence of pain. The first step in diagnostic approach for oral swelling is to check whether the swelling is arising from surface epithelium or, alternatively, arising from pathologic changes from the underlying soft tissue (ie, fibrous, fat tissue, blood vessels, lymphatic vessels, nerves, cartilage, or bone), resulting in secondary elevation of surface epithelium/tissue. The second step is to categorize them into benign, malignant, inflammatory, or reactive growth. The swellings that are movable, firm, and not indurated are in the benign category. The swellings that are fixed, indurated, ulcerated, or ulceroproliferative are likely to be malignant type.² The swellings that are either tender or painful with or without sinus/fistulation are probably of an inflammatory origin. The swellings that occur secondary to injury are reactive lesions and may be associated with or without pain. Third step is to evaluate clinical parameters such as surface appearance of swelling, location, consistency, and the presence or absence of pain. The swelling observed on oral tissues may be a regular swelling or one of the following types: papillary, verrucous, dome-shaped, papule, polypoid, diffuse, or multifocal (**Table 2**). Fourth step is evaluating status of lymph nodes. Lymph nodes that are palpable and hard and fixed to underlying structure are likely to be an association of malignant condition. Lymph nodes that are palpable and soft and movable favor benign growth association. Tenderness/pain associated with lymph node palpable is the characteristic of inflammation.^{3,4} The presence and absence of lymph node cannot differentiate benign from malignant conditions.

The fifth step is to formulate differential diagnosis based on clinical details and plan for investigation for arriving at a final diagnosis through microscopic examination, that is, biopsy (**Fig. 1**).

Potentially Malignant Disorders

Potentially malignant disorders convey that “not all lesions and conditions described under this term may transform to cancer, rather there is a family of morphologic alterations among which some may have an increased potential for malignant transformation.” Potentially malignant disorders serve as a clinical marker for future malignancy in oral mucosa.⁵ The risk factors for oral cancer and precancer are broadly categorized as established, strongly suggestive, possible, and speculative factors⁶ (**Table 3**).

Oral lichen planus

Lichen planus affects both skin and oral mucosa due to multiple factors (**Box 1**), with prevalence of 0.5% to 1%. Lichen planus is characterized by symmetric appearance

Table 1
List of benign and malignant oral lesions

Potentially Malignant Disorders	Reactive Lesions	Epithelial Tumors	Connective Tissue Tumors	Oral Lesions Associated with Child Abuse
Leukoplakia	Pyogenic granuloma	Benign tumors	Benign tumors	Herpes simplex,
Erythroplakia	Peripheral giant cell granuloma	Squamous papilloma	Fibroma	Epstein-Barr Virus,
Oral submucous fibrosis	Peripheral ossifying fibroma	Verruca vulgaris (common wart)	Lipoma	cytomegalovirus, gonorrhoea
Lichen planus	Epulis fissuratum (inflammatory fibrous hyperplasia)	Focal melanosis (oral melanotic macules)	Neurofibroma	Molluscum contagiosum
	Inflammatory papillary hyperplasia	Mole (acquired melanocytic nevus)	Neurilemoma	Condyloma Accuminatum
		Keratoacanthoma	Hemangioma	
		Malignant tumors:	Lymphangioma	
		Oral squamous cell carcinoma	Osteoma	
		Verrucous carcinoma	Malignant tumors:	
		Basal cell carcinoma	Fibro sarcoma	
		Melanoma	Osteosarcoma	


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Appearance of Swelling	Description
Papillary	The swellings that appear as fingerlike surface projections.
Verrucous	The swellings that have multiple fingerlike appearance but characterized by more irregular surface.
Dome-shaped	These are oval-shaped swellings with rolled margin and a central pit that may or may not be plugged with keratinaceous material.
Papules	These are small swellings that are <0.5 cms and are usually multiple in number.
Polypoid	These are similar to papules but exceed 1 cm in growth size and tend to be multifocal.
Nodular	These are raised solid lesions that are >5 mm in diameter.
Macule	Focal area of color change, which is neither elevated nor depressed to adjacent mucosa.
Diffuse	Swellings that are characterized by their multifocal appearance.

of bilateral white/white-grey striations or plaque.⁷ The lesion is predominantly observed on buccal mucosa, tongue, and gingival. The lesion tends to show erythematous, erosion- or blister-like appearance.

Clinical features The lesion is characterized by bilateral symmetric radiating white or greyish white striae, small angular, flat-topped papules only 0.5 mm to 2 cm with slight female predilection. The striae have lacelike appearance, which is termed as “Wickham striae.”⁸ The clinical types of lichen planus are reticular (Fig. 2), atrophic, papular, bullous, plaque, erosive, or ulcerative.⁹ Erosive type has potential for malignant transformation to oral squamous cell carcinoma (OSCC).¹⁰

Diagnosis Clinical appearance is diagnostic; however, microscopic examination is necessary for securing final diagnosis.

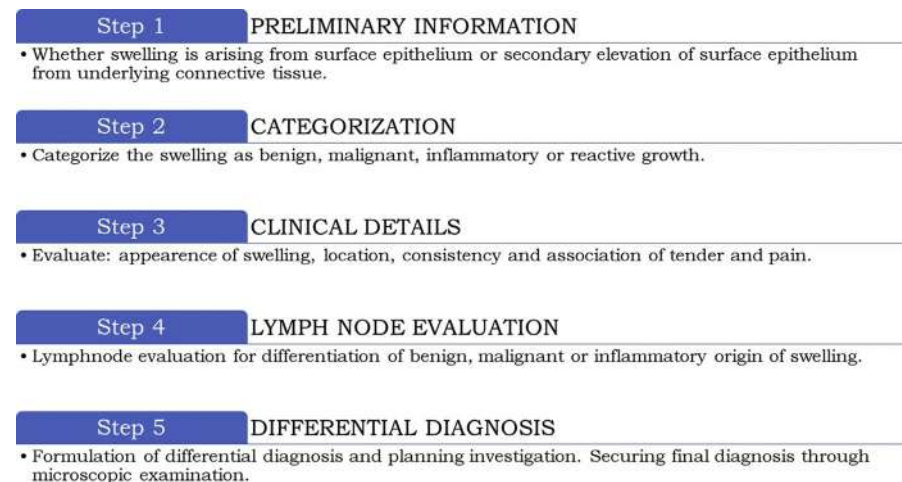


Fig. 1. Steps in diagnostic approach of swellings.

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Table 3
Risk factors for oral cancer and precancer

Established	Strongly Suggested	Possible	Speculative
Smoking	Sunlight (lip)	Viruses	Mouthwashes
Chewing tobacco	Radiation	Immune deficiency	Mate drinking
Snuff dipping		Dentition?	Periodontal disease
Alcohol misuse		Ethnicity?	Familial
Betel quid syphilis			

Adapted from Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol.* 2009;45(5):309–16; with permission.

Management Corticosteroids and topical retinoid are helpful in relieving symptoms. Associated causative factors require elimination.

Erythroplakia

Erythroplakia is an uncommon potentially malignant disorder characterized by red patch that cannot be clinically or pathologically diagnosed as any other condition with a prevalence of 0.001% to 0.83%, whereas most of the erythroplakia diagnosed is 1.2 per 100,000 population in the United States. The causative factors for erythroplakia are tobacco, alcohol, candidial infection, hematinic deficiency, and chronic trauma.

Clinical features Erythroplakia is characterized by well-defined erythematous patch or plaque with soft and velvety texture. Intermixed red and white patch is termed as erythroleukoplakia. Erythroplakia is predominantly observed in geriatric population (65–75 years) with no gender predilection. Frequently reported locations are floor of the mouth, tongue, and soft palate. Microscopic examination is usually indicated to rule out OSCC.¹¹

Management Cessation of habit is the most important strategy in the patient management. Surgical excision is used due to association of dysplastic features in the tissue. The recurrence rate is lesser than 5% and postoperative follow-up is required.

Leukoplakia

Leukoplakia is a white patch or plaque that cannot be characterized clinically or pathologically as any other diseases. Tobacco smoking is the most common cause, with prevalence of leukoplakia ranging from 1.5% to 4.3%.¹²

Box 1

Causative factors for oral lichen planus

Tobacco smoking and chewing
Stress
Dental materials
Drugs
Infectious components
Autoimmunity
Food allergies
Immunologic alterations



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Fig. 2. Reticular pattern of lichen planus involving left buccal mucosa and left commissure of lip. (From Olson MA, Rogers RS, Bruce AJ. Oral lichen planus. Clin Dermatol 2016;34(4):495–504; with permission.)

Clinical features Leukoplakia is characterized by flat to slightly elevated, non-scrapable white or gray patches that are usually seen among middle-aged adult men with smoking habit. Leukoplakia can be seen at the vermillion border of the lip, buccal mucosa, floor of the mouth, gingiva, or tongue. Causes of leukoplakia include tobacco smoking or chewing; betel quid; alcohol; trauma; infections such as syphilis and *Candida albicans*; chemicals such as Sanguinaria; ultraviolet radiation; iron-deficiency anemia; immune deficiency state; human papillomavirus (HPV) 16 and 18; and deficiency of vitamin A, B12, and C. Various clinical forms of leukoplakia are thin, thick, homogeneous, granular, nodular, verrucous, verruciform, speckled, or proliferative verrucous leukoplakia. Intermixed white-red patches are called as erythroleukoplakia. Speckled leukoplakia has a higher potential of malignant transformation to OSCC.¹³

Diagnosis Differential diagnosis of leukoplakia includes aspirin burn, candidiasis, frictional keratosis, leukoedema, linea alba, lupus erythematosus, cheek bite, syphilis, smoker's palate, and white sponge nevus.⁵ History and clinical details are adequate for ruling out differential diagnosis. Microscopic examination should be done to confirm diagnosis of leukoplakia and evaluate the association of dysplasia. Biopsy is also important to rule out squamous cell carcinoma.

Management Cessation of habit is the most important strategy in the patient management. Surgical excision is used due to association of dysplastic features in the tissue. An approach for management of leukoplakia depends on elimination of cause and biopsy report^{14,15} (Fig. 3).

Reactive Lesions

Reactive lesions are growths resulting from trauma or irritation and characterized by slow-growing, painless, pedunculated or sessile mass with or without bleeding tendency. The surface of the growth may vary from smooth to ulcerated tissue. Lymph node may be palpable and tender due to inflammatory origin.¹⁶

Pyogenic granuloma

Pyogenic granuloma is a tumorlike growth exuberant resulting from tissue response to local irritation or trauma.

Clinical features Pyogenic granuloma is characterized by painless, smooth, lobulated, pedunculated mass with bleeding tendency. However, cases have been reported with sessile growth or without bleeding tendency. Lesion appears as a pinkish

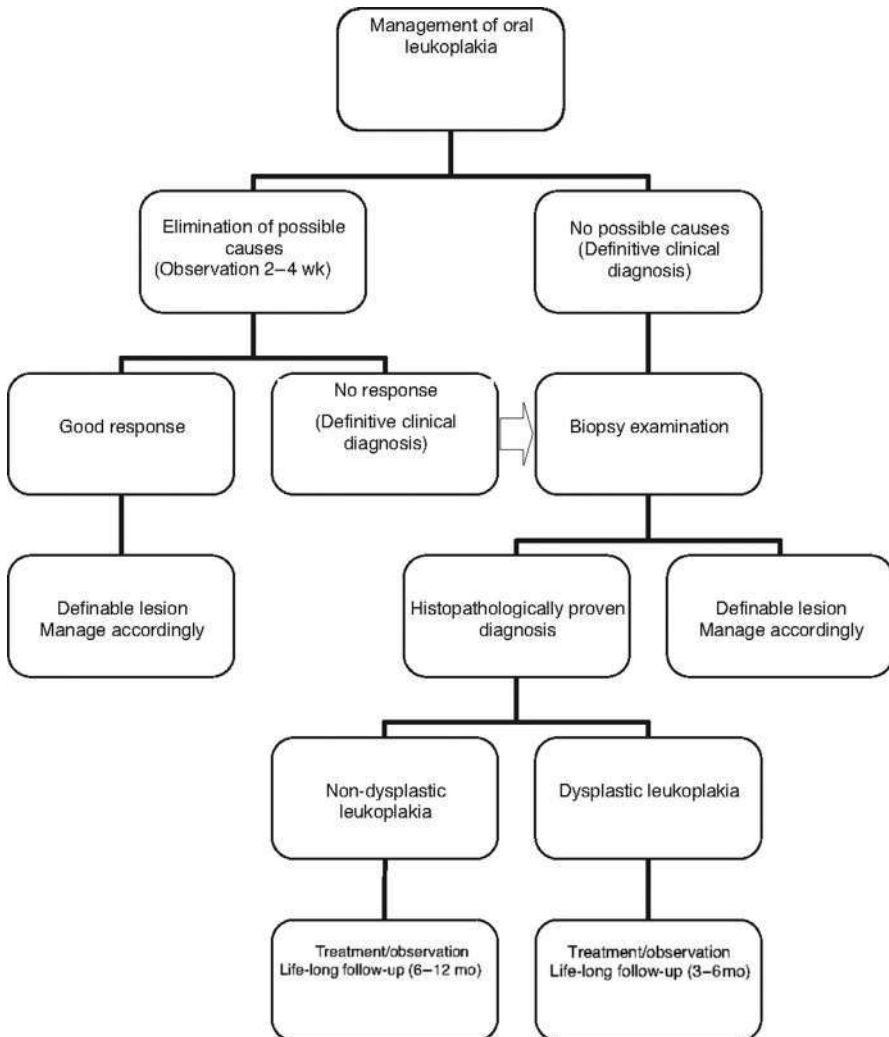


Fig. 3. Management of leukoplakia. (From Kumar A, Cascarini L, McCaul JA, et al. How should we manage oral leukoplakia? Br J Oral Maxillofac Surg. 2013;51(5):377-83; with permission.)

varying size (Fig. 4). The lesion occurs due to trauma, calculus, plaque, overhanging restoration, implantitis, pregnancy or hormonal change. Rapid growth of tissue alarms with malignant appearance. Lesion is predominantly seen as gingival swelling more commonly from labial side.¹⁷

Diagnosis and management Microscopic examination is usually done to rule out malignancy. The treatment options include surgical excision, curettage, cryotherapy, chemical and electric cauterization, lasers, and intralesional corticosteroids.¹⁸

Peripheral ossifying fibroma

Peripheral ossifying fibroma is one of the common reactive growths of soft tissues, and mineralized component of the lesion possibly originates from periosteum or



Fig. 4. Exophytic growth extending labiolingually through interdental space. (A) Labial view. (B) Lingual view. (From Deore GD, Gurav AN, Patil R, et al. Sclerotherapy: a novel bloodless approach to treat recurrent oral pyogenic granuloma associated with port-wine stain. *Ann Vas Surg.* 2014;28(6):1564; with permission.)

periodontal ligament. This lesion is considered to be a resultant of reparative response to intrabony hemorrhage and inflammation.¹⁹

Clinical features Peripheral ossifying fibroma is characterized by nodular, red/pink, pedunculated/sessile mass occurring at papilla of the gingiva with surface ulceration. The lesion mimics pyogenic granuloma and is predominantly seen among teenagers and women. Most of the cases were reported in incisor and canine region of maxillae.²⁰

Diagnosis Radiographic examination of the tissue shows minimal cortical plate expansion with or without severe root resorption. Microscopic examination is usually indicated to rule out pyogenic granuloma and peripheral giant cell granuloma.

Management Conservative surgical excision with curettage to prevent recurrence. Approximately 8% to 10% of cases show recurrence.

Peripheral giant cell granuloma

Peripheral giant cell granuloma is another reactive lesion that is categorized as tumor-like growth. Trauma is the most common cause for occurrence and seen predominantly in root canal-treated tooth. Radiographic changes are helpful in recognition.²¹

Clinical features Peripheral giant cell granuloma is a red or blue, nodular gingival or alveolar sessile/pedunculated mass that measures less than 2 cm. There is no specific age predilection and is frequently reported on women. Mandibular anterior is the most common region of occurrence.²²

Diagnosis and management Radiologically the lesion shows cupping resorption of alveolar bone. The cupping effect is reflection of base of the lesion. Microscopic examination is required to rule out peripheral ossifying fibroma and pyogenic granuloma. Conservative surgical excision with thorough oral prophylaxis, scaling, and root

planning are used for removal of source irritation that is, supra/sub-gingival calculus. Approximately 10% to 18% of cases showed recurrence.

Inflammatory papillary hyperplasia

This is a hyperplastic connective tissue growth that is seen among edentulous patients with ill-fitting partial or complete dentures. Ill-fitting denture, sharp edges, trauma, and poor denture hygiene are the most common causes.²³

Clinical features Papillary hyperplasia is characterized by asymptomatic papillary/fingerlike growth of tissue beneath denture (Fig. 5) predominantly seen in hard palate surface and remains asymptomatic until secondary infection.²⁴ The secondary infection can cause redness, soreness, pain, or burning sensation.

Diagnosis and management Clinical appearance of the lesion is diagnostic. The growth is usually removed surgically. Denture hygiene instructions must be followed to prevent recurrence.

Inflammatory fibrous hyperplasia

Inflammatory fibrous hyperplasia is a reactive growth resulting from poor denture hygiene practices and chronic injury/irritation from the denture. Concurrent occurrence of candidiasis may be observed.

Clinical features The lesion is characterized by painless, pink to red, nodular, circumscribed polypoid mass with bleeding tendency. The lesion may be symptomatic, that is, pain when associated with irritation or erythematous changes. Female predilection is observed. Hard palate is the most common site and occasionally seen over mandibular alveolar ridge. Few cases of inflammatory fibrous hyperplasia have been reported with candidiasis and human immunodeficiency virus infection.^{25,26}

Diagnosis and management Clinical appearance is diagnostic. Surgical removal of hyperplastic tissue and fabrication of new denture is advised. Individuals with candidiasis are managed with antifungal medications.

Tumors of Epithelial Tissue Origin

The tumors of this origin are characterized by the swelling that arises from surface epithelium. The recognition of tumors as epithelial versus mesenchymal




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Fig. 5. Inflammatory papillary hyperplasia of the palate. (From Infante-Cossio P, Martinez-de-Fuentes R, Torres-Carranza E, Gutierrez-Perez JL. Inflammatory papillary hyperplasia of the palate: treatment with carbon dioxide laser, followed by restoration with an implant-supported prosthesis. *Br J Oral Maxillofac Surg.* 2007;45(8):658–60. with permission.)

tissue origin is not just academic importance but to understand relevance of clinical, histologic behavior, aggressiveness and prognosis. The relevance of above-mentioned areas greatly varies in malignant neoplasms. Hence the topics covered here are discussed under 2 headings: benign and malignant lesions.

Benign epithelial tumors of oral mucosa

Squamous papilloma Squamous papilloma is a viral-induced benign proliferation of stratified squamous epithelium of oral tissue. The swelling is characterized by solitary occurrence of finger-like projection of surface epithelium. The occurrence of this lesion is presumably associated with low-risk type of HPV (types 6 and 11). The mode of transmission of HPV for inducing this lesion is not clear.²⁷

Clinical presentation Squamous papilloma is characterized by painless, slow-growing, fingerlike projection of surface epithelium that are either sessile or pedunculated with normal-appearing color or whitish change. Soft palate is the most commonly encountered site involvement; however, it can occur on tongue, lips, or buccal mucosa. No specific gender predominance is observed. The dimension of the lesion is often varying with their size being less than 0.5 mm to 3 cm. Lymph nodes are not affected in this condition.²⁸

Diagnosis Microscopic examination of biopsied tissue is helpful in achieving final diagnosis. The differential diagnosis of this condition can include verruca vulgaris when the lesion appears rougher and irregular surface tissue growth; condyloma accuminatum should be considered when the lesion is associated with whitish appearance and blunt surface projections.

Management Surgical excision of lesion along with base of the surface tissue will provide good prognosis. Recurrence of lesion is uncommon.

Verruca vulgaris Verruca vulgaris is characterized by white papillary growth of surface epithelium that is either sessile or pedunculated. The condition is predominantly observed in cutaneous surfaces, whereas in oral cavity it is observed in vermilion border of lip. Because of higher predilection on epidermis (skin) the condition is often noted as common/cutaneous wart. The lesion is often associated with low-risk type of HPV (HPV 2, 4, 6, and 40). The mode of transmission of HPV for inducing this lesion is autoinoculation of virus from affected skin or mucous membrane.²⁹

Clinical presentation Verruca vulgaris is characterized by painless, white papillary growth of surface epithelium that is either sessile or pedunculated. Although it is uncommon in oral mucosal surfaces, the cases have been reported on labial mucosa and anterior tongue. The lesion appears in small size and grows rapidly to attain its maximum size. Multiple lesions can occur in oral cavity.

Diagnosis Microscopic examination of biopsied tissue is necessary for arriving final diagnosis. Differential diagnosis of squamous papilloma may be considered when the lesion has less white color.

Management Surgical excision of the lesion should include base of the lesion. Very few cases have reported with recurrence.

Focal melanosis Benign pigmented lesion is characterized by well-circumscribed, painless, flat, brown mucosal discoloration with increase in pigmentation possibly due to increase in number of melanocytes at basal cell layer of epithelium. Focal

melanosis is frequently observed on lips. The lesion is managed by surgical excision specially to rule out malignant melanoma.

Clinical presentation Focal melanosis is a painless, slow-growing pigmented macule that is a predominantly brown change of oral mucosa on the affected region. However, cases with blue, brown, or black have been reported. Female predilection is observed and can be seen in any age group. Although lip is the most commonly encountered site, the lesion can be seen on gingiva, buccal mucosa, or palate (**Fig. 6**).^{30,31}

Diagnosis Although clinical appearance of the lesion is adequate for diagnosis, incisional biopsy should be done to rule out malignant melanoma. Hence microscopic examination of biopsied tissue is necessary for final diagnosis.

Management The lesion undergoes spontaneous resolution after incisional biopsy; hence surgical management is not required. Surgery may be planned based on aesthetic needs of patients. Lesion has less tendency for recurrence. Reports mentioned that focal melanosis has no malignant transformation potential.³²

Acquired melanocytic nevus This is a common malformation of the skin and mucosa and generally termed as “mole.” Acquired melanocytic nevus is a benign, localized proliferation of cells from neural crest called as nevus cells. Acquired melanocytic nevus is the most common benign cutaneous tumor but uncommon intraoral lesion.³³

Clinical presentation Acquired melanocytic nevus is a most common pigmentation that occurs in cutaneous surface, frequently reported in childhood with slight female predilection. Intraoral occurrence is uncommon, mostly observed in palate, gingiva, tongue, or other areas of oral mucosa.³⁴

Diagnosis Clinical appearance of the lesion is characteristic and does not require biopsy and microscopic examination unless if acquired melanocytic nevus shows secondary characters such as rapid growth and ulceration.




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Fig. 6. Melanosis over palate and buccal mucosa. (From Ho AW, Sato R, Ramsdell A. JAAD grand rounds quiz. A case of oral melanosis. J Am Acad Dermatol. 2014;71(5):1030–3; with permission.)

Management Treatment is usually not required unless patient has aesthetic demands. When treatment is planned, conservative surgical excision is the treatment of choice. Recurrence is unlikely to occur and tends to regress with age.

Keratoacanthoma Keratoacanthoma is a relatively common benign epithelial proliferation of pilosebaceous glands with a self-healing potential. The lesion is considered as low grade and a variant of squamous cell carcinoma. The exact cause for appearance of this lesion is unclear but sunlight damage and HPV are possible reasons. Intraoral occurrence of this lesion is rare.³⁵

Clinical presentation Keratoacanthoma is characterized by painless, slow-growing, well-demarcated, firm, sessile, dome-shaped swelling with keratin plugging in center. The central plug could be yellowish, brown, or black. A predominant number of cases were reported on lips. The lesion commonly occurs on a sun-exposed area and reported more frequently among midaged men. The progression of this lesion is divided into 3 phases: (1) growth, (2) stationary, and (3) involution phase. The lesion has a tendency for resolution and hence it is termed as “self-healing carcinoma.” Regression usually occurs in 6 to 12 months from the time of onset.

Diagnosis Age, location, and clinical presentation are helpful in achieving provisional clinical diagnosis. However, keratoacanthoma must be differentiated with OSCC. Hence biopsy is usually required to rule out malignancy. Microscopic examination of biopsied tissue is necessary for arriving final diagnosis.

Management Excisional biopsy is usually indicated due to size of lesion. The lesion has a tendency for spontaneous regression and rarely recurs.

Malignant epithelial tumors of oral mucosa

Oral squamous cell carcinoma OSCC is the malignant neoplasm of oral epithelium. It is the most common oral cancer arising from oral mucosa. Smoking and smokeless tobacco are the most common causes for OSCC. Tongue and lips are the frequently affected areas. It can also affect the lips, cheek mucosa, tongue, palate or teeth-bearing alveolar segment, and gums.³⁶ Microscopic examination of the affected region is the key for diagnosis. Staging and grading are the most important steps in assessing the cancer. OSCC requires both surgery and radiotherapy.³⁷

Clinical presentation The clinical presentation of OSCC is varying and can present as simple as white patch or can present with complex features such as swelling, ulcer, or ulceroproliferative swelling. Tobacco smoking, smokeless tobacco, sunlight damage, alcohol, betel quid, iron deficiency, radiation, phenolic agents, syphilis, vitamin A deficiency, oral candidiasis, oncogenic viruses (HPV), immunosuppression, and chronic injury are considered as risk factors for developing OSCC.³⁸ The lesion is predominantly characterized by exophytic and ulcerated growth. Ulceration varies in size with indurated margin and fixed to adjacent soft tissues. Metastasis of the malignant lesion can be observed in regional lymph nodes. Palpable regional lymph nodes may be identified with one or few of the characters: increase in size, number, and hard in consistency and immobile while palpation.³⁹

Diagnosis OSCC is a clinically recognizable condition because it affects surface mucosa. Although the lesion is affecting oral mucosa, it can perforate bone and manifest in osseous tissue. Hence changes in bone can be observed in radiographs. Radiologically, the lesion shows multilocular appearance with radiolucent changes. Small-sized multiloculation shows honeycomb appearance, whereas large-sized

multiloculation shows soap bubble appearance. Microscopic examination of incisional biopsy is mandatory for arriving final diagnosis. Histopathologic examination is helpful in grading the malignancy into well, moderate, or poorly differentiated OSCC based on dysplastic features, keratin pearls, and parental cell resemblance.

Management OSCC must be treated to achieve favorable prognosis. Cases diagnosed with OSCC are managed both surgically and by radiation therapy. OSCC has a tendency to recur.⁴⁰ Depending on the stage and grade of OSCC, neck dissection procedure is performed. Patient requires routine postoperative follow-up to monitor and prevent recurrence. Referral of the patient to Oncosurgeons and Faciomaxillary Surgeons at right time is very important in the patient management.

Verrucous carcinoma Verrucous carcinoma is a low-grade variant of squamous cell carcinoma. This condition is often associated with the use of smokeless tobacco, chewing tobacco, or snuff users. However, cases have been reported among nonusers of above-stated tobacco products. The lesion has least tendency to metastasis and hence surgical excision without neck dissection is a choice.

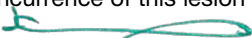
Clinical presentation Verrucous carcinoma is characterized by diffuse, painless, well-demarcated, exophytic proliferative growth of oral mucosa predominantly observed on vestibular space and midaged men. The lesion shows papillary or verruciform surface projections giving rough surface texture and appears white due to excessive keratin deposits. Gingiva, buccal mucosa, hard palate, and tongue are also reported as sites of occurrence. Site of verrucous carcinoma in oral cavity corresponds to the site of tobacco placement. Long-standing untreated cases of verrucous carcinoma can destroy underlying connective tissue structures such as cartilage, bone, muscle, or salivary glands. Lymph node enlargement is secondary to inflammatory changes and not due to metastatic deposits.⁴¹

Diagnosis Clinical appearance of the lesion is characteristic for achieving provisional diagnosis. However, microscopic examination is necessary for arriving final diagnosis. Microscopic examination of biopsied tissue is necessary to differentiate between verrucous carcinoma and verrucoid variant of oral squamous carcinoma. Differential diagnosis of verrucous carcinoma includes papillary hyperplasia and squamous cell carcinoma.

Management Verrucous carcinoma is managed through wide surgical excision. The lesion has a tendency for recurrence. Chemotherapy is used as a temporary management of verrucous carcinoma for inoperable cases.

Basal cell carcinoma Basal cell carcinoma (BCC) is a common malignancy of skin that is slow-growing, locally invasive neoplasm originating from basal cell layer of skin. BCC occurs on sun-exposed surface of the face, skin, and scalp of elderly persons. The lesion is managed through surgical procedures.

Clinical presentation BCC is characterized by solitary ulcer or nodular growth. The lesion may seem as solitary or multiple. Early lesions may show a pigmentation or nodular appearance. The ulcer tends to show rolled-out and indurated margins. The ulcer is locally invasive and shows furrowing appearance, hence the name "rodent ulcer." The lesion shows predilection for middle-aged men. This malignant neoplasm has a unique aggressive behavior but does not have metastatic property. Few cases are associated with nevoid BCC syndrome where the concurrence of this lesion with odontogenic keratocysts.⁴²



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Diagnosis Nonulcerated BCC must be differentiated from nevi, sebaceous cyst, or mesenchymal dermal tumors. Ulcerated lesions must be differentiated from squamous cell carcinoma. Hence microscopic examination of biopsied tissue is usually indicated to rule out differential diagnosis and arriving at a definitive diagnosis.

Management Small lesions are treated by surgical excision with laser or electrodesiccation and curettage. Large lesions require wide excision, and radiation/chemotherapy is required for aggressive lesions. During surgical procedure, normal-appearing adjacent mucosa is also excised for about 1 cm to avoid recurrences. Because of local invasion property of BCC, microscopic examination of excised tissue is important for evaluation of cancer-free cells at surgical margins.

Melanoma Malignant melanoma is a neoplasm of melanocytes that has unpredictable biological behavior. Melanoma is the third common malignancy of skin and occurs where melanocytes are present. Intraoral melanomas are reported. Ultraviolet radiation is a major risk factor for initiation of this malignancy. Surgical excision is the mainstay of treatment, and prognosis of melanoma is poor.

Clinical presentation Melanoma is characterized by pigmented (black, bluish black, or dark brown) lesions that begin as a focal area of macule. The lesion is rapidly progressive causing diffuse pigmentation with or without induration. Ulcerations may or may not be seen. Cases have been reported with nonpigmented appearance, which mimics inflammatory lesion or other benign neoplasm. Sun exposure, ultraviolet radiation, and xeroderma pigmentosum are the known risk factors.⁴²

Diagnosis Differential diagnosis of pigmented lesions is important due to clinicopathologic similarity with other conditions. Differential diagnosis of melanoma includes traumatized nevus, pigmented variant of BCC, blue nevus, lentigo, traumatic hematoma, and pigmented actinic keratosis. The ABCDE diagnostic criteria for melanoma are instrumental in achieving a clinical diagnosis⁴³ (**Box 2**). Microscopic examination is indicated to rule out other conditions and arriving at a definitive diagnosis.

Management Malignant melanomas are treated by surgical procedure. Chemotherapy, radiotherapy, and immunotherapy have been used in the management of melanoma. Survival rate for melanomas is very low.

Connective Tissue Tumors

Benign connective tissue tumors of oral mucosa

Fibroma Fibroma is a benign proliferation of fibroblast resulting from trauma or irritation. Trauma from occlusal forces and irritation from dental materials are the most common reason for invitation of fibroma.

Box 2

ABCDE diagnostic criteria for melanoma

- A—Asymmetry (one-half of the lesion does not symmetrically match with another half)
- B—Border irregularity (Borders of melanoma are blurred, notched, or ragged)
- C—Color irregularity (pigmentation shows varied appearance, ie, brown, black, tan, red, white, and blue; all can be seen in the lesion)
- D—Diameter (greatest diameter of the lesion is larger than 6 mm)
- E—Elevation (surface of the lesion is elevated)



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Clinical features Fibroma is characterized by painless, slow-growing nodular mass that is either sessile or pedunculated.⁴⁴ The size of the lesion is usually less than 1 or 2 cm. The surface may be ulcerated due to trauma. However, surface ulceration is not a consistent feature. The lesion has no specific location predominance but is seen frequently at the line of occlusion, that is, buccal mucosa or at the area of chronic trauma.⁴⁵

Diagnosis and Management Clinical presentation is generally diagnostic. Conservative surgical excision is the treatment of choice with elimination of causative factor. Recurrences are uncommon.

Lipoma Lipoma is a benign tumor of adipose tissue and predominantly observed in trunk and proximities. Intraoral lipomas were reported in buccal mucosa. The exact cause remains unclear; however, few studies reported genetic influence in their occurrence.

Clinical features Lipoma is characterized by painless, slow-growing, smooth surface, sessile or pedunculated lobular mass. Superficial lipoma appears in yellow color, lipoma in deeper tissue appears normal mucosal color. The lesion is predominantly reported in adult population.⁴⁶

Diagnosis and management Clinical appearance is generally diagnostic. Conservative surgical excision is the treatment of choice. Biopsied tissue usually floats in the fixative solution due to density of adipose tissue. Recurrences are uncommon.

Neurofibroma Neurofibroma is a benign peripheral nerve neoplasm that consists of 2 elements namely Schwann cells and fibroblasts. Neurofibromas can occur as multiple or solitary lesions. Intraoral lesions are predominantly solitary lesions.

Clinical features Neurofibroma is characterized by painless, slow-growing, small, and soft to firm nodules. Intraoral neurofibromas are often rare. However, when it occurs it is predominantly seen over tongue or buccal mucosa as solitary lesions. Intraosseous neurofibromas arise from central portion of bone.⁴⁷

Diagnosis and management Radiological appearance of intraosseous neurofibromas are either well demarcated or poorly demarcated and uni- or multilocular. Surgical excision is the treatment of choice. Recurrence is not common. Individuals with solitary neurofibromas should be evaluated periodically for the multiple lesions.

Schwannoma (neurilemmoma) Neurilemmoma is a benign encapsulated neoplasm of nerve sheath that arises from Schwann cell origin. Approximately 25% to 48% neurilemmomas are from head and neck region.

Clinical features Neurilemmoma is a painless, slow-growing, encapsulated tumor that typically arises in association with nerve trunk.⁴⁸ Size of the swelling may range from few millimeters to centimeters. It is most commonly seen in young and middle-aged adults. Tongue is the most common site (**Fig. 7**).⁴⁹ Intraoral neurilemmomas are commonly reported in posterior mandible. Pain and paresthesia are not unusual for intraoral lesions.

Diagnosis and management Intraoral neurilemmomas radiologically appear as either unilocular or multilocular radiolucencies. Solitary lesions are managed by surgical excision. Recurrence is uncommon.⁵⁰

Hemangioma Hemangioma is a developmental vascular anomaly or hamartoma rather than benign neoplasm. It occurs due to rapid growth of endothelial cells lining blood vessels and is frequently reported on infants and manifest within the first month of life.



Fig. 7. Exophytic growth on the side lateral surface of the tongue. (From López-Jornet P, Bermejo-Fenoll A. Neurilemmoma of the tongue. *Oral Oncol Extra* 2005;41(7):154–7; with permission.)

Clinical features Hemangioma is characterized by small, raised, brick red areas with firm and rubbery consistency. Superficial hemangiomas appear mucosal color, whereas deep lesions appear bluish. Approximately 4% to 5% of children younger than 1 year are affected. Female predilection is reported. They are frequently seen on white than other ethnic groups. Eighty percent of hemangiomas occur as solitary lesions, and 20% cases have concurrent occurrence with other tumors. Syndromes associated with hemangioma are given in **Box 3**.⁴³ Four variants of hemangiomas are capillary, cavernous, lobular, and arterial. Hemangiomas tend to regress.⁵¹

Diagnosis and management Clinical appearance is generally diagnostic. Management is employed based on aesthetic or functional needs of patient. Treatment options of hemangioma include surgery, radiotherapy, injection of sclerosing agents, cryotherapy, carbondioxide snow, and intralesional corticosteroids.⁵²

Lymphangioma Lymphangioma is a benign hamartoma of lymphatic vessel than a true tumor. It arises due to developmental disturbance causing sequestration of lymphatic vessels. It is frequently in head and neck region. Intraoral lesions are reported on tongue.

Clinical features Lymphangiomas are predominantly reported on tongue. The tongue is enlarged, that is, macroglossia. Superficial lymphangioma is characterized by pebbled surface with numerous translucent vesicles of varying size. Men are commonly affected.⁵³ Three variants of lymphangioma are macro, microcystic, and mixed type (**Box 4**).

Box 3

Syndromes associated with hemangiomas

Rendu-Osler-Weber syndrome
 Struge-Weber-Dimitri syndrome
 Kasabach-Merritt syndrome
 Maffucci syndrome
 Von Hippel-Lindau syndrome
 Klippel-Trenaunay-Weber syndrome

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Box 4**Variants of lymphangioma**

Macrocytic lymphangioma: cysticlike spaces more than 2 cm (eg, Cystic hygroma)

Microcytic lymphangioma: smaller vascular channels less than 2 cm in size

Mixed lymphangioma: combination of both macrocytic and microcytic

Diagnosis and management Clinical appearance is generally diagnostic. Management is employed based on aesthetic or functional needs of patient. Recurrences are common in lymphangioma. Treatment options include sclerosant, sodium tetracycline sulfate and bleomycin. Head and neck lymphangiomas have a potential to cause obstruction to respiratory channel leading to death.

Osteoma Osteoma is a benign neoplasm of mature compact or cancellous bone. Cases have been reported on craniofacial and skeletal bones. Osteomas may arise from periosteal, endosteal, or extra-skeletal region. Osteomas result from injury, inflammation, or hamartomatous growth. Multiple osteomas are associated with Gardner syndrome.⁵⁴

Clinical features Osteoma is characterized by slow-growing, well-defined, hard and asymptomatic swelling. Facial deformities are seen in unusually large osteomas. They are predominantly seen in adults. Condyle and body of mandible are common locations; other areas are angle of mandible, coronoid process, and ramus region. Lingual surface adjacent to premolars and molars are frequently observed with osteomas.

Diagnosis and management Radiologically osteoma appeared as well-defined and uniform-appearing radiopaque mass. Small and asymptomatic osteomas do not require any treatment, but periodic watch is important. Surgery is preferred when functional and aesthetic needs are important. Mouth opening and movement may be restricted due to osteomas at condyle or coronoid process.

Fibrosarcoma Fibrosarcoma is a malignant neoplasm of fibroblasts. Fibrosarcoma frequently occurs in extremities, and approximately 10% cases have been reported in head and neck region.

Clinical features Fibrosarcoma is characterized by slow-growing mass but reaches considerable size to cause disfigurement and pain. This lesion is common among young adults and children. Paranasal sinuses are common site in head and neck region. Intraoral lesions are uncommon.⁵⁵

Diagnosis and management Microscopic examination of biopsied tissue is essential for final diagnosis. Wide surgical excision is the treatment of choice. Recurrence is seen in almost 50% of cases. The survival rate is approximately 5 years.

Osteosarcoma Osteosarcoma is a malignant neoplasm of mesenchymal with the ability to produce mineralized tissue. Osteosarcomas are the second common malignancy to arise from bone. Cause of osteosarcoma remains unclear; however, strong association was reported on genetics, radiation exposure, alkylating agents, and Paget disease. Distal femoral and proximal tibia metaphyses are commonly affected. Approximately 6% of cases constitute to gnathic osteosarcomas with predilection to mandible.

Table 4	
Criteria for suspecting child abuse	
	Yes No
Physical findings: are there:	
Fresh bruises? Unusual locations or shapes?	
Old scars? Unusual locations or shapes?	
Past or current bumps? Unusual locations or shapes?	
Signs of rectal, genital, or oral injury or infection?	
Medical experience:	
Is abuse or neglect suggested by:	
Current medical problems?	
Prior medical problems?	
Prior emergency visits—ingestions or trauma?	
Prior hospitalizations?	
Prior surgical interventions?	
Current or past venereal disease or pregnancy?	
Poor compliance with prior medical care or treatment?	
Incomplete immunizations for age?	
Poor mental or physical growth and development for age?	
Behavioral abnormalities: is there evidence of:	
Withdrawal or hyperactivity?	
Over compliance with physical examination?	
"Compliant posturing"?	
Phobias?	
Sleeping problems?	
Recent onset of enuresis or encopresis?	
"Sexualized play"?	
Excessive interest in genitalia?	
Psychosocial conditions: is there evidence of:	
Disturbed parent-child interaction?	
Violent interaction between parents?	
Violent interaction between siblings?	
Violent interaction with friends and relatives?	
Parents being abused as children?	
Parents being victims of sexual abuse?	
Extra stresses on the family?	
Marital discord?	
Unemployment?	
Alcoholism?	
Substance abuse?	
Recent death or illness in the family?	
Inappropriate custodial care of the child?	
Daytime?	
After school?	
Evening?	



(continued on next page)

Table 4
(continued)

	Yes	No
Nights?		
Weekends?		
Inappropriate responsibilities for a child?		
Heavy chores such as cooking and housekeeping?		
Care of siblings?		
Family isolation?		
Lack of telephone?		
Lack of supportive relatives, friends, or neighbors to whom they can turn in a crisis situation?		
Previous referrals for abuse or neglect?		

From Schachner L, Hankin DE. Assessing child abuse in childhood condylomaacuminatum. J Am Acad Dermatol. 1985;12(1 Pt 1):157-60; with permission.

Clinical features Osteosarcomas are seen in both young and elderly age range. Osteosarcoma is characterized by swelling, pain, loosening of teeth, nasal obstruction, or paresthesia. Osteosarcomas of maxillae may be seen on alveolar ridge, sinus floor, palate.⁵⁶

Diagnosis and management Radiologically osteosarcomas are recognized by radiopaque, mixed radiolucency with ill-defined borders. The elevation of the periosteum may give sun-ray or sun-burst appearance. Root resorption with symmetric generalized widening of the periodontal ligament space is a diagnostic clue for osteosarcoma. Management includes surgery, chemotherapy, and radiotherapy. Approximately 60% to 80% cases showed 5-year survival rate. Prognosis is somewhat better in gnathic osteosarcomas. Metastasis is frequently reported.

Oral Lesions Associated with Child Abuse

Child abuse is a one of the major social and health problems and often results from physical, sexual, and emotional abuses or neglect. It affects physical, cognitive, or emotion growth of child that may extend into adulthood. Child abuse is a wide topic for discussion; however, the context of explanation in this article is to recognize oral lesions that serve as possible markers for child abuse. Oral lesions associated with child abuse are herpes simplex, Epstein-Barr virus, cytomegalovirus, gonorrhoea, molluscum contagiosum, and condyloma accuminatum. The discussion of condyloma accuminatum is made here due to linkage with benign neoplasm of oral cavity.

Condyloma accuminatum

Condyloma accuminatum is a benign epithelial neoplasm that is associated with HPV types 2, 6, 11, 53, and 54 often seen predominantly in genital, perianal region. HPV types 16, 18, and 31 are associated with anal lesions. The lesion can be seen in oral and laryngeal mucosa. The lesion is a sexually transmitted disease with development of lesion at the area of sexual contact.⁵⁷ Lawrence and colleagues stated that "Childhood condyloma acuminatum may often, but not always, be a manifestation of sexual abuse." They had provided a check-list criterion for suspecting child abuse, which cover 4 main areas: physical findings, medical

experience, behavioral abnormalities, and psychosocial conditions. Any “yes” response to the checklist (**Table 4**) mandates a Child Protective Services consultation.⁵⁸ Condyloma accuminatum is diagnosed in young adults and frequently observed in labial mucosa, soft palate, and labial frenum.⁴³ Condyloma accuminatum is characterized by painless, slow-growing, well-demarcated, nontender, sessile, pink-colored exophytic growth with short and blunt surface projects. The size of the lesion varies between 0.5 and 2.0 cm. Microscopic examination is required for arriving final diagnosis. Immunohistochemical analysis of biopsied tissue is necessary for identification of HPV types in the pathology specimen. The lesion is managed by conservative surgical excision. The lesions associated with HPV types 16 and 18 have a potential for malignant transformation to OSCC.⁵⁹

SUMMARY

General dentist has the training to identify a wide variety of oral conditions. Unfortunately, diagnostic skills are likely to fade due to uncommon prevalence of these conditions. The important step in approaching patients with these conditions is to provide critical insight to signs and symptoms presented. Comprehensive clinical examination will enable the dentist to identify whether the condition is reactive, benign, or malignant oral disease. The dentist should take a special initiative while handling oral disease cases for diagnosis and management. In doubt, or when the lesions are challenging, the patient must be referred timely to oral medicine, oral pathologists, or oral surgeon for further evaluation and management.

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Development and Evaluation of a New Oral Health Literacy Instrument among Telugu Speaking Population: The Indian Oral Health Literacy Measure

Abstract

Purpose: Oral health literacy (OHL) is a relatively unexplored construct in the Indian context. Most of the few previous studies estimating OHL among different populations of India used instruments that were either previously validated in a different country or not tested for psychometric properties. With this background, the objective of this study was to develop an Indian oral health literacy measure and to evaluate the psychometric properties of the instrument among Telugu speaking population of Andhra Pradesh (IOHLM-T). **Materials and Methods:** The initial version of the instrument after evaluated for face and content validity was tested for psychometric properties among 200 adult patients visiting the outpatient department of SIBAR Institute of Dental Sciences. Predictive validity of the instrument was checked by the association between IOHLM-T score and oral health impact profile (OHIP-14), decayed-missing-filled teeth (DMFT) scores. Convergent validity was tested by assessing the correlation between IOHLM-T score and rapid estimate of adult literacy in dentistry (REALD-30) score. **Statistical Analysis:** SPSS version 20 software was used to analyse the data. Independent samples *t*-test, Pearson's correlation, one-way ANOVA and stepwise multiple linear regression were done to analyse the data. **Results:** IOHLM-T demonstrated good internal consistency reliability (Cronbach's alpha 0.75), convergent validity ($r = 0.34$ between Reald-30 and IOHLM-T scores) and predictive validity (significant negative correlation of IOHLM-T with OHIP-14 and DMFT scores). **Conclusion:** IOHLM-T demonstrates good face validity, content validity, predictive validity, convergent validity and internal consistency reliability and thus can be used among different populations in India after translation to the corresponding languages and evaluation of psychometric properties.

Keywords: Health literacy, India, oral health, oral health literacy estimation, psychometrics

Introduction

Literacy is a multidimensional expression and there is no universal definition for literacy which encapsulates all its dimensions. Different countries have different fundamental understanding of literacy. In India, those who can read and write in any of the languages are considered to be literate.^[1] Literacy is among the basic indicators of the level of development achieved by a society. The term 'health literacy' was first used in 1974 by Professor Scott k Simonds in his paper 'Health Education as Social Policy'. According to the World Health Organisation (WHO), health literacy has been defined as 'the cognitive and social skills which determine the motivation and ability of individuals to gain access

to, understand, and use information in ways which promote and maintain good health'.^[2] This definition is distinct from others in that it focuses not only on cognitive skills but also incorporates social skills which are required to implement decisions into practice, besides referring to both motivation and ability.^[3] Limited health literacy compromises an individual's ability to access health care services and lead a healthy life. With regard to oral health, there is substantial evidence that the oral health status of Indian population is poor, and the impact of oral health problems on the quality of life of an individual is well known.^[4] However, the utilisation of dental services continues to be poor.^[5,6] Though there are various factors influencing utilisation of dental services, inadequate oral health literacy is discussed

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as an important factor contributing to low utilisation of dental services and consequently poor oral health.^[7]

Oral health literacy (OHL) is a relatively unexplored construct in the Indian context. Most of the few previous studies estimating OHL among different populations of India used instruments which were either previously validated in a different country or not tested for psychometric properties.^[8-10] The solitary attempt to develop an OHL questionnaire for Indian population in 2011 could not establish the psychometric properties of the instrument and was not subsequently tested or used.^[11] Another attempt in this direction was made in 2016 through the Hindi version of oral health literacy adult questionnaire (OHL-AQ), an instrument originally developed in Tehran.^[12] While it is more practical to adopt previously validated questionnaires from different languages in diverse areas of scientific research, this practice raises questions about a multitude of facets relating to cross-cultural equivalence.^[13] With this background, the objective of this study is to develop an OHL instrument suitable for Indian population and to evaluate the psychometric properties of the instrument among Telugu speaking population of Andhra Pradesh, subsequently identified in this article as Indian oral health literacy measure in Telugu (IOHLM-T).

Materials and Methods

This study, to develop an OHL instrument in the context of Indian population, involved building consensus on the conceptual understanding of OHL, development of an item pool and evaluation of psychometric properties of the instrument. The study was conducted between March, 2018 and August, 2018. The methodology adopted in the process of developing IOHLM-T was described under the following four sections.

Development of item pool

All the previously validated OHL instruments were accessed and reviewed before the formulation of the instrument. Twenty-one OHL instruments were identified from electronic literature search.^[14-33] After a thorough review of the available instruments and other relevant articles, the conceptual framework proposed by the Institute of Medicine's health literacy expert panel was considered relevant and the components of health literacy enumerated in the framework were reviewed.^[34] Following many rounds of consensus building, it was agreed upon by the investigators to include the domains of reading comprehension, numeracy, knowledge and the ability to communicate and navigate the health care system. Once the above-mentioned domains were identified, the next task was to locate the potential topics with regard to these domains from where the questions could be formulated.

Reading comprehension

To select the topics for assessing reading comprehension, an oral health education video developed by All India

Institute of Medical Sciences (AIIMS) and Ministry of Health and Family Welfare, Government of India as a part of the National Oral Health Care Program (NOHCP) was regarded as a credible source of information by a group of experts, which was directed towards the oral health promotion of the citizens of the nation.^[35] The principal investigator watched the video, which lasts for 32 min, several times in both the languages before the notes were taken down in English. Information relating to tooth morphology, deciduous and permanent dentition and dental caries was selected to be included in the reading comprehension section of the instrument. Two reading comprehension passages as described above were included in this section with 14 items in the form of multiple-choice questions.

Numeracy skills

Two health numeracy questionnaires, namely, general health numeracy test (GHNT) and numeracy understanding in medicine instrument (NUMI) were reviewed along with OHL instruments.^[36,37] Scenarios from these questionnaires were adopted in the formulation of the numeracy-related items. Six items were included in this section designed with differing degrees of difficulty to ably distinguish between those with limited numeracy skills and good numeracy skills.

Oral Health Knowledge

In this sub-section, six items regarding conceptual knowledge were adopted from the literature.^[38]

Critical and communicative literacy

After a thorough review of literature relating to communicative and critical health literacy, it was decided to formulate questions based on the previously validated communicative and critical health literacy instrument among diabetic patients.^[39] For the items on critical and communicative literacy, responses 'often' and 'very often' were considered as reflective of the participants' ability to gain access to and critically evaluate oral health information.

Thus, the initial version of the instrument consisted of 32 items with 14 items from reading comprehension, 6 items each from numerical knowledge, non-numeric conceptual knowledge and communicative, critical oral health literacy. The initial version of the instrument was translated to the regional language Telugu (IOHLM-T) by one of the investigators, which was again back-translated to English by three experts. Minor changes were made in IOHLM-T following consultation with the back translators.

Evaluation of psychometric properties of IOHLM-T

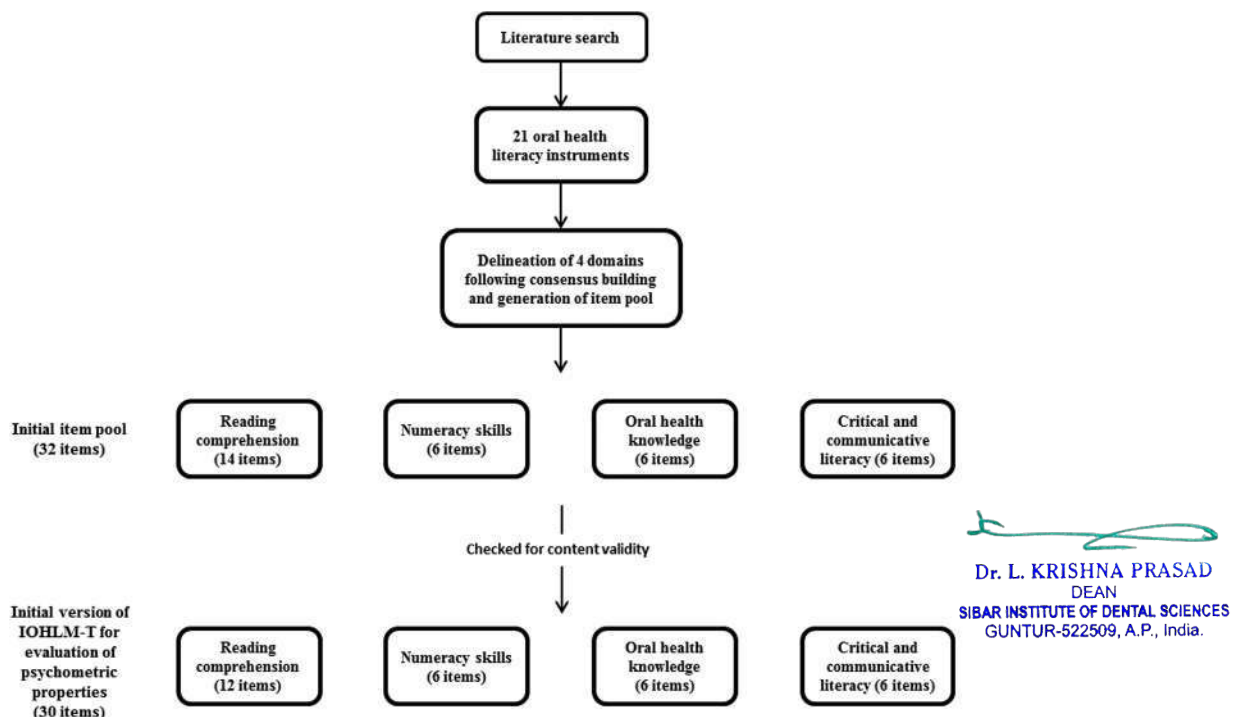
The initial questionnaire in the local language Telugu was administered to 20 participants to check for both feasibility and face validity of the instrument. Few changes

in the wording of the items were made as necessary. The questionnaire was given to 6 experts to check for content validity. Content validity index (CVI) was used to assess the content validity of the questionnaire.^[40] 30 questions were rated as ‘quite relevant’ or ‘highly relevant’ by the raters, and the item level content validity index (I-CVI) was found to be >0.83 after deletion of two items from the reading comprehension section. The scale level content validity index average method (S-CVI/AVG) and universal agreement (S-CVI/UA) were found to be 0.994 and 0.964, respectively. The flow chart depicting the development of IOHLM-T is given in Figure 1.

IOHLM-T tested for face and content validity (Annexure 1) was administered to a convenience sample of 200 patients attending the outpatient department of SIBAR Institute of Dental Sciences, the only postgraduate teaching dental institution in Guntur district, Andhra Pradesh. The study protocol received approval from the Institutional ethics committee of SIBAR Institute of Dental Sciences (08/IEC/SIBAR/2014). Inclusion criteria used in this study were older than 18 years of age and the ability to read and write in Telugu. Patients with acute oral health problems requiring immediate care, those with disabilities, mental illnesses were excluded. 200 subjects satisfied the eligibility criteria and demonstrated willingness to participate in the study from the approached 310 patients. The purpose of the study was explained to the participants in detail and informed consent was obtained prior to their participation in the study. Along with IOHLM-T, the participants were given a self-administered questionnaire

seeking the information relating to age, gender, educational qualification, per capita monthly family income and previous dental visits. Socioeconomic status of the participants was determined according to BG Prasad scale for socioeconomic classification.^[41] All the participants were assessed for oral health-related quality of life using OHIP-14.^[42] The prevalence (number of participants giving a response of ‘often’ or ‘very often’ to at least one of the items on the scale), extent (number of items on the scale for which the participant has responded with ‘often’ or ‘very often’) and severity (the composite score obtained by the participant on OHIP-14 scale) of OHIP were calculated. Clinical examination of the participants was carried out in the comprehensive dental clinics of the teaching dental institution. Coronal caries experience was assessed using DMFT Index (WHO 1997 modification).^[43] A single trained and calibrated dentist conducted the clinical oral examination [95% CI for intra-class correlation coefficient (ICC) estimate 0.86–0.93].

Predictive validity of the questionnaire was assessed by juxtaposing the score obtained with OHIP-14 and DMFT scores. The hypothesis was that participants with low OHL scores would have poor oral health-related quality of life and high caries experience. An attempt was made to establish the convergent validity of the questionnaire, a sub-type of construct validity, by comparing the OHL score with REALD 30^[14] scores among 98 participants from the study sample of 200 who can understand both English and the regional language Telugu.





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Figure 1: Flowchart depicting the development of initial version of IOHLM-T for evaluation of psychometric properties

Scoring of IOHLM-T

In order to make the scale comparable with the previously validated OHL measures and to facilitate the determination of cut off scores in the categorisation of OHL, it was decided to express the final value as a weighted score ranging from 0–100. With a notion that reading comprehension, numeracy, knowledge and critical, communicative abilities are equally contributing to OHL, equal weight (25%) was assigned to each of the four domains of the OHL scale. Within each domain, the maximum possible score of 25 was divided by the total number of questions in that domain to derive the weight to be assigned for an individual question. The final OHL score was calculated as follows:

$$\text{IOHLM-T score} = [2.083(\theta_{RC}) + 4.167(\theta_{NS} + \theta_{OHK} + \theta_{CC})]$$

where θ denotes the number of correct responses, RC denotes reading comprehension, NS denotes numeracy skills, OHK denotes oral health knowledge and CC denotes critical and communicative literacy. The cut-off points suggested for the categorisation of subjects into three levels of OHL in the previously validated OHL measures[17] were employed in the present study: inadequate OHL (0–59); marginal OHL (60–74); adequate OHL (75–100).

Statistical analysis

SPSS Version 20 software (IBM SPSS statistics for Windows version 20, Armonk, NY, USA) was used for data analysis. Independent samples *t*-test, one-way ANOVA and Pearson’s correlation tests were used to analyse the data, owing to the normal distribution of OHL scores (Kolmogorov–Smirnov test; $P = 0.083$). Stepwise multiple linear regression analysis was performed with IOHLM-T score as the dependent variable to understand the contributions of each of the predictor variables in explaining the variance in IOHLM-T scores.

Results

The mean age of the study participants was 34.37 ± 13.74 years, with a range of 19 to 70 years. Majority

of the study participants were male, completed primary education and belong to upper middle socioeconomic status. The average time taken in the present study for completion of IOHLM-T was 18 min (17.88 ± 4.6). The mean IOHLM-T score among the study sample was 57.85 with the highest mean score in the sub-section of oral health knowledge. Table 1 shows the mean scores obtained in each of the sub-sections of IOHLM-T and the mean composite score. 65.5% of the participants had inadequate (IOHLM-T 0–59), 20% had marginal (IOHLM-T 60–74) and 14.5% had adequate OHL (IOHLM-T ≥ 75).

The mean caries experience of the study population was 2.98. 169 (84.5%) participants reported oral health problems to be negatively influencing their quality of life often or very often. The mean score for OHIP extent and severity was 2.56 and 14.10, respectively [Table 1]. Table 2 shows the association of IOHLM-T score with different categorical predictor variables at bivariate level. Educational background of the study participants was found to be significantly associated with IOHLM-T score. The scores obtained by participants with high school education or less were found to be significantly lesser across all sub-sections of IOHLM-T compared to their counterparts. While there were no differences in OHL between different socioeconomic strata, participants with dental visit within the last 24 months demonstrated higher IOHLM-T scores. There was no difference in the IOHLM-T scores based on age and sex.

Predictive validity of the IOHLM-T was established through the following observations. There was a significant difference in OHL based on the OHIP prevalence. Participants reporting oral health as significantly influencing their quality of life had lower OHL scores. Table 3 shows the correlation between IOHLM-T scores and continuous predictor variables. While OHIP extent, severity and DMFT score were negatively correlated with IOHLM-T score, the number of filled teeth was observed to be positively correlated. IOHLM-T scores on a sub-sample of 98 participants who could read and write in English and Telugu showed a moderately strong positive correlation

Table 1: Descriptive statistics for the IOHLM-T and the continuous predictor variables of IOHLM-T (n=200)

Measure	Mean	Standard deviation	95% CI	Range (min-max)
Reading comprehension Score	16.23	4.75	15.57-16.88	18.75 (6.25-25)
Numeracy score	15.08	6.7	14.15-16	20.83 (4.16-25)
Oral health Knowledge score	18.08	5.62	17.3-18.85	16.67 (4.16-20.83)
Critical and Communicative Literacy score	8.45	3.47	7.96-8.93	12.5 (4.16-16.67)
IOHLM-T score	57.85	14.19	55.88-59.81	68.75 (18.75-87.5)
DMFT score	2.98	2.22	(2.67-3.28)	16 (0-16)
Decayed teeth (Dt)	1.76	1.35	(1.57-1.94)	10 (0-10)
Missing teeth (Mt)	0.95	1.84	(0.69-1.2)	16 (0-16)
Filled teeth (Ft)	0.27	0.64	(0.18-0.35)	3 (0-3)
OHIP extent	2.56	1.24	(2.38-2.73)	5 (0-8)
OHIP severity	14.10	4.53	(13.47-14.72)	24 (0-24)

CI-Confidence interval

Table 2: Association between categorical predictor variables and IOHLM-T score

Variable	Category	n	Reading comprehension score (SD)	Numeracy score (SD)	Oral health knowledge score (SD)	CCL score (SD)	IOHLM-T score (SD)
Sex	Males	109	16.19 (4.62)	15.02 (5.98)	17.98 (5.14)	8.43 (3.62)	57.62 (13.96)
	Females	91	16.43 (4.76)	15.28 (7.1)	18.23 (6.02)	8.52 (3.19)	58.46 (14.41)
	<i>P</i>		0.718	0.77	0.69	0.853	0.67
Educational Background	High school education or less	103	14.92 (4.1)	13.87 (5.43)	16.55 (5.6)	7.75 (3.54)	53.09 (12.08)
	More than high school education	97	17.13 (5.04)	15.91 (6.24)	19.06 (5.92)	8.93 (3.2)	61.03 (13.82)
	<i>P</i>		0.008*	0.014*	0.002*	0.01*	0.0001*
Socioeconomic status	Upper	51	16.36 (4.81)	15.15 (5.9)	18.74 (5.46)	8.81 (3.14)	59.06 (11.97)
	Upper middle	74	17.01 (4.53)	15.74 (5.61)	17.91 (4.92)	8.38 (2.86)	59.02 (11.33)
	Middle	42	15.94 (5.04)	15.28 (4.97)	17.85 (5.12)	7.98 (3.07)	57.05 (12.18)
	Lower middle	33	15.37 (4.26)	14.19 (5.04)	17.44 (5.81)	8.26 (3.46)	55.26 (11.41)
	<i>P</i>		0.3074	0.638	0.67	0.662	0.321
Latest dental visit	Within the last 24 months	111	17.03 (5.87)	15.69 (6.4)	18.91 (5.9)	9.34 (3.8)	60.97 (12.01)
	More than 24 months or first visit	89	15.46 (3.68)	14.47 (5.76)	17.09 (5.37)	7.83 (2.96)	54.85 (11.67)
	<i>P</i>		0.029*	0.163	0.025*	0.002*	0.004*
OHIP Prevalence	Yes	169	15.84 (5.76)	14.54 (5.18)	17.84 (7.21)	8.28 (3.6)	56.5 (13.23)
	No	31	18.35 (6.24)	17.66 (6.02)	19.38 (6.62)	9.37 (3.81)	64.76 (16.74)
	<i>P</i>		0.028*	0.003*	0.269	0.126	0.002*

Independent samples *t*-test. †One-way ANOVA. * denotes statistical significance ($P \leq 0.05$)

Table 3: Correlation between IOHLM-T score and continuous predictor variables

Variable	Pearson's correlation coefficient (r)	95% CI (r)
OHIP extent	-0.46*	(-0.33--0.52)
OHIP severity	-0.58*	(-0.41--0.69)
DMFT score	-0.376*	(-0.24--0.46)
Ft score	0.52*	(0.41-0.59)
REALD 30 score	0.34*	(0.25-0.48)

Pearson's Correlation; * denotes statistical significance ($P \leq 0.05$)

with the REALD-30 score indicating the convergent validity of IOHLM-T. IOHLM-T demonstrated good internal consistency reliability with Cronbach's alpha 0.75, and the internal consistency statistic for the sub-scales ranged from 0.68 to 0.84. At the multivariate level, educational background of the participants, last dental visit, OHIP prevalence, OHIP severity and Ft score were found to be significantly associated with IOHLM-T scores. Table 4 shows the results from stepwise multiple linear regression with IOHLM-T score as the dependent variable, the final model showing an adjusted R^2 (coefficient of determination) of 0.53.

Discussion

IOHLM-T is the first OHL instrument developed for the Indian context, the psychometric properties of which were established. It is also unique in the fact that this is the first OHL measure that included critical and communicative

literacy as an integral component of OHL. IOHLM-T, adopting the views documented by Institute of Medicine's panel on health literacy, measures the subject's ability to read and understand oral health-related information, to assimilate numerical information required to follow oral health-related advice, to communicate and gain access to oral health advice/delivery system, to critically review the oral health information received, besides evaluating his/her oral health knowledge.

It is intuitive that education has an inextricable link with literacy. However, educational attainment has often been discussed as a poor proxy for literacy and it was emphasised that literacy needs to be theorised and measured in ways distinct from that of education. Educational background of the subjects was found to be significantly associated with IOHLM-T scores at both bivariate and multivariate levels. Few of the previous studies reported educational level to be a significant predictor either exclusively at bivariate level^[44-47] and multivariate levels,^[48] or at both bivariate and multivariate levels.^[18] However, studies conducted by Sabbahi *et al.*^[17] and Wong *et al.*^[23] documented results inconsistent with the present study with no differences in OHL based on educational background.

Subjects who had their latest dental visit within the past 2 years demonstrated higher IOHLM-T scores compared to their counterparts. This finding could be a result of the relatively recent exposure of these subjects to oral

Table 4: Stepwise multiple linear regression for prediction of variance in IOHLM-T scores

	Unstandardised Coefficients (β)	95% CI (β)	Standard Error	P
Education (High school or less)	2.585	1.19-4.97	0.568	0.029*
Last dental visit (≤ 24 months)	-1.716	-2.72--0.710	0.510	0.014**
OHIP prevalence (yes)	-0.630	-0.116--0.84	0.431	0.041*
OHIP severity	-3.64	-5.23--2.04	0.811	0.001*
Ft score	1.89	0.64-3.13	0.63	0.02*

F (5, 194)=86.36; $P < 0.001$; $R^2 = 0.581$; Adjusted $R^2 = 0.53$; *denotes statistical significance ($P \leq 0.05$)

health care facilities. Literature shows mixed results for the association between dental visits and OHL. Studies conducted by Sabbahi *et al.*^[177] and Jones *et al.*^[46] found a significant association between frequency of dental visits and OHL, whereas studies by Atchison *et al.*,^[18] Wong *et al.*^[23] and Lee *et al.*^[47] showed no differences in OHL levels based on dental attendance.

Predictive validity of IOHLM-T was established based on the association between IOHLM-T scores and oral health-related quality of life, caries experience. The prevalence, extent and severity of oral health impact profile were documented in this study. A significant association was found between OHIP prevalence and OHL scores at both bivariate and multivariate levels. While OHIP extent and severity were also found to be significantly negatively correlated with OHL scores at bivariate level, at multivariate level, only OHIP severity remained a significant predictor. OHIP extent and severity were entered alternatively in different models to avoid multicollinearity, as there was a strong positive correlation between these two variables ($r = 0.81$). These results were consistent with the findings from previous studies.^[15-16,47,49,50] In the Indian scenario, a study done by Sharma *et al.*^[51] among undergraduate students in Bangalore city showed positive correlation between oral health-related quality of life and OHL scores. The overall DMFT score (caries experience) was found to have a significant weak negative correlation with OHL scores at bivariate level, but no significant association was found at multivariate level. While decay component of DMFT and missing component of DMFT were negatively correlated with the OHL scores, the filled component showed a highly significant moderate positive correlation. The missing component of DMFT was not entered in the regression model owing to its strong positive correlation with OHIP severity ($r = 0.79$). The filled component of DMFT remained a significant predictor of IOHLM-T scores at the multivariate level. High caries experience among subjects with low OHL levels was previously reported in the literature.^[10,52-54]

Conclusion

IOHLM-T demonstrates good face validity, content validity, predictive validity, convergent validity and internal consistency reliability and hence can be used among different populations in India after translation to the corresponding languages and evaluation of psychometric properties. This

also ensures comparability of OHL levels between different populations of the huge and diverse Indian geographic. All the previously validated OHL instruments, including IOHLM-T, were developed to estimate the OHL of those subjects who could read and write. However, based on the World Health Organisation's description of health literacy, it is obvious, though a paradox, that individuals could be 'health literate' in being able to gain access to, understand and use health information, without actually being literate (possessing reading and writing skills). The authors opine that an important direction for future OHL research could be the development of an instrument that could capture the OHL of people who could not read and write.

Declaration of participant consent

The authors certify that they have obtained all appropriate participant consent forms. In the form, the participants have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Annexure- 1

Indian Oral Health Literacy Measure

Reading Comprehension

Passage 1: Read the following information

Teeth are very important part of our inner and outer personality. For good health our teeth also needs to be healthy. But for most people dental health is the last priority. They are not even aware of how to take good care of teeth. So first let us get introduced to our teeth and gums. A tooth can be divided into two parts, “crown” and “root”. Crown is the part you can see in the mouth. But the root part is not visible. It is hidden in the jaw bone. The outermost layer of crown is called “enamel”. It is the strongest structure in the entire human body. The yellowish layer below the enamel which looks like bone is called “dentin”. The dentin surrounds the vessels and nerves of the teeth called “pulp”. The blood vessels supply food, while the nerves carry sensation to the teeth.

There are two dentitions in human being. First is during the childhood when we call them “milk teeth”. Milk teeth do the job of chewing and help in the development of jaw bone and permanent teeth. Milk teeth start emerging in the infant’s mouth at the age of six months, and grow fully by the age of two and half years. When the child is six years old, the first permanent teeth emerge. Gradually the milk teeth start falling, and by the age of twelve, permanent teeth emerge in the place of milk teeth. The number of permanent teeth in human beings is 32.

Section 1: Answer the following questions based on the information given above.


- 1) The part of tooth present in jaw bone and covered with gums is called:
a) Enamel b) Crown c) Root d) Don’t know
- 2) Strongest structure in human body:
a) Enamel b) Jaw bone c) Dentin d) Don’t know
- 3) Number of milkteeth:
a) 24 b) 32 c) 20 d) 16
- 4) First permanent tooth erupts at which age:
a) Six months b) Six years c) Three years d) Three months
- 5) By what age permanent teeth emerge in place of milk teeth?
a) 8 years b) 6 years c) 10 years d) 12 years
- 6) If stands for general health and stands for oral health, which of the following pictures correctly demonstrate the relation between oral health and general health?
a) b) c) d) Don’t know

Passage 2: Read the following information

Identification of dental caries and having awareness on how to protect ourselves from dental caries are very important. Accumulation of plaque in mouth is a major reason for dental caries. Acids are produced when microorganisms come in contact with the left-over food in the mouth. Dental caries initially start as black / brown spot on the teeth. This slowly extends to create a hole in the tooth. When tooth decay extends to pulp, it results in pain. From there, the infection spreads to tooth root and jaw bones and causes swelling. This swelling can spread to face and neck. This can result in fever and is life threatening sometimes. Tooth decay is mainly found on chewing surfaces or between the back teeth, the places where food usually gets stuck. Therefore, in order to prevent dental caries keeping the mouth clean and free from accumulation of plaque is very important. Brushing twice daily with tooth paste, mouth rinsing, and tongue cleaning are best practices in preventing tooth decay. During the night, if in infants are left for long time with milk bottle in the mouth, that can also cause dental caries. To prevent this, children must be given a spoonful of water in sitting position, after having milk. It is important to regularly clean infant’s teeth with small tooth brush from the time the first tooth emerges in the mouth.

Section 2: Answer the following questions based on the information given above.

- 7) Dental caries start as color spot on the teeth:
a) Black / Brown b) White / Purple c) White / Brown d) Don’t know
- 8) ----- are produced when microorganisms come in contact with the left-over food in the mouth:
a) Sweets b) Acids c) Bases d) Don’t know
- 9) Pain starts when decay extend to:
a) Enamel b) Dentin c) Pulp d) Don’t know
- 10) There is no chance for dental caries to cause infection of jaw bones.
a) Yes b) No c) Don’t know
- 11) Milk teeth of children should be cleaned with tooth brush starting from which age:
a) When children can brush on their own
b) Six months c) Eighteen months d) No need to brush milk teeth
- 12) What are the precautions that have to be taken to prevent dental caries after giving milk to children? (Write in your own words)


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Numeracy Skills

Section 3: Answer the following questions.

- 13) Dentist advised a patient Ramaiah who complained of toothache to get his tooth extracted. However, since Ramaiah had high blood sugar levels, he was asked to consult back after attaining blood sugar level between 80 and 150. At achievement of which of the following blood sugar levels, can Ramaiah consult the dentist?
 a) 60 b) 160 c) 130 d) 200 e) Don't know
- 14) Dentist who examined Kumar identified that there was swelling and pus discharge near the gum. He advised Kumar to take a tablet once in every eight hours. How many tablets should Kumar take in 3 days?
 a) 12 b) 24 c) 9 d) 6 e) Don't know
- 15) New tooth brush can remove 30% more plaque than a tooth brush which has been in use for three months. What does this mean?
 a) Tooth brush which has been in use for three months can remove only 30% of plaque.
 b) New tooth brush can remove more plaque than a tooth brush which has been in use for three months only on 30% of occasions.
 c) If a tooth brush which has been in use for three months can remove 60% plaque, new tooth brush can remove 90% plaque.
 d) Don't know
- 16) A dentist advised Seethamma who underwent extraction to take a tablet twice daily for three days. If she takes the first tablet at 9 AM on Monday, when should she take the next tablet?
 a) 12 noon on Tuesday b) 10 AM on Monday c) 9 PM on Monday d) Don't know
- (17-18) Answer the following Questions based on the information given in the table.

Problem	% of population suffering
Gum diseases	90%
Malocclusion	30%
Dental caries	60%

- 17) According to the information given in the table:
 a) Malocclusion is more common than dental caries b) Malocclusion is more common than gum diseases
 c) Gum diseases are more common than malocclusion d) Don't know.
- 18) According to the information given in the table:
 a) 60% of the population has dental caries. b) Nine out of every hundred people are suffering from gum diseases.
 c) There are people who have more than 30 teeth with malocclusion. d) Don't know.

Oral Health Knowledge

Section 4: Answer the following questions.

- 19) After how much time of use does a tooth brush needs to be changed?
 a) No need to change till it wears off b) 2 weeks c) 3 months d) 6 months
- 20) From what age children can start brushing their teeth without adult supervision?
 a) 3 years b) 12 years c) 24 months d) 6 years
- 21) Use of fluoridated tooth paste can prevent dental caries.
 a) Yes b) No c) Don't know
- 22) What is the ideal time to be spent on brushing teeth?
 a) 1-3 minutes b) 4-6 minutes c) > 6 minutes d) Don't know
- 23) Can dental plaque stick to your tongue?
 a) Yes b) No c) Don't know
- 24) Consuming sweets in between meals decreases the risk for tooth decay.
 a) Yes b) No c) Don't know

Critical and communicative literacy

Section 5: Answer the following questions.

- 25) Were you able to obtain oral health related information when needed?
 a) No b) Sometimes c) Often d) Very often
- 26) Were you able to understand the information obtained relating to oral health?
 a) No b) Sometimes c) Often d) Very often
- 27) Did you implement the information you obtained relating to oral health in your daily life?
 a) No b) Sometimes c) Often d) Very often
- 28) Did you analyze the information obtained?
 a) No b) Sometimes c) Often d) Very often
- 29) Did you share the information with others?
 a) No b) Sometimes c) Often d) Very often
- 30) Which of the following is the most likely thing you would do in case of toothache?
 a) Home remedies/self-care b) Take pain killers c) Consult a dentist d) Wait for the pain to relieve



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Measuring the Impact of COVID-19 on Mental Health: A Scoping Review of the Existing Scales

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ABSTRACT

Background: The coronavirus disease (COVID-19) pandemic, associated with the economic consequences of non-pharmaceutical interventions such as lockdown, has led to mental health consequences among people worldwide. Protecting the mental well-being of populations is an imperative component of fighting the COVID-19 pandemic. This scoping review attempts to present an overview of the existing tools to measure COVID-19-related mental health problems.

Methods: Literature search was conducted in the PubMed electronic database using developed key search terms. Reference lists of the identified eligible articles were reviewed to locate relevant articles missed from the electronic database search. Fifteen scales measuring COVID-19-associated mental health problems, validated among diverse populations across the world, were included in this review.

Results: The majority of these scales were validated among middle-aged adults in Turkey. Only a few validated scales encompass the negative socioeconomic consequences of COVID-19. None of the

available scales focused on the aspects of suicidal ideation or behavioral responses/ coping strategies, neither were they inclusive of participants from diverse age, geographic, and COVID-19 exposure groups.

Conclusion: This scoping review highlights the need for future research to develop and validate comprehensive psychometric tools to assess COVID-19-associated mental health problems. Also, in view of the vulnerable nature of healthcare professionals for developing mental health concerns in the course of providing services for COVID-19-affected individuals, future psychometric research needs to concentrate on the development of measures specific for these professionals.

Keywords: Clinical psychology, epidemiology, psychotherapy, qualitative

Key Messages: Various validated tools are available to measure the mental health implications of COVID-19. The available psychometric instruments must be used in an informed manner in different settings to comprehend the psychological burden posed by COVID-19 on populations. Identification of COVID-19-associated mental health problems with an informed

use of the available psychometric tools facilitates a thorough preparation of healthcare systems in dealing with the psychological aftermath of COVID-19.

Coronavirus disease (COVID-19) emerged as a devastating pandemic of unprecedented magnitude pushing the world into a looming health crisis. While the physical implications of this infectious disease are apparent, it is undeniable that the condition also holds the potential for mediating negative socioeconomic and psychological implications.¹ Across the globe, it has substantially been established by now that the mental health problems are on the rise through the COVID-19 pandemic.²⁻⁷ Hossain et al. reviewed the mental health outcomes of nonpharmaceutical preventive interventions such as quarantine and reported depression, anxiety, insomnia, and mood disorders among isolated individuals.⁸ A few of the many stressors leading to mental health concerns among the general population in the COVID-19 era are fear of infection,

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financial depletion, deficient supplies, and stigmatization.⁹ Suicidal tendencies among COVID-19-affected individuals are also reported.¹⁰ Another important concern is the impact of COVID-19 on the psychological well-being of healthcare workers (HCW). Around the world, the health systems are under tremendous pressure catering to the needs of an ever-increasing number of COVID-19 cases. The situation demands HCW to work for longer periods in settings different from their regular work while staying away from their families and dealing with an infectious disease with a lot of uncertainties around. This context reflects the possibility of mental health concerns such as fear of infection, the guilt of providing suboptimal care, burnout, insomnia, anxiety, and depression among HCW.¹¹⁻¹⁵ It has to be acknowledged here that mental health is a broad construct encompassing emotional, psychological, and social dimensions, making it an elusive construct to measure. Though diagnostic interviews were identified to be the gold standard and definitive diagnostic ways for appraisal of mental health status, the amount of time they consume precludes their application in conventional health settings, especially when the assessment has to be made at a mass level. It is for this reason that the reliance on screening tools for measuring mental health status has increased over the years.¹⁶ During the COVID-19 pandemic, new scales have been validated to assess the COVID-19-related mental health problems. A preliminary scan of the existing COVID-19 psychometric scales revealed that the focus predominantly had been placed on COVID-19-related fear and anxiety. However, drawing on the suggestions made by experts that the psychological implications of COVID-19 are manifold, tools that comprehensively capture COVID-19-related mental health concerns are warranted. The present scoping review, therefore, was conducted to address any existing knowledge gap and in order to provide a condensation of the available scales assessing COVID-19-related mental health problems.

Materials and Methods

This scoping review was conducted to examine the available psychometric tools to

measure COVID-19-related mental health problems. While the primary purpose of a scoping review is to provide an overview of the available evidence by summarizing the available literature pertaining to a concept, there exists no concrete consensus on the indications for the conduct of a scoping review.¹⁷ Besides comprehending the fundamental characteristics of a phenomenon, identification and analysis of knowledge gaps, which forms the basis for this study, is purported to be one of the key indications of scoping reviews.¹⁸ The framework proposed by Arksey and O'Malley for scoping reviews was adopted in this study.¹⁹

1. *Identification of the research question:* Since the scoping review attempts to comprehensively document the existing literature, a broad question that facilitates the inclusion of different constructs relating to mental health are warranted. The research question for this review was identified as: In spite of a preliminary scan of the results showing predominant emphasis on the constructs of anxiety and fear, "What are the existing scales to measure COVID-19-related mental health problems?"
2. *Formulating the search strategy to identify relevant studies:* A literature search was conducted in the PubMed electronic database after a series of consensus building among the investigators for identification of key search terms. The following key terms were included in the search for eligible studies: Coronavirus*, COVID-19, COVID, "COVID 19", "SARS CoV-2", "SARS CoV 2" for coronavirus disease; "mental health," anxiety, fear, phobia, stress for mental health problems; and scale, tool, instrument, questionnaire for scale. The final search string was (Coronavirus* OR COVID-19 OR COVID OR "COVID 19" OR "SARS CoV-2" OR "SARS CoV 2") AND ("mental health" OR anxiety OR fear OR depression OR stress OR phobia) AND (scale OR instrument OR questionnaire OR tool). Those studies measuring the constructs related to mental health specific to COVID-19, published in the English language in peer-reviewed journals in the year

2020, after the emergence of the COVID-19 pandemic, were included in the review. The search was inclusive of all the relevant publications available till June 10, 2020.

3. *Selection of relevant studies:* Figure 1 shows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram for the scoping review. The search strategy yielded 183 results. The EndNote bibliographic software package was used to import and manage all references. The title, abstracts, and key words of all the articles were independently reviewed by three investigators to check the relevance of these articles based on the selection criteria of the review. Scales measuring constructs not related to mental health and those that are not specific for COVID-19, scales which have not been subjected to psychometric validation, and general surveys using the validated scales to assess COVID-19-related mental health constructs were excluded from the review. The full texts of the 14 articles that were agreed upon by all the reviewers to be satisfying the study criteria after the initial review were reviewed. References from all these 14 articles were also reviewed to check if any relevant publications were missed in the electronic database search. The final review included 15 articles.²⁰⁻³⁴
4. *Study charting and summarizing:* All the 15 articles included in the final review were comprehensively charted and summarized. Summarization included details on the authors, journal, country, number of items, dimensionality, time of conduct of the study, and other psychometric properties of the scale. While summarizing the scales, the constructs and dimensions were identified based on the American Psychiatric Association *Diagnostic and Statistical Manual of Mental Disorders* (Fifth Edition, DSM-5).³⁵

Results

An overview of the existing scales measuring COVID-19-related mental health problems have been provided in **Table**



S1. The majority of these scales were validated among middle-aged adults (range for mean age across scales: 26.5–49.8 years). There was a great variation in the sample size between the studies, ranging from 249 for the “Italian version of Fear of COVID-19 Scale” to 8550 for the “Bangla version of Fear of COVID-19 Scale.” Most of the scales, 4 of the 15 included in the review, were validated in Turkey, followed by 3 in the United States and 2 in Italy. The Fear of COVID-19 Scale (FCV-19S) was validated and available in seven languages (Farsi, Hebrew, Bangla, Turkish, Russian, Italian, Arabic), while the Coronavirus Anxiety Scale was available in English and Turkish. The majority of the studies included in this review (40%) were published in the *International Journal of Mental Health and Addiction*, followed by the *Death Studies* journal (20%). It is evident from **Table S1** that a majority of scales were validated before the peak incidence was reached in the corresponding nations. “COVID Stress Scales” (CSS) was observed to be the lengthiest scale, with 36 items, and “Obsession with COVID-19 Scale” was the briefest scale, with only 4 items. Cronbach’s alpha was identified as the most common measure for internal consistency reliability, ranging from 0.73 to 0.93 among the 15 scales considered. Factor structure was presented for all the scales except the “Italian version of COVID-19 Peritraumatic Distress Scale.” A total of 10 out of the 15 scales were unidimensional with a single-factor solution. The majority of the scales were validated among the general population. Hospital Anxiety and Depression Scale (HADS), Perceived Vulnerability to Disease Scale (PVDS), Patient Health Questionnaire (PHQ), Brief Resilience Scale Turkish (BRS-T), Severity Measure for Specific Phobia-Adult (SM-SP-A), self-rated mental health, Impact of Event Scale-Revised, and Depression Anxiety Stress Scales (DASS) were among the measures used for establishing of the scales included in this review.

Table S2 presents a detailed account of the items from the distinct instruments included in the review and the constructs and dimensions they measure; translated instruments are not included in this table to avoid repetition of content. “Obsession with COVID-19

Scale” is a brief four-item scale focusing on the cognitive dimension. Coronavirus Anxiety Scale is a five-item brief mental health screener with an emphasis on the physiological symptoms of COVID-19 anxiety. FCV-19S is a seven-item scale measuring the construct of fear and predominantly focuses on the emotional dimension, with representative items from physiological and cognitive dimensions. COVID-19 Phobia Scale (C19 P-S) had 19 items loaded on 4 factors representing corona phobia: psychological, psychosomatic, economic, and social. CSS demonstrated a five-factor solution focusing on danger and contamination, socioeconomic consequences, xenophobia, traumatic stress, and compulsive checking related to COVID-19; moderate to strong positive correlation (0.41–0.73) was observed between the five factors. COVID-19 Anxiety Scale is a seven-item scale with representative items from cognitive, physiological, and emotional dimensions of anxiety. COVID-19 Peritraumatic Distress Index (CPDI) is a 24-item scale measuring COVID-19-associated psychological distress on a 5-point Likert scale, with overall scores ranging from 0 to 100.

Discussion

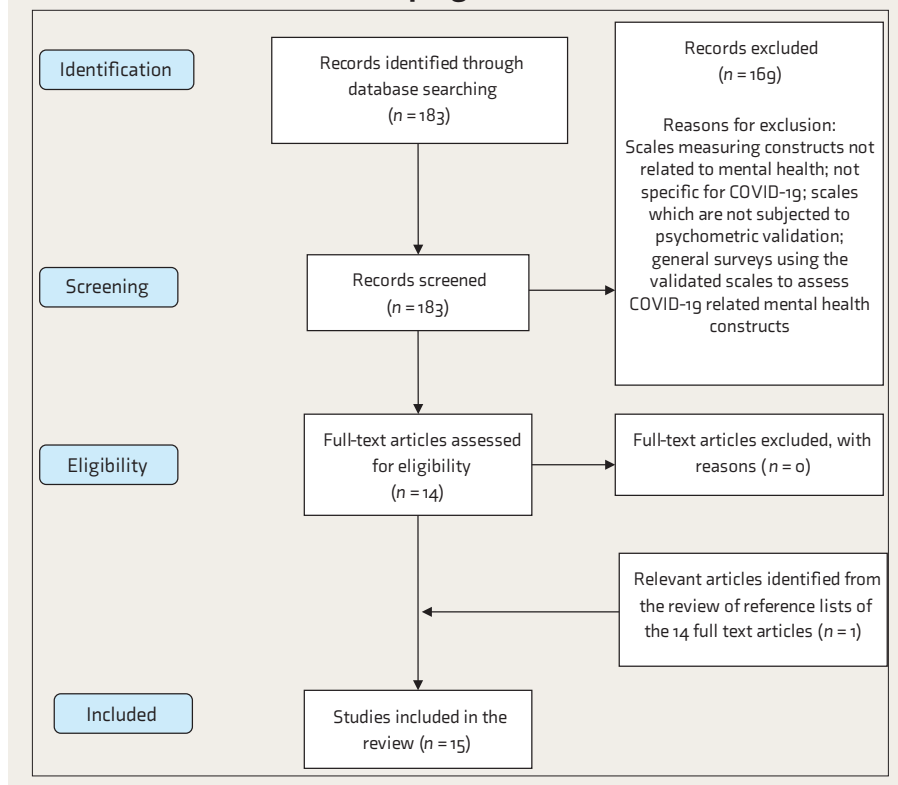
It is evident from the literature that along with the general stressors, a fear of prolonged outbreak, fear of infection vulnerability, and exposure or close contact with COVID-19-affected individuals negatively influence the mental health and well-being.³⁶ The psychiatric symptoms encompass depression, anxiety, somatic symptoms, panic attacks, psychosis, and suicidal tendencies.^{37,38} CSS and C19 P-S consider the negative economic consequences of COVID-19 as contributing towards the COVID-19-associated mental health problems. Items relating to active search on the internet and social media for COVID-19 information are included in the CSS, along with practical concerns like worrying about cash transactions and future supplies of essentials, which makes CSS a comprehensive instrument among the available scales. However, CSS does not include behavioral responses to COVID-19, which found a place in C19 P-S, where items relating to avoidance of people who sneeze and

spending extensive amounts of time on cleaning hands were included. Though it is not the aim of a scoping review to critically appraise/synthesize the findings, it is apparent that CSS, C19 P-S, and CPDI are more comprehensive measures compared to other scales included in this review. However, as the choice of an instrument depends on a lot of other factors such as feasibility and relevance and since all the validated scales demonstrated good psychometric properties, it is difficult to suggest the single best available instrument to measure COVID-19-related mental health problems.

According to American Psychiatric Association, concurrent validity is the extent to which one measurement is backed up by a related measurement obtained at about the same point in time, while criterion validity is reflective of the correlational nature of a measurement with an established standard of comparison. Convergent validity is defined as the extent to which responses on a test or instrument exhibit a strong relationship with responses on conceptually similar tests or instruments.³⁹ However, these expressions do not have consistent interpretations in the literature and may overlap.³⁰ For instance, it was reported that the concurrent validity of the Turkish version of FCV-19S was checked with Depression, Anxiety and Stress Scale-21 (DASS-21). DASS-21 is a short form of DASS-42 with three subscales: depression, anxiety, and stress.⁴¹ Since DASS-21 measures constructs of depression, anxiety, and stress, which are different from the construct of interest of FCV-19S, which is fear, and as there is a fine distinction between these constructs as evident from DSM-5, the expression “convergent validity” could have been preferred while referring to the correlation between the Turkish version of FCV-19S and DASS-21. Similar observations relating to overlapping use of the expressions of validity were made by us with regard to measuring concurrent validity of FCV-19S by comparing with DASS-21 and PVDS,⁴² which is a 15-item scale with two subscales of perceived infectability and germ aversion; assessment of concurrent validity of the Bangla version of FCV-19S with Bangla PHQ-9 depression measure⁴³; checking criterion validity of

FIGURE 1.

PRISMA Flowchart of the Scoping Review



FCVS-T with BRS-T,⁴⁴ an instrument that assesses the ability to overcome difficult situations; assessment of concurrent validity of the Italian version of FCV-19S with HADS⁴⁵ and SMSP-A⁴⁶; assessing the concurrent validity of the Arabic version of FCV-19S with HADS-A, HADS-D, and HADS-T, which are the anxiety subscale, depression subscale, and combined scales, respectively.

Though it is an established notion that anxiety/fear may lead to suicidal tendencies and inclination towards substance use, there are no available scales that attempted to document these facets.^{47,48} Also, none of the available scales included items on adopted coping strategies. It is noteworthy that the validity sample for only a few scales was inclusive of the COVID-19-affected people. The mean age of the validity samples across the scales ranged from 26.5 to 49.8 years; this finding is reflective of the necessity to consider population from different age groups in the validation process owing to the established contributory role of age in individuals' progression towards severe disease.⁴⁹ The time of validation of the scales in correspondence to the emo-

tional epidemic curve does play a significant role in influencing the responses of the participants. All the scales were validated during the first peak of the emotional epidemic curve; therefore, validation studies during the dipping phase and second peak phase of this curve are warranted.⁵⁰ A striking similarity between the majority of the scales in the present review was the predominant emphasis given to the somatic symptoms experienced by people on thinking about COVID-19. It is to be noted that the items on different scales included in the review concur to a considerable extent; this overlap must be understood in terms of the interchangeable use of the terms "anxiety," "fear," and "phobia" in common parlance despite the precise differences in meaning.³⁹ C19P-S and CPDI are the two distinct scales that differ from the other scales by integrating items representative of the behavioral dimension. Except for the COVID-19 Anxiety Scale, which consisted of items on a semantic differential scale, all the other instruments chose Likert response options, which is an interesting observation in light of the potential acquiescence bias

with Likert rating scales.⁵¹ COVID-19-imposed lockdown and quarantine measures may necessitate some individuals to stay away from their families. However, none of the available scales focused on separation anxiety. Failure to document the aspects of substance use and suicidal ideation is the other limitation observed in the existing scales, though these constructs were established to be possible consequences of fear and anxiety.^{47,48} Another fundamental lapse identified was the inability of any of the existing scales to discern COVID-19-associated depression. Except for the OCS,⁵² CAS,²⁰ and the Italian version of CPDI,³³ all the remaining scales included in the review inquire about the current status of the subject but do not necessitate the patient to respond by reflecting on their experiences from the past few weeks. Because it has been six months since the identification of the first COVID-19 case, there is a necessity to focus on acute stress disorder and post-traumatic stress disorder associated with COVID-19 among the affected and recovered. A health-systems-related concern that could have an impact on the mental health of people is the unavailability or inaccessibility of healthcare facilities, which was not included in any of the validated scales included in this review. The aforementioned lapses in the scales considered in this review were discussed by us after thoroughly reviewing the catalogue of WHO psychiatric instruments⁵³ and DSM-5.³⁴ Moreover, drawing on the insights provided in the WHO Assessment Instrument for Mental Health Systems (WHO-AIMS), the following were identified to be the domains of mental health systems that require attention and close monitoring, learning lessons from COVID-19: the articulation of agencies for public education and awareness campaigns on mental health, development of community mental health services, development of mental health component in primary healthcare, active functioning of mental health mobile clinic teams, the increased proportion of undergraduate training hours devoted to psychiatry and mental-health-related subjects, and refresher training for HCW in mental health facilities.⁵⁴ In line with these directives, Das discussed the need for telepsychiatry in

active identification of individuals with psychological infirmity and provision of awareness education and psychological interventions in the Indian context.⁵⁵ There is substantial literature available on the feasibility and effectiveness of online mental health services during the COVID-19 pandemic across the globe.⁵⁶⁻⁵⁸

Spoorthy et al.⁵⁹ reported in their review that HCW across the globe had encountered a substantial degree of anxiety, stress, and insomnia in the course of the COVID-19 pandemic. Shanafelt et al.⁶⁰ summarized the possible sources of fear and anxiety among HCW during the COVID-19 pandemic; they also discussed the conventional self-reliant nature of health fraternity as an important contributor to anxiety while dealing with a previously un-encountered disease that is outside the professionals' area of clinical expertise. Despite the increased vulnerability of HCW in developing mental health concerns in the process of battling a pandemic the health systems are not prepared for, none of the available scales were exclusively developed for HCW. The following are a few of the concerns that render HCW more vulnerable with regard to COVID-19 impact on mental health: limited availability of personal protective equipment, necessity to work for longer durations, fear among HCW about the possibility of transmitting the infection to family members, reports on COVID-19-associated deaths of HCW around the globe, possible discrimination out of fear of infectability from society owing to the involvement of these professionals in the care provision of COVID-19-affected, etc.⁶⁰⁻⁶² Given these concerns, it is also imperative that psychometric tools meant to assess COVID-19-posed mental health concerns among HCW be developed and validated.

FCV-19S was observed to be the most commonly translated and cross-cultural-ly adapted instrument, which is available in seven languages. The probable reason for this instrument to be preferred for cross-cultural adaptation in different countries was the fact that this is the earliest available COVID-19-related psychometric tool, published on March 27, 2020. The next psychometric tool²⁰ assessing coronavirus anxiety was published only three weeks later. Though CPDI was

published on March 6, 2020, the full instrument was not made available in the publication.³³ The 7-item COVID-19 Anxiety Scale was observed to be the only COVID-19 psychometric tool validated in the Indian context.³¹ While the adaption of validated measures developed in other countries is possible with a simple translation of the instruments to regional language, it is advisable to verify if the items in these tools meet the intended needs of Indian population, owing to the possible difference in psychological aspects between the populations.⁶³ Even within the country, rural and urban people can psychologically be different with regard to their COVID-19-related mental health problems; therefore, scale validity samples must include participants from diverse geographical regions so that differences in item responses across different geographic categories may be determined. Also, checking the psychometric and dimensional stability of translated measures is warranted.

The limitations of this review include confinement to the English language and the PubMed database. Nevertheless, an overview of the existing scales and identification of the knowledge gap in the evaluation of the desired constructs provides a direction for future psychometric research with regard to COVID-19-related mental health problems.

The directives for future studies include the development of more comprehensive measures to document mental health problems posed by COVID-19 pandemic by incorporation of the aspects of suicidal ideation, post-traumatic distress, coping strategies, etc. These studies must also aim for representation from diverse age, geographic, and COVID-19 exposure groups.

Conclusion

This scoping review identifies that majority of the existing scales are heavily biased towards the somatic symptoms of COVID-19-imposed mental health problems. C19 P-S, CPDI, and CSS are among the scales which attempted to capture COVID-19-associated mental health problems comprehensively. FCV-19S, while being a unidimensional scale with only seven items, offers an opportunity for screening in busy healthcare settings

and also allows cross-country comparisons, owing to its validation and availability in multiple languages worldwide. The seven-item COVID-19 Anxiety Scale was identified as the only psychometric measure validated in the Indian context and could be adapted in other Indian languages.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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Development and Initial Validation of the COVID-19 Anxiety Scale

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Abstract

Background: Safeguarding the psychological well-being of the public is also an integral component of fighting COVID-19. However, there is limited availability of psychometric measures to document COVID-19-related anxiety among the general public. **Objectives:** This study was aimed at developing a validated scale to measure COVID-19-related anxiety. **Methods:** Three hundred and seven subjects from different gender, educational categories participated in the study. Exploratory factor analysis for the determination of factor structure, Pearson's correlation test, and Kruskal–Wallis ANOVA were employed in data analysis using SPSS version 20 software. **Results:** COVID-19 Anxiety Scale (CAS) demonstrated a two-component structure identified as: “fear of social interaction;” “illness anxiety.” The final scale with seven items demonstrated good internal consistency reliability (Cronbach's Alpha 0.736). CAS exhibited good construct validity showing moderately negative correlation (Pearson's $r = -0.417$) with the self-rated mental health and resulted in higher scores among individuals with lower educational qualification (Kruskal–Wallis ANOVA $\chi^2 [2, 303] = 38.01; P = 0.001$). **Conclusion:** CAS is a rapidly administrable, valid, and reliable tool that can be used to measure COVID-19-related anxiety among the Indian population.

Key words: Coronavirus, principal component analysis, psychometrics

INTRODUCTION

Coronavirus Disease (COVID-19) has been designated as a public health emergency of international concern by the World Health Organization on January 30, 2020.^[1] While the early beginning of the COVID-19 outbreak occurred in December 2019 in the Hubei province of China, there has been unprecedented rise, since then, in the number of COVID-19 affected people across the globe leading to the declaration of COVID-19 as a pandemic on March 11, 2020.^[2] While the primary focus has been placed on the development of COVID-19 specific vaccine, identification of COVID-19 positive cases, quarantining suspected individuals and close contacts of the affected, and developing strategies for mass screening, the psychological impact of COVID-19 on the general public was given little attention. With COVID-19 swiftly evolving globally, a substantial degree of fear is induced among the public. It is estimated that the magnitude of anxiety and stress, depression levels, and suicidal inclinations would rise as a consequence of the psychological burden posed by COVID-19 associated fear.^[3] In the context of potential disease transmission, emotional

discomfort among concerned populations is inevitable. In these circumstances, it is very important to alleviate people's fear and anxiety of acquiring COVID-19. However, we have limited availability of validated COVID-19-related anxiety scales, which preclude the assessment of COVID-19 related concerns among the public and the planning of informed support programs. This study was done to develop a scale to measure the anxiety relating to COVID-19 and evaluate its psychometric properties among the Indian population.

MATERIALS AND METHODS

This study was conducted during February and March 2020 among the general population belonging to the southern Indian

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state of Andhra Pradesh. Ethical approval for this study was obtained from the Institutional Ethical Committee of SIBAR Institute of Dental Sciences (pr. 70/IEC/SIBAR/2020). Based on the objectives of the study, which include the identification of the underlying latent constructs by exploratory factor analysis (EFA), the rating scale for adequate sample size in factor analysis proposed by Comrey and Lee focusing on the necessity to render standard errors of correlation coefficients sufficiently small was considered, according to which a sample size of 300 was deemed to be good.^[4] The study sample constituted 307 participants, aged 18 years and above, with representation from different age, educational, and gender categories. All the participants provided implied consent with voluntary participation in the study. For convenience, the methodology adopted in this study is discussed under the following subheadings.

- i. Generation of item pool: various measures related to the construct of fear and disease vulnerability were reviewed before the generation of the item pool. Twelve items articulated by the primary investigator (VCC) were reviewed by the other investigators before the evaluation of the initial scale for content validity and face validity. All the items were designed to assess the anxiety associated with COVID-19 on a four-point (1–4) semantic differential scale, where the evaluative dimension was explicitly mentioned in the choices, with higher scores reflective of increased COVID-19-related anxiety
- ii. Evaluation of content validity and face validity: the initial scale with 12 items was reviewed by a five-member expert panel. The scale level inter-rater agreement was assessed using the Intra-class Correlation Coefficient (Two-way mixed model, mean of 5 raters, absolute agreement). ICC was found to be 0.87 for the 10 item scale after the deletion of two items owing to limited content relevance as opined by the expert panel
- iii. Assessment of item-total correlations (ITCs) and internal consistency reliability: Corrected ITCs to check the association of each of the scale items with the total score obtained were used for initial scale purification. Corrected ITC of <0.3 indicates the limited association of the corresponding item to the overall construct measured by the scale.^[5] Cronbach's alpha was used to assess the internal consistency reliability of the scale, and a value >0.7 was deemed as a reflection of the optimum degree of correlation between the scale items
- iv. Determination of factor structure and scale purification: EFA was done with principal components as the extraction method to determine the items which are good indicators of the construct of interest. Factor rotations were done as necessary, and items demonstrating significant cross-loadings, with a difference in loadings of <0.2, across factors were intended to be removed as a part of scale purification
- v. Establishment of construct validity and temporal stability of the scale: A single self-rated mental health (SRMH) question, on a five-point scale, was used to test the

construct validity of the scale.^[6] Furthermore, the differences in total scale scores obtained based on the educational qualification of the subjects were considered to establish construct validity for the scale. Temporal stability was assessed by re-administering the scale to 30 participants 5 days after the initial administration.

Statistical analysis

SPSS version 20 software (IBM SPSS statistics for windows version 20, Armonk, NY, USA) was used to analyze the data. Descriptive statistics, EFA (principal components extraction) using Promax rotation with Kaiser Normalization for determination of factor structure, Pearson's correlation test for checking the correlation between SRMH and CAS scores, Kruskal–Wallis ANOVA for identifying differences in CAS scores based on educational qualification were employed in data analysis.

RESULTS

The background characteristics of the study population are described in Table 1. The mean age of the study participants was 35.32 ± 10.9 years. Corrected ITCs for the ten-item scale suggested removal of two items having ITC <0.3. The retained eight items demonstrated ITCs from 0.33 to 0.63; Cronbach's alpha if item deleted values ranged from 0.67 to 0.75 [Table 2]. Cronbach's alpha for the eight-item scales was 0.77. KMO measure of 0.731 indicated underlying factor structure and significance from Bartlett's test of sphericity reflected the correlation among item responses indicating the appropriateness of factor analysis. A two-factor solution was obtained from EFA with principal components as the extraction method based on the Eigenvalue >1 criterion. The unrotated matrix showed cross-loadings for three items (Items 3, 4, 10). Therefore, Promax rotation with Kaiser normalization, owing to the correlated nature of items loaded on the two factors, was employed to simplify the factor structure. Item 10 demonstrated cross loading in the rotated matrix too and hence was removed from the scale. The final scale (CAS) with seven items, with the possible range of scale scores being (7–28), yielded a two-factor solution: “fear of social interaction;” “illness anxiety.” Table 3 shows the factor loadings, communalities,

Table 1: Background characteristics of the study participants (n=307)

Variable	Category	n (%)
Gender	Male	205 (66.8)
	Female	102 (33.2)
Age group (years)	18-30	93 (30.29)
	31-40	107 (34.85)
	41-50	61 (19.86)
	51 and above	46 (14.98)
Educational qualification	Secondary education	65 (21.2)
	Bachelor's degree/diploma	109 (35.5)
	Master's degree	129 (42)
	Doctoral degree	4 (1.3)

Table 2: Item level descriptive statistics, item-total correlation, and internal consistency reliability estimates (n=307)

Item	Mean (SD)	Median (IQR)	Cronbach's alpha*	Corrected item-total correlations ^[5]	Corrected item-total correlations [‡]
How afraid are you of acquiring COVID-19 when going into the public? (Item 1)	2.78 (0.91)	3 (1)	0.67	0.576	0.601
In your opinion, how susceptible you are for COVID-19? (Item 2)	2.21 (0.95)	2 (2)	0.74	0.155 [§]	Eliminated
How frequently are you feeling worried that you have acquired COVID-19? (Item 3)	1.64 (0.67)	2 (1)	0.71	0.378	0.334
How frequently is your sleep getting affected because of thoughts relating to COVID-19? (Item 4)	1.64 (0.8)	1 (1)	0.7	0.398	0.352
How frequently are you avoiding conversations on COVID-19-related information out of fear/anxiety? (Item 5)	2.67 (0.8)	3 (1)	0.67	0.596	0.636
How worried are you of acquiring COVID-19 when an unknown person is coming closer to you? (Item 6)	2.95 (0.84)	3 (2)	0.68	0.553	0.583
How anxious are you getting when knowing information on COVID-19? (Item 7)	2.57 (0.86)	3 (1)	0.7	0.382	0.401
In your opinion, how much COVID-19 has affected your personality (Item 8)	1.88 (0.95)	2 (1)	0.75	0.122 [§]	Eliminated
How concerned are you when people cough or sneeze because of the fear that you may acquire COVID-19? (Item 9)	2.68 (1.01)	3 (2)	0.72	0.326	0.379
How often you are thinking about COVID-19 on a routine day? (Item 10)	2.57 (0.85)	2 (1)	0.68	0.526	0.575

*Depicts internal consistency estimates of remaining items if the corresponding item is removed from the total score, ^[5]Corrected item-total correlations for the 10 item scale, [‡]Corrected item-total correlations after eliminating items 2, 8, [§]Corrected item-total correlations of <0.3. SD: Standard deviation, IQR: Interquartile range, COVID: Coronavirus disease

Table 3: Pattern matrix from exploratory factor analysis showing the factor loadings and communalities for the seven items of the coronavirus disease-19 anxiety scale

Item	Factor 1	Factor 2	Communality
How afraid are you of acquiring COVID-19 when going into the public? (Item 1)	0.661		0.579
How frequently are you feeling worried that you have acquired COVID-19? (Item 3)		0.862	0.718
How frequently is your sleep getting affected because of thoughts relating to COVID-19? (Item 4)		0.813	0.64
How frequently are you avoiding conversations on COVID-19 related information out of fear/anxiety? (Item 5)	0.788		0.675
How worried are you of acquiring COVID-19 when an unknown person is coming closer to you? (Item 6)	0.65		0.528
How anxious are you getting when knowing information on COVID-19? (Item 7)	0.749		0.503
How concerned are you when people cough or sneeze because of the fear that you may acquire COVID-19? (Item 9)	0.693		0.434
Eigen value	2.806	1.271	
Percentage of total variance explained	40.08	18.15	

KMO measure of sampling adequacy (0.731), Bartlett's test of sphericity - χ^2 (df)=503.42 (21), $P<0.001$, Extraction method: Principal components, Rotation: Promax rotation with Kaiser normalization, Factor 1: Fear of social interaction, Factor 2: Illness anxiety, KMO: Kaiser-Meyer Olkin, COVID: Coronavirus disease

and the total variance explained by the components in EFA. The mean CAS score of the study participants was observed to be 16.93 ± 3.71 , and the scale demonstrated good internal consistency reliability (Cronbach's alpha 0.736). Significant moderately negative correlation was identified between SRMH and CAS scores ($r = -0.417$; $P = 0.001$). An apparent difference was noted in the mean CAS scores based on educational qualification, with participants having higher educational qualifications demonstrating significantly lower CAS scores as analyzed using Kruskal-Wallis ANOVA ($\chi^2 [2, 303] = 38.01$; $P = 0.001^*$). The temporal stability of the instrument was established with an ICC (Two-way mixed-effects model, single rater, absolute agreement) of 0.91 between test and re-test. The seven-item CAS was provided in Appendix 1.

DISCUSSION

CAS is a brief, rapidly administrable, 7-item instrument demonstrating face and content validity, internal consistency reliability, structural validity, construct validity, and test-retest reliability. The semantic differential scale was considered over the Likert scale to eliminate the potential acquiescence bias.^[7] When the mean inter-item correlations were examined factor wise, items loaded on "fear of social interaction" factor had average inter-correlation of 0.5, where as the items on "illness anxiety" factor had an average inter-correlation of 0.42 which are within the suggested range of (0.4-0.5) by Briggs and Cheek.^[8] The mean inter-item correlation for the seven-item CAS was 0.45, suggesting that the two sets of items are better represented as a summary measure. It is for this reason of inter-correlation



between items from both the factors that the decisions of using promax rotation and representing CAS score as an aggregate measure of “fear of social interaction” and “illness anxiety” scores were made. It was identified that subjects with higher educational qualifications had lesser CAS scores, the rationale for which could be better information seeking and accessing behaviors, more scientific outlook compared to people with lesser educational qualifications. Furthermore, a significant negative correlation was identified between CAS and SRMH scores. In spite of the aforementioned associations of CAS scores with educational qualification and SRMH scores, more thorough approaches could be adopted to establish the construct validity of the scale, such as determining the correlation between CAS and perceived vulnerability to disease scale scores.^[9]

One of the limitations of this study includes the loading of only two items on the “illness anxiety” factor. Although a minimum of three items for factor is suggested, it is not uncommon in literature for subscales to have two items.^[10,11] It is also important to acknowledge here that the construct of anxiety relating to COVID-19 is dynamic in nature, and the additional dimensions of “fear of losing livelihood post COVID-19” and “uncertainty about future” have emerged with prolonged lockdown since the beginning of this study. Therefore, future directives for psychometric research with regard to COVID-19 would be to strengthen the factor structure by the addition of relevant items and to encompass the ever-changing construct with emerging dimensions. Based on the results from this initial validation, we conclude that CAS is a rapidly administrable, valid, and reliable tool that can be used to measure COVID-19-related anxiety among the Indian population.

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APPENDIX

Please rate your perceptions on the following questions. Select the circle that closely reflects your perception.

1) How afraid are you of acquiring COVID-19 when going into the public?
 Extremely afraid Not at all afraid

2) How frequently are you feeling worried that you have acquired COVID-19?
 Always Never

3) How frequently is your sleep getting affected because of thoughts relating to COVID-19?
 Always Never

4) How frequently are you avoiding conversations on COVID-19 related information out of fear/anxiety?
 Always Never

5) How worried are you of acquiring COVID-19 when an unknown person is coming closer to you?
 Extremely worried Not at all worried

6) How anxious are you getting when knowing information on COVID-19?
 Extremely anxious Not at all anxious

7) How concerned are you when people cough or sneeze because of the fear that you may acquire COVID-19?
 Extremely concerned Not at all concerned

Appendix 1: COVID-19 Anxiety Scale.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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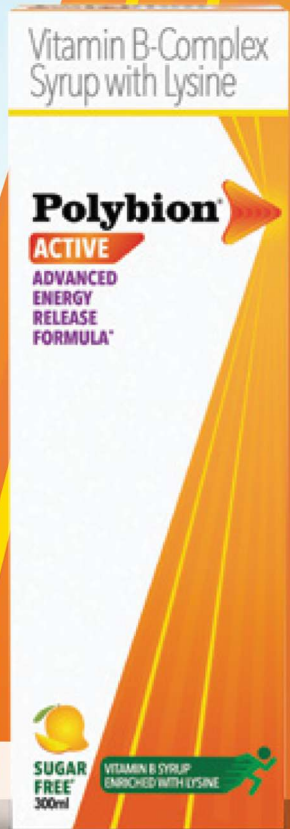
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Aspiring a Paradigm Shift in the Current Understanding of Oral Health Promotion by Testing the Possibility of Deriving Requisite Manpower Using Basic Clinical Data: An Epidemiological Investigation

Abstract

Introduction: Majority of rural dental outreach programs focus on screening the subjects attending the programs and providing oral health education for them. There has only been limited emphasis on the provision of care as a part of the outreach activities for the geographically disadvantaged rural population. The objective of this study is to check whether the basic clinical data as collected in the form of number of decayed teeth can be a valid and reliable predictor in calculation of requisite time for provision of restorative services by developing a predicting equation from the data obtained on 400 subjects in outreach activities and subsequently testing the predicting general linear equation for predictive accuracy. **Materials and Methods:** The number of decayed teeth in each of the participants was recorded along with collection of demographic data from the study participants. Data obtained from the 400 participants was used to generate a predicting equation after running a backward stepwise multiple linear regression. The equation was subsequently tested among a subsample of 200 participants from the exploratory sample and an independent validatory sample of 200. SPSS version 20 software, multiple linear regression, Wilcoxon signed rank test, Mann Whitney U test were used in data analysis. **Results:** Number of decayed teeth was observed to be a single, significant predictor of the man hours required in provision of restorative care. The predicting equation generated had good predictive accuracy and predictive stability as observed from the non-significant differences between the requisite time calculated using the predicting equation and that clinically determined by the calibrated examiner among both the subsample of exploratory sample and the validatory sample. **Conclusion:** The predicting equation generated in this study accurately and consistently estimated the requisite man hours necessary for provision of restorative oral health care in outreach programs.

Keywords: *Community outreach, health workforce, linear models*

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Introduction

Despite the lack of contemporary, comprehensive oral health survey records in India, various oral health surveys in different parts of the country suggest that oral health status continues to be poor over the years. Oral health promotion has long been recognised as the solution for improvement of oral health status. Many authors have proposed various strategies for oral health promotion and discussed in depth on how oral health promotion differs from oral health education in important ways.^[1,2] Provision of oral health services for people is at the heart of oral health promotion. Community outreach programs in the rural and remote areas without

access to oral health care services, while being an important prospective way for improvement in oral health, often restrict to oral health screening and bringing awareness among public about the importance of oral health. Though raising awareness on oral health is laudable, national and international experiences suggest that improved knowledge does not necessarily contribute towards better outcomes. Therefore, outreach programs need to focus on provision of care without curtailing the scope of such programs to screening and oral health education. Mere screening programs have been remarked to

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be one way tickets to disheartenment by letting the people know about their problems without provision of services which is against the cardinal ethical principles.^[3] However, provision of services in outreach programs demands thorough preparation on the part of care providers in terms of identification of required manpower, material, and formulation of strategic plans. While few programs have been in existence for quite some time, majority of oral health outreach programs are sporadic in nature without a concrete basis for recruitment of resources.^[4]

With the aforementioned background, the objective of this study is to check whether the basic clinical data as collected in the form of number of decayed teeth can be a valid and reliable predictor in calculation of requisite time for provision of restorative services by developing a predicting equation from the data obtained on 400 subjects in outreach activities and subsequently testing the predicting general linear equation for predictive accuracy in two ways: a) on a randomly split sample of 200 from the “exploratory group” b) on a “validatory” sample of 200, independent of the exploratory sample.

Materials and Methods

This study to develop a predicting equation of requisite man hours and subsequent validation for predictive accuracy and stability was conducted in the outreach programs organized by a teaching dental institution in the neo capital region of Andhra Pradesh between March and May, 2019. Ethical approval for the study was obtained from the institutional ethical committee of SIBAR Institute of Dental Sciences (Pr. 183/IEC/SIBAR/2018) on December 12th, 2018. All the subjects attending the outreach programs for oral examination and treatment were informed about the study and consent was obtained from those willing to participate. The number of decayed teeth was recorded using World Health Organization (WHO) 1997 recommendations.^[5]

After recording basic clinical data, the subjects were clinically examined by a single examiner, calibrated for clinical acumen in decision of requisite man hours for rendering restorative care with a gold standard, to document the time required for provision of care. Investigations were advised, wherever felt necessary, by the examiner for which the subjects were not charged and were provided with conveyance facilities to the teaching dental institution. The calibration exercise was done on 25 subjects attending the outpatient department of the teaching dental institution and an Intra class Correlation Coefficient (ICC) of ≥ 0.95 was deemed as sufficient agreement.

The development of predicting equation was done on a purposive exploratory sample of 400 subjects in outreach activities ensuring representation of people from different age groups, social classes, genders, and smoking habits. The sample size of 400 was arrived at based on the minimum number of events per variable (EPV) rule suggesting EPV of

50 would yield an adequate sample for development of predicting equation.^[6] SPSS version 20 software (IBM SPSS statistics for Windows version 20, Armonk, USA) was used to analyze the data. Backward stepwise multiple linear regression method was used to obtain the most appropriate model with highest Coefficient of determination (R^2) with requisite time for provision of restorative care as the regressand and number of decayed teeth as the continuous regressor, socioeconomic status^[7] as the multichotomous categorical regressor for which dummy variables were created as necessary.

Subsequently, the predictive accuracy and predictive stability of the predicting equation was tested on a subsample of 200 participants of the exploratory sample and on an independent validatory sample of 200 respectively. Lack of significant difference between the requisite man hours calculated using the predicting equation and that documented by the examiner by virtue of clinical acumen signifies the predictive accuracy of the developed model. Wilcoxon signed rank test and Mann Whitney U test were done to assess the predicting model accuracy and stability i.e. to check how well the predicting equation performed in the sub sample of exploratory sample and validatory sample respectively.

Results

Of the 400 study participants, 224 were females and the mean age of the study sample was 41.6 ± 10.25 years. The mean number of decayed teeth among study participants was 2.89 ± 2.1 with no significant differences based on gender. Significant difference in the mean number of decayed teeth was found based on socioeconomic status, while no difference was observed between subjects with and without the habit of smoking [Table 1]. Age was observed to have a strong positive correlation ($r=0.78$) with the number of decayed teeth, and therefore was not entered in the multiple linear regression model to avoid multicollinearity.

The predicting equation obtained from stepwise multiple linear regression is given below [Table 2]. Backward stepwise linear regression revealed that the number of decayed teeth is the single, significant predictor of requisite time to provide restorative care (Adjusted $R^2=0.446$); Predicted requisite time for provision of restorative care (mins) = $16.38 + 40.13 \times$ number of decayed teeth. There were no significant differences between the requisite time calculated using the predicting equation and that clinically determined by the calibrated examiner among both the subsample of exploratory sample and the validatory sample [Table 3]. These findings highlight the predictive accuracy and the predictive stability of the equation developed.

Discussion

Geographical inequalities in the distribution of dentists,^[8] lack of concrete policy on proper utilization of available oral

Table 1: Differences in mean number of decayed teeth based on gender, socioeconomic status and tobacco consumption habits

Variable	Category	n	Mean number of decayed teeth ±SD	95% CI	P-value
Gender	Male	224	2.73±1.84	2.489–2.92	0.07
	Female	176	3.09±2.13	2.77–3.4	
Socioeconomicstatus [†]	Lower	164	3.04±2.06	2.72–3.35	0.041*
	Lower middle	108	2.78±1.92	2.41–3.14	
	Middle	128	2.79±1.7	2.49–3.08	
Tobacco habit	Yes	68	2.71±1.98	2.24–3.18	0.062
	No	332	2.92±2.2	2.68–3.15	

Mann Whitney U test;[†]Kruskal Wallis ANOVA; CI, Confidence Interval;*P ≤ 0.05 considered statistically significant.

Table 2: Backward stepwise linear regression with requisite time for restorative care (in minutes) as the dependent variable

Model(n = 400)	Regressioncoefficients	Standard error	P-value
Constant	16.38	2.06	
Number of decayed teeth	40.13	3.48	0.0001*

*Statistical significance; Adjusted R²=0.446.

Table 3: Testing the predictive accuracy and predicting stability of the generated equation

Sample	Mean time (mins) required according to clinical examination	Mean time (mins) required according to predicting equation	Mean difference(S. E)	P-value
Subsample of exploratorysample (n = 200) [†]	149.34 ± 78.6	146.18 ± 74.32	3.16 (0.3)	0.13
Validatory sample (n= 200) [‡]	130.63 ± 62.2	128.57 ± 63.1	2.06 (0.27)	0.09

[†]Wilcoxon signed rank test; [‡]Mann Whitney U test; P ≤ 0.05 considered statistically significant.

health care professionals in public health systems,^[4] and lack of comprehensive documentation of oral health status on a regular basis with the national oral health survey conducted in 2002–2003 remaining the solitary comprehensive documentation of the nation’s oral health status^[9] are among the primary reasons for the observation of poor oral health status among Indian populace. The Indian context of oral health promotion is paradoxical in the fact that the nation possesses more number of dentists than the estimated requisites, yet the oral health status remains poor.^[10] Community outreach programs are considered as a pragmatic solution to address oral health inequalities to some extent. However, the referral services offered at teaching dental institutions through outreach programs are underutilized. Only subjects with oral health needs perceived by themselves tend to utilize the referral services, while those whose needs were disclosed to them after identification by the oral health professionals tend to neglect the oral health problems as most of the oral health care services provided across the globe are curative in nature after the subjective perceive his/her oral health problem. Preventive services and care for problems with negligible impact on quality of life are

often untended.^[11] It is also for this reason that treatment services at outreach sites are extremely important. The clinical data recorded as part of surveys and outreach programs tend to be too basic that it is challenging to compute the number of man hours required in provision of requisite care for a person and the community as a whole. For instance, recording Decayed, Missing, Filled teeth index (DMFT) gives us valuable insights into the magnitude of caries experience in a community. But the index does not facilitate computation of man hours required in provision of restorative care for the subject as the severity of the carious lesion would not be recorded as a part of the index. This brings up the need for more sensitive clinical examination which bears the drawback of consuming too much time than what is practically possible in outreach scenarios. It is not always possible to spend the same amount of time with a subject in outreach activities, as much as the time spent per patient in a clinical setting. This discrepancy in the amount of time spent per person must be understood in light of reach versus intensity. Therefore it would be worthwhile if the computation of man hours required to provide oral health care can be done based on the fundamental clinical data

recorded in surveys and outreach programs. Such computation of man hours from basic clinical data would eliminate unnecessary wastage of time in identification of requisite manpower and aid service providers in preparing for the provision of oral health services by estimating the requisite man hours in advance.

The findings from this study were observed to be coherent with the hypothesis that the severity of carious lesions increases with an increase in the number of decayed teeth in light of the magnitude of increase in requisite man hours with an increase in number of decayed teeth. This study provides an important direction in the estimation of required resources in outreach programs. Outreach activities are usually conducted with major participation from house surgeons of the corresponding institutions. Therefore, it would be extremely beneficial if the accurate estimation of requisite time for provision of care could be derived based on the basic clinical data documented by these dental students. It is to be acknowledged here that lack of insights into the requisite resources, especially manpower, often times is responsible for the idea of provision of care in outreach programs getting aborted even before its conception. Prior estimation of these resources would facilitate provision of care in outreach programs without confining the scope of the programs to mere screening.

Conclusion

The predicting equation generated in this study accurately and consistently estimated the requisite man hours necessary for provision of restorative oral health care in outreach programs. Directions for future research include estimating the resources required for provision of comprehensive oral health care for the communities based on different clinical parameters.

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Conflicts of interest

There are no conflicts of interest.

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The influence of tobacco consumption on periodontal health: A stratified analysis based on type of tobacco use

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ABSTRACT

Introduction: Though the negative influence of tobacco consumption on the periodontal status of an individual is established, the magnitude of this association based on the type of tobacco used is seldom investigated. **Aim:** The aim of this study is to check the differences in the periodontal status of individuals based on their current tobacco consumption status and type of tobacco consumption. **Materials and Methods:** This study was conducted among subjects attending a teaching dental institution in Andhra Pradesh. Subjects were divided into three groups based on their current tobacco consumption status. The following parameters were recorded after examination by two calibrated investigators: number of sites with bleeding on probing; number of teeth with periodontal pockets; number of teeth with clinical attachment loss of ≥ 5 mm; number of teeth lost. SPSS version 20 software was used to analyze the data. **Results:** While current users demonstrated poor periodontal status than the former and nonusers, there was a significant difference in periodontal health between former users and nonusers in all the study parameters except the mean number of teeth lost. Among current users, the mean percentage of sites with bleeding and attachment loss > 5 mm were found to be least among smokers compared to tobacco chewers and those who consume both smoke and smokeless forms of tobacco. **Conclusion:** The study confirms the negative influence of tobacco consumption on periodontal health and also establishes the increased destruction of clinical attachment levels among tobacco chewers compared to smokers.

Keywords: Periodontal attachment loss, smokeless tobacco, tobacco use

Introduction

Though the tobacco consumption habits among the Indian population have been reported to be decreasing, statistics relating to the prevalence of tobacco use confirm that more

than one-quarter of the nation's populace still consume tobacco in one form or the other. Khaini and Beedi are among the most commonly used smokeless and smoke forms of tobacco used in India, respectively. Clear gender variations are seen in the adoption of this deleterious habit, with 42.4% of men and 14.2% of women reporting the use of tobacco. There are statewide variations in the prevalence of tobacco consumption with Andhra Pradesh (20%) demonstrating considerably lesser prevalence compared to the national average (28.6%).^[1] It is

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an established fact that the incidence of tobacco use is higher among people belonging to lower socioeconomic strata and a cyclical relationship has been reported between tobacco consumption by poorer sections and further deterioration of their socioeconomic status owing to the negative health-related consequences of tobacco use which include coronary artery disease, emphysema, chronic bronchitis, peptic ulcer, lung, oral cavity, and gastrointestinal tract cancers.^[2,3] Periodontal diseases refer to the disintegration of connective tissue that anchors the tooth and could result in tooth mortality. Poor periodontal health is one of the many adverse health consequences of tobacco use which did not receive enough emphasis, especially in light of the significant negative effect of periodontal diseases on the quality of life of the affected.^[4] It is this increased incidence of periodontal diseases among tobacco users that offers an opportunity for the oral health care professionals to inquire about the tobacco consumption habits of their patients, discuss with them the deleterious effects of tobacco, and motivate them towards quitting the habit.^[5]

Despite the high self-reported rate of attempting to quit the habit, the majority of people, who attempt quitting, resume using tobacco due to a variety of reasons.^[6] The poor supporting environment is one of the major reasons which preclude an individual from quitting the habit of tobacco use. Previous studies highlighted the socioeconomic inequalities in the success of quit attempts, with people belonging to poorer socioeconomic strata being less successful.^[7] Considering the fact that oral health care services at dental institutions are more commonly utilized by economically disadvantaged sections and the inextricable association between tobacco use and negative oral health outcomes. Ministry of Health and Family Welfare, Government of India in collaboration with various stakeholders including Dental Council of India published a book “Establishment of Tobacco Cessation Centers in Dental Institutes—An Integrated Approach in India—Operational Guidelines 2018.”^[8] All the dental institutions in the country have been directed to establish tobacco cessation clinics following the aforementioned directives, and it was noted by the council that 12 dental institutions have not established tobacco cessation centers as directed, as on 27th of September, 2019.^[9] In this context, it becomes important to just not to assess the association between tobacco use and periodontal disease but to establish the magnitude of influence of tobacco on periodontal status of an individual based on the type of use to better get informed and motivate the subjects with tobacco consumption habit towards quitting.

Materials and Methods

This cross-sectional study was conducted among subjects attending the outpatient department of a teaching dental institution in coastal Andhra Pradesh. The teaching dental institution serves a huge demographic and people from different socioeconomic strata. The study protocol was approved by the institutional ethical committee (2_IEC-KIMS-19) and informed consent was obtained from all the study participants before the

clinical examination. The sample size was determined to be 98 in each group using G*power 3.1.9.2 software and 100 subjects were included in each of the three study groups. The three study groups are as follows: current tobacco users who reported use of 100 cigarettes or other smoking forms of tobacco in their lifetime and are currently smoking every day or some days and/or chewing tobacco products at least 20 times in their life and are currently chewing tobacco products every day or some days; former tobacco users who reported use of 100 cigarettes or other smoking forms of tobacco in their lifetime and had quit smoking at the time of the study and/or chewing tobacco products at least 20 times in their life and had quit chewing tobacco products at the time of the study; and nonusers who had never smoked or chewed tobacco products in their life or who reported use of less than 100 cigarettes or other smoking forms of tobacco and less than 20 times use of chewing tobacco products.^[10,11] The intention of the investigators was to recruit current users first and then identify the age and gender-matched subjects in the former user and nonuser groups. However, since none of the subjects in the current user group were females, there was no question of gender matching and subjects were only matched on their age. The study was conducted from February 2019 to July 2019. All the subjects underwent an interview seeking information about oral hygiene practices, systemic health, followed by a clinical examination to record the following parameters: number of sites with bleeding on probing; number of teeth with periodontal pockets; number of teeth with clinical attachment loss of ≥ 5 mm; number of teeth lost. Two investigators participated in the data collection process. The intraclass correlation coefficient values for the two examiners ranged from 0.89 to 0.94 for various parameters demonstrating a good interexaminer reliability. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 20 software (IBM SPSS Statistics for Windows version 20, Armonk, NY, USA). One way analysis of variance with Tukey's post-hoc tests and Chi-square test were used to analyze the data.

Results

The mean age of the study participants was 52.13 years with a standard deviation of 10.82. Current users were observed to be older compared to former users and nonusers. There was a significant difference in the recorded clinical parameters between the three study groups [Table 1]. While current users demonstrated poor periodontal status than the former and nonusers, there was a significant difference in periodontal health between former users and nonusers in all the study parameters except the mean number of teeth lost. Gingival recession was documented to be more common among current users. Pairwise comparisons revealed significant differences among all possible pairs in the clinical parameters considered with current users having poor periodontal status than former users and nonusers [Table 2]. The only exception was the lack of difference in the mean number of teeth lost between former users and nonusers. When the current users were stratified based on the type of tobacco use, a significant difference was observed between these groups with regard to the mean percentage of sites with a bleeding, mean

Table 1: Comparison of clinical parameters between the three study groups

Parameter	Group	n	Mean±SD	Between group sum of squares	Mean square	F	P
% of bleeding sites	Current user	100	25.1±5.34	4337.247	2168.62	72.685	0.001*
	Former user	100	21.03±6.46				
	Nonuser	100	15.8±4.38				
Mean number of sites with periodontal pockets	Current user	100	5.37±3.16	534.32	267.16	35.55	0.001*
	Former user	100	3.95±2.98				
	Nonuser	100	2.11±1.89				
Number of sites with attachment loss ≥5 mm	Current user	100	7.32±3.43	1187.94	593.97	84.94	0.001*
	Former user	100	4.4±2.49				
	Nonuser	100	2.48±1.72				
Number of teeth lost	Current user	100	4.8±2.57	331.74	165.87	36.53	0.001*
	Former user	100	2.79±2.02				
	Nonuser	100	2.4±1.7				

One way ANOVA; P≤0.05 considered statistically significant; *denotes statistical significance

Table 2: Multiple pairwise comparisons of the clinical parameters between the study groups

Parameter	Reference Group	Comparison Group	Mean difference	P
% of bleeding sites	Current user	Former user	4.07	0.001*
		Nonuser	9.29	0.001*
		Former user	5.22	0.001*
Mean number of sites with periodontal pockets	Current user	Former user	1.42	0.001*
		Nonuser	3.26	0.001*
		Former user	1.84	0.001*
Number of sites with attachment loss ≥5 mm	Current user	Former user	2.92	0.001*
		Nonuser	4.84	0.001*
		Former user	1.92	0.001*
Number of teeth lost	Current user	Former user	2.01	0.001*
		Nonuser	2.4	0.001*
		Former user	0.39	0.4

Tukey's post hoc tests; P≤0.01 considered statistically significant; *denotes statistical significance

number of teeth with periodontal pockets, and the mean number of teeth with attachment loss greater than 5 mm. There was no significant difference in the number of teeth lost based on the type of tobacco use [Table 3]. Multiple pairwise comparisons revealed that tobacco chewers had a higher mean percentage of bleeding sites compared to smokers and those who reported using both smoking and smokeless tobacco forms. Participants who reported the use of both smoking and smokeless forms of tobacco were observed to be having more attachment loss compared to exclusive chewers and smokers [Table 4]. Graphical representation of the differences in clinical parameters between current and former tobacco users, non users was provided in Figure 1. Figure 2 shows the differences in clinical parameters among current tobacco users based on the type of tobacco use.

Discussion

In light of the established association between periodontal status and the systemic health of an individual, it is understood that tobacco consumption deteriorates systemic health both directly and in an indirect manner, with poor periodontal status assuming a mediating role. Primary care physicians and oral health care professionals play a key role in advising and supporting the subjects in the process of quitting tobacco. Also, oral health is increasingly being emphasized as an essential component

of primary care.^[12] Often oral health care facilities serve as the primary contact points for people in the diagnosis of chronic conditions and in engaging the subjects to seek requisite medical care.^[13] Literature suggests that dentists administering an efficient tobacco cessation program could achieve a 10–15% quit rate in a year.^[14] In this context, oral health care professionals are expected to play a key role in improving the health status of the communities they are serving in, by proactively involving in tobacco cessation counseling.^[15]

It is unfortunate that the habit of tobacco consumption is on the rise in developing nations.^[16] The acquisition of tobacco chewing habits in different forms is more alarming. In the present study, it was found that 82% of the current tobacco users have tobacco chewing habits, while 49% reported using smoking forms of tobacco. These numbers are in accordance with the Global Adult Tobacco Survey 2016–17 data.^[1] The reasons for increasing the use of smokeless forms of tobacco are the relatively cheaper cost of these products and less revealing the nature of their use compared to smoking.^[16] The study results reinforce the negative influence of tobacco use on periodontal status. Though there are numerous studies evaluating the impact of tobacco use on the periodontal health of an individual, this is one of the first studies which attempted to comprehensively document periodontal status by considering different clinical

Table 3: Comparison of clinical parameters among current users based on the type of use

Parameter	Group	n	Mean±SD	Between group sum of squares	Mean square	F	P
% of bleeding sites	Chewer	51	28.1±4.006	1227.18	613.59	37.96	0.001*
	Smoker	18	18.78±2.88				
	Both	31	23.83±4.56				
Mean number of sites with periodontal pockets	Chewer	51	6.33±3.36	99.13	49.56	5.37	0.06*
	Smoker	18	4.19±2.84				
	Both	31	4.67±2.52				
Number of sites with attachment loss ≥5 mm	Chewer	51	7.29±3.6	29.04	14.52	1.23	0.04*
	Smoker	18	6.27±3.49				
	Both	31	7.74±3.29				
Number of teeth lost	Chewer	51	4.88±2.71	11.05	5.52	0.82	0.4
	Smoker	18	4.11±1.99				
	Both	31	5.06±2.64				

One way ANOVA; P≤0.05 considered statistically significant; *denotes statistical significance

Table 4: Multiple pairwise comparisons of the clinical parameters among current users based on the type of use

Parameter	Reference group	Comparison group	Mean difference	P
% of bleeding sites	Chewer	Smoker	9.32	0.001*
		Both	4.25	0.001*
		Smoker	-5.06	0.001*
Mean number of sites with periodontal pockets	Chewer	Smoker	1.67	0.038*
		Both	2.14	0.071
		Smoker	0.47	0.85
Number of sites with attachment loss ≥5 mm	Chewer	Smoker	1.02	0.046*
		Both	-0.45	0.83
		smoker	-1.47	0.04*

Tukey's post-hoc tests; P≤0.01 considered statistically significant; *denotes statistical significance

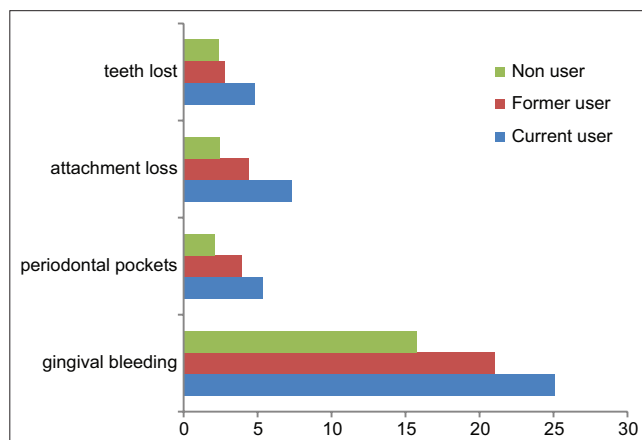


Figure 1: Bar chart showing the differences in clinical parameters between the study groups

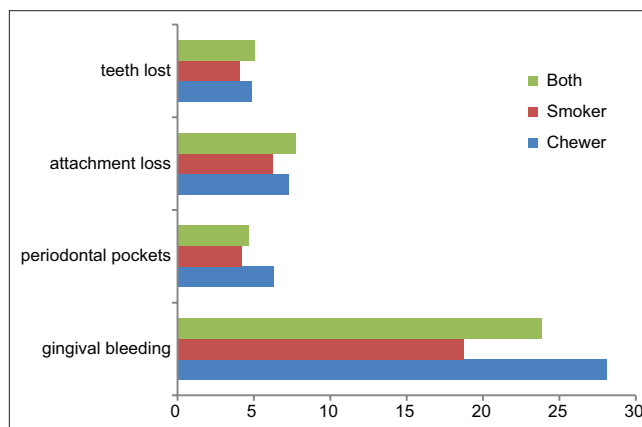


Figure 2: Bar chart showing differences in clinical parameters based on the nature of tobacco use

parameters. It was identified in the present study that current users have a significantly higher percentage of sites with bleeding compared to former users and nonusers. Among current users, exclusive smokers had the least incidence of gingival bleeding. These results are in compliance with those reported by Gautam *et al.*^[17] and Feldman *et al.*^[18] The possible reason for this finding is the inhibitory effect of nicotine, one of the many harmful by-products of tobacco smoke, on the manifestation of the early signs of periodontal disease, because of its local vasoconstrictive action.

The observation made in the present study that current users have a significantly higher number of teeth with periodontal pockets is inconsistent with the studies done by Gautam *et al.*^[17] Akaji *et al.*, and^[19] Katuri *et al.*^[20] Among current users, chewers demonstrated a significantly higher number of teeth with periodontal pockets which are in accordance with the studies conducted by Robertson *et al.*^[21] and Chu *et al.*^[22] It was observed in this study that the number of teeth with attachment loss greater than 5 mm was more in those participants who reported the use of both forms of tobacco compared to exclusive smokers and chewers. This is in accordance with the studies reported by

Katuri *et al.*,^[20] Monten *et al.*,^[23] Amarasena *et al.*,^[24] and Mittal *et al.*^[25] Verma *et al.*^[26] reported greater attachment loss among tobacco chewers in a study conducted among gutkha chewers in the Delhi NCR region. The rational explanations for the more severe destruction of periodontal attachment apparatus among subjects consuming smokeless tobacco forms are manifold. Few justifications in this direction could be comparatively high blood nicotine levels among chewers than smokers, mechanical injury and irritation caused by holding smokeless forms of tobacco in close proximity to the gingival tissue, and higher incidence of gingival hyperemia among chewers caused by the aggravation of inflammatory reactions by smokeless tobacco.^[26] The results from this study inform the oral health care professionals about the form-dependent association between tobacco and periodontal health and help them better play their roles in the arena of primary health care. There is a necessity for these care providers to undergo training on tobacco cessation counseling, since the majority of tobacco users already know the negative influence of tobacco on oral and general health, and it is not only the knowledge that needs to be shared but also the attitude that needs to be changed.^[27,28]

Conclusion

The study confirms the negative influence of tobacco consumption on periodontal health and also establishes the increased destruction of clinical attachment levels among tobacco chewers compared to smokers. In light of the proven negative association between periodontal disease and systemic health of an individual, these findings give a heads up for the dental fraternity to get more actively involved in tobacco cessation counseling and directly participate in health promotion, in general, and oral health promotion in particular.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Attitudes and Perceived Barriers in Geriatric Dental Care among Undergraduate Dental Students in Capital Region of Andhra Pradesh

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Abstract

Background: This is the era of the elderly. In India, there is an exponential increase in the aging population. Increase in longevity means that the community will have to bear a greater burden of disease. The general health and well-being of older adults are related to their oral health. **Aim:** The to study the perceived barriers and attitudes of undergraduate dental students towards geriatric dental care. **Materials and Methods:** Four hundred and eighty senior dental students participated in the present cross-sectional questionnaire study. The first sought background information including gender, age, prior treating in geriatric dental care, and prior experience in treating the elderly in their education. The responses were categorized in a Likert scale. Data were analyzed using SPSS version 20. $P < 0.05$ was considered as statistically significant. Fisher exact test was used to know the attitude to work with elderly and the barriers in elderly dental care. Gender-wise comparison of mean attitude score toward the elderly was done. **Results:** The perceived barriers of dental students' showed significant association with respect to financial ability of the elderly ($P = 0.017$), follow-up of elderly patients ($P = 0.04$), elderly patient compliance ($P = 0.05$), and inadequate communication skills in treating elderly patients ($P = 0.05$). Females (2.500 ± 0.48) showed positive attitude when compared to male (1.166 ± 0.19) study subjects. The total attitudes mean score was 1.87 ± 0.34 . **Conclusions:** Even though dental students were willing for elderly dental care, student's inadequate knowledge of geriatric care, poor communication skills, and lack of confidence in management of elderly patients are the identified barriers.

Keywords: Ageing, attitudes, barriers, dental students

INTRODUCTION

Geriatric dental education can be defined as that portion of the predoctoral dental curriculum that deals with special knowledge, attitude, and technical skills required in provision of oral health care to older adults.^[1,2] According to the population census 2011, there are nearly 104 million elderly persons (aged 60 years and above) in India. Of them, 53 million were female and 51 million were male. A report released by the United Nations population fund and help age India suggests that the number of elderly persons is expected to grow to 173 million by 2026. Both the share and size of the elderly population is increasing over time. From 5.6% in 1961, the population has increased to 8.6% in 2011. For males, it was marginally lower at 8.2%, while for females, it was 9.0.^[3] Aging is a natural process. Old age should be

regarded as a normal, inevitable biological phenomenon. During the latter half of the 20th century, the age composition of the population changed dramatically, with more people living to older ages and the older population getting older. This demographic change will have a major impact on the delivery of general and oral-health care, as well as on the providers of these services. Although some older adults have physical and/or psychological conditions that require special attention in the dental office setting, one should not assume that all older

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people share common conditions.^[4,5] To evaluate a specific patient, the dentist must understand the cultural, psychological, educational, socioeconomic, dietary, and chronologically specific experiences that may have influenced his or her life. Oral health and status are affected by similar factors, and they are the accumulation of a person's life experiences with dental care, as well as with caries, periodontal diseases, and iatrogenic diseases.^[6-8] Increased knowledge of aging has previously been found to have only a modest effect on improving attitudes toward older people.^[9] To develop competence in managing geriatric patients, dental students must undergo educational experiences that result in development of special clinical skills and a caring attitude toward the elderly.^[10] Training in geriatric dentistry would enable the dentists to understand and empathize with the psychological behavior of the elderly, especially those suffering from some chronic medical illness.^[11] Negative attitudes toward the elderly are not unique to dental professionals. The apparent acceptance of edentulous state as the ultimate result of aging rather than as a pathological process has resulted in the lack of seriousness associated with the treatment of dental problems of the aged.^[12] Many studies have shown the attitudes of health professionals, in general, to be negative toward the elderly.^[13-16] Such studies have not been conducted in India to measure the attitudes of dental students toward the elderly. The present study was conducted with the aim to know the association of perceived barriers in elderly care and to assess the attitude of undergraduate dental students toward the geriatric population.

MATERIALS AND METHODS

A cross-sectional questionnaire study was carried out among the students from clinical years of two teaching dental institutions in the Capital Regional Development Authority of the state of Andhra Pradesh. Ethical clearance (reference no 132/IEC/SIBAR/2017) was obtained from the Institutional Ethical Committee of the teaching dental institution. The study sample consisted of 480 undergraduate dental students who deal with geriatric patients in their clinical postings. The sample size was estimated using the formula $n = Z\alpha^2 Z\beta^2 pq/d^2$. Here, $Z\alpha$ was taken under 95% confidence level, $Z\beta$ was taken under 80% power of the test, and the given permissible error as 6% was allowed. All the students who were available in the period of March 2017 to February 2018 were included in this study. Among those, 199 were final-year students and 281 were interns. A structured, hand-delivered, and self-administered questionnaire was used for data collection. The validity of the questionnaire was checked by test-retest method and the Cronbach's alpha value was 0.8. The contents of the questionnaire were according to the geriatric dentistry guidelines and their objectives of dental education.^[17-21] Information obtained from the students included personal data such as age, gender, training regarding geriatric care, and their perceived barriers in the provision of dental care for geriatric patients. Responses regarding perceived barriers were recorded using 5-point Likert scale ranging from 1 = not important,

2 = very important, 3 = no idea, 4 = important and to 5 = very important. The attitudes of students toward the elderly were measured using the geriatric attitudes scale. The responses were recorded using a Likert scale with five categories ranging from strongly disagree = 1 to strongly agree = 5. A response of "strongly disagree or disagree" indicates a negative response and "strongly agree or agree" is indicative of positive response. A response of "not sure" was considered to be reflective of uncertainty or indecision. Informed consent was obtained from all the participants before the questionnaire was administered. Statistical analysis was done using SPSS Version 20 software (IBM SPSS statistics for Windows version 20, Armonk, NY, USA) and descriptive statistics and Chi-square test were done for analyzing the data. Owing to the limited number of responses in few categories of independent variables, categories were merged to facilitate the ease and relevance of the statistical analysis. In those, Fisher's exact test was used for the analysis. $P < 0.05$ was considered as significant.

RESULTS

Of 480 undergraduate dental students, 64 (13.3%) were male and 416 (86.7%) were female. The mean age of the study subjects was 21.58 ± 1.143 . The minimum age was 19 years and maximum 25 years. Among those, 199 were final years and 281 were interns. Sixty percentage reported not receiving any training in the provision of dental care for geriatric subjects. About 78.3% of dental students were showed a willingness to work for elderly patients [Table 1]. Most of the students (72.9%) positively answered the statements: "Looking after old people is a social duty," "listening to past experiences of old people is interesting and I pay more attention and I behave more understandingly to my old patients than my young ones." A total of 68.8% disagreed with the statement that old people do not add much to society [Figure 1]. Regarding barriers to provide elderly care, approximately half of the dental students believed that the financial ability of the elderly patient to pay for services, lack of appropriate facilities in dental offices, and

Table 1: Distribution of the study subjects according to gender and their responses

Demographic variable	n (%)
Gender	
Male	64 (13.35)
Female	416 (86.7)
Year wise study subjects	
Final years	199 (41.45)
Interns	281 (58.54)
Received any training in geriatric care	
Yes	192 (40)
No	288 (60)
Willingness to work for elderly	
Yes	376 (78.3)
No	22 (4.6)
No difference	82 (17.1)



insufficient knowledge of geriatric dental care are common barriers. More than half of the students opined that follow-up of the elderly patients is difficult and is an important barrier for the provision of dental care [Figure 2]. Attitudes of the dental students showed significant results with respect to financial ability of the elderly ($P=0.017$), follow-up of elderly patients ($P=0.04$), elderly patient compliance ($P=0.05$), and inadequate communication skills in treating elderly patients [$P=0.05$ and Table 2]. The mean attitudes based on gender, females (2.500 ± 0.48) showed a positive attitude when compared to male (1.166 ± 0.19) study subjects. The total attitude mean score was 1.87 ± 0.34 [Table 3].

DISCUSSION

This study was designed to explore undergraduate dental students' knowledge of geriatric dental care and their attitudes toward the elderly. The rapid growth of the elderly population emphasizes the need for geriatric dental care knowledge in dental professionals.^[22] Dental providers' beliefs, attitudes, and comfort level with treating older patients are major factors in encouraging or discouraging dental care utilization by the elderly.^[9] Some studies addressed dentists' and dental students' opinions about treatment of the elderly in long-term care facilities, but few studies have focused on dental students' barriers for providing dental care for elderly patients.^[18,23-26] The first- and second-year

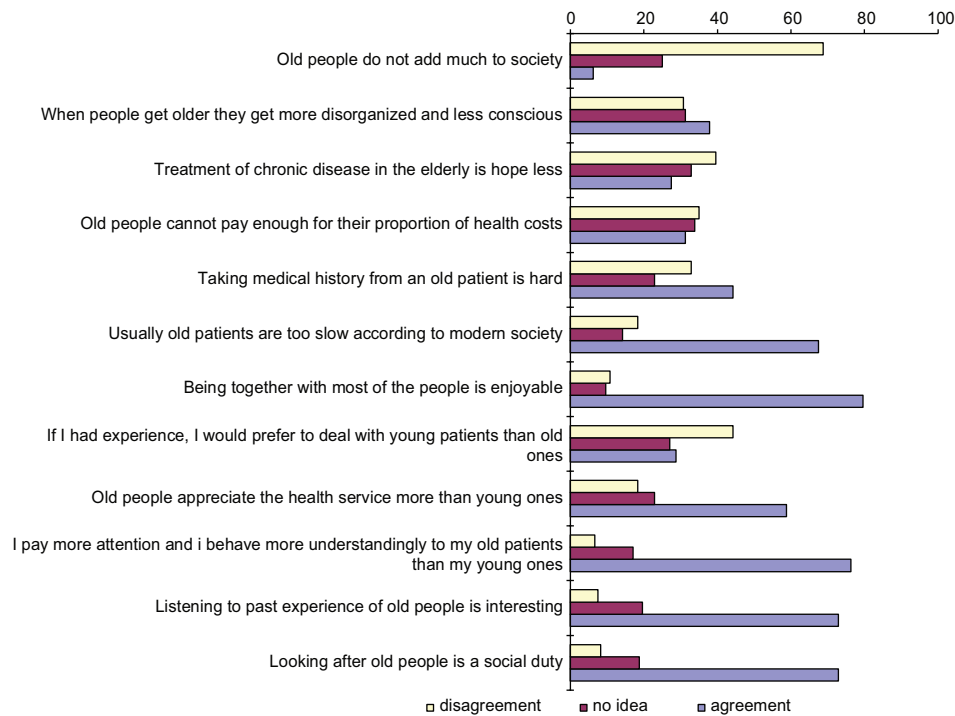


Figure 1: Percentage of dental students' agreement with statements given about elderly people

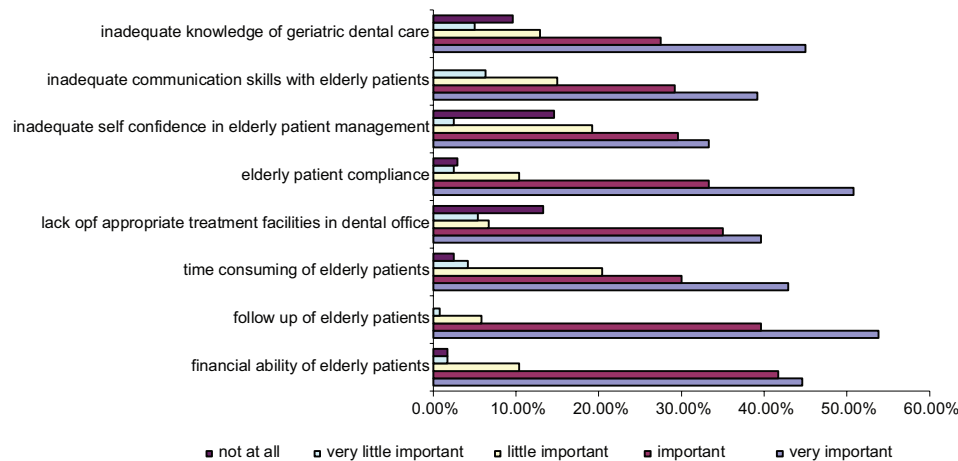


Figure 2: Undergraduate dental students' responses to important barriers for the provision of dental care to elderly patient for each of eight items (ranged from very important to not at all)

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Table 2: Attitude of dental students to work with the elderly and the barriers in elderly dental care

Barriers	Willingness for elderly dental care			P
	Yes, n (%)	No, n (%)	No difference, n (%)	
Financial ability of elderly patients				
Very important	148 (39.4)	4 (18.2)	62 (75.6)	0.017*
Important	174 (46.3)	14 (63.6)	12 (14.6)	
Little important	38 (10.1)	4 (18.2)	8 (9.8)	
Very little important	8 (2.1)	0 (0.0)	0 (0.0)	
Not at all	8 (2.1)	0 (0.0)	0 (0.0)	
Follow up of elderly patients				
Very important	234 (62.2)	14 (63.6)	10 (12.2)	0.004*
Important	122 (32.4)	8 (36.4)	60 (73.2)	
Little important	16 (4.3)	0 (0.0)	0 (0.0)	
Very little important	4 (1.1)	0 (0.0)	0 (0.0)	
Time consuming of elderly patients				
Very important	162 (43.1)	14 (63.6)	30 (36.6)	0.062
Important	120 (31.9)	8 (36.4)	16 (19.5)	
Little important	70 (18.6)	0 (0.0)	28 (34.1)	
Very little important	16 (4.3)	0 (0.0)	4 (4.9)	
Not at all	8 (2.1)	0 (0.0%)	4 (4.9)	
Lack of appropriate treatment				
Very important	162 (43.1)	0 (0.0)	28 (34.1)	0.034
Important	128 (34.0)	14 (63.6)	26 (31.7)	
Little important	20 (5.3)	8 (36.4)	4 (4.9)	
Very little important	12 (3.2)	0	14 (17.1)	
Not at all	54 (14.4)	0	10 (12.2)	
Elderly patient compliance				
Very important	194 (51.6)	14 (63.6)	36 (43.9)	0.058
Important	134 (35.6)	8 (36.4)	18 (22.0)	
Little important	32 (8.5)	0 (0.0)	18 (22.0)	
Very little important	12 (3.2)	0 (0.0)	0 (0.0)	
Not at all	4 (1.1)	0 (0.0)	0 (0.0)	
Inadequate self confidence				
Very important	138 (37.1)	4 (18.2)	18 (22.0)	0.001*
Important	106 (35.6)	4 (18.2)	32 (39.0)	
Little important	70 (18.8)	14 (63.6)	8 (9.8)	
Very little important	12 (3.2)	0 (0.0)	0 (0.0)	
Not at all	46 (12.4)	0 (0.0)	24 (29.3)	
Inadequate communication				
Very important	164 (43.6)	4 (18.2)	20 (24.4)	<0.001*
Important	116 (30.9)	4 (18.2)	20 (24.4)	
Little important	44 (11.7)	14 (63.6)	14 (17.1)	
Very little important	20 (5.3)	0 (0.0)	10 (12.2)	
Not at all	32 (8.5)	0 (0.0)	18 (22.0)	
Inadequate knowledge				
Very important	180 (47.9)	4 (18.2)	32 (39.0)	0.012*
Important	106 (28.2)	8 (36.4)	18 (22.0)	
Little important	34 (9.0)	10 (45.5)	18 (22.0)	
Very little important	24 (6.4)	0 (0.0)	0 (0.0)	
Not at all	32 (8.5)	0 (0.0)	14 (17.1)	

Fisher's exact test, *Statistically significant

students were not taken because they were not in touch with patients. In this study, most of the undergraduate dental students' showed positive attitudes toward the elderly which was similar to the study done by Gupta *et al.*^[10] In the present study, the female students had comparatively more positive attitudes to the male

students which was statistically significant $P=0.000$, which was coinciding with the statement given by Gupta *et al.*^[10] According to their study, male participants had a less positive attitude when compared to the female participants. Old people do not add much to society with this almost 71.2% female participants' and 53.1%

Table 3: Gender wise comparison of the study subjects' attitude toward the elderly

Study subjects	n	Attitude score mean±SD	P
Male	64	1.166±0.19	0.746
Female	416	2.500±0.48	
Total	480	1.87±0.34	

Mann–Whitney U-test. SD – Standard deviation

of male participants' disagreement with this statement, which shows that they had much more positive attitude toward the elderly which was statistically significant $P=0.001$. Inadequate communication skills with elderly patients with this barrier, 65.6% of males and 35.1% females were considering as very important barrier which was statistically significant $P=0.001$. Majority of males (65.6%) and females (80.6%) said that they had inadequate knowledge of geriatric dental care. The study participants' inadequate knowledge of geriatric dental care, poor communication skills, and lack of confidence in management of elderly patients are the important barriers to dental provision to the elderly care. Regarding attitudes, females are more positive than males, and it seems that female dental students' were more caring and empathetic toward the elderly in the provision of dental care. Limitations of the study observed were the study sample should include a large number of subjects, then the results were more generalizable.

CONCLUSION

The study results conclude that the positive attitudes of undergraduates toward geriatric patients, even though they willing to give geriatric dental care, the lack of knowledge in giving care was one of the observed barriers for the elderly care. The key findings like 376 dental students were willing to work with elderly patients, only 22 students were not willing to work with elderly, and 82 students said that there was no difference between younger and older patients care that shows a positive attitude of the participants in the dental college toward the elderly care. The female undergraduate students were more empathetic when compared to male undergraduate students. There is a need to improve the knowledge and skills in the management of the elderly so that positive attitudes can be utilized properly to improve the quality of life of the elderly population in India. The level of knowledge and attitudes found in the present study can be improved by conducting regular educational programs.

Recommendations

The dental colleges must take responsibility for training students to meet growing elderly dental care needs. The first and foremost suggestion is that the addition of geriatric dental education to the undergraduate dental curriculum. A separate subject in the training of dental students must be introduced to provide quality dental care to the elderly population.

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Conflicts of interest

There are no conflicts of interest.

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Oral healthcare-seeking behavior and perception of oral health and general healthcare among WHO indexed age groups in East-Coast India

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ABSTRACT

Introduction: In a country where 30,570 dentists graduate per year, it is staggering to acknowledge that only 10% of dentists serve the rural people, who constitute around 68.8% of the country's population. **Aim:** To find out the oral healthcare-seeking behavior, profile, and pattern in Tenali Mandal among WHO recommended index age groups. **Materials and Methods:** A cross-sectional, multilocality, single-visit study was done to acknowledge the oral healthcare-seeking behavior, profile, and pattern in Tenali Mandal, Guntur, Andhra Pradesh. There are 9 villages, 2 peri-urban, and 1 town present in the Tenali Mandal and the study was conducted following the National Pathfinder Survey. **Results:** Around 35.4% of the study participants utilized dental services while most of them sought care in the last 1-6 months (23.4%) and the main reason for the last dental visit was tooth pain (13.1%), followed by decayed tooth without pain (6.9%). Dental care-seeking behavior among the age group of 12 years was 1.611 times more when compared to 65-74 years age group which was statistically significant ($P \leq 0.010$). For dental care-seeking behavior among gender, the males utilized 0.982 times less when compared to females which was not statistically significant ($P \leq 0.881$), while for the place of residence, urban place was 2.707 times more utilizing the services when compared to rural place which was statistically significant ($P \leq 0.001$). **Conclusion:** The results of the study indicates that the barriers of oral healthcare-seeking behavior among dental health conditions poses public health problems in the study area, as well as it is an important baseline indicator against which target for oral health improvement can be set and monitored.

Keywords: Behavior, care-seeking, India, oral health

Introduction

Oral health has long been recognized as an integral part of general health, particularly given the Indian population's growing life expectancy, oral health plays a critical role in improving an

individual's quality of life. Unfortunately, the trend indicates a rise in oral health problems in developing countries such as India.^[1] A multitude of problems exist when it comes to rural population in terms of developing oral healthcare. For a country where 30,570 dentists graduate annually, it is astonishing to note that only 10% of dentists represent rural citizens, who make up about 68.8% of the country's population.^[2] Though inaccessibility to dental care is one of the major challenges, inadequate use of facilities is yet another formidable challenge that mitigates rural India's potential

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for improving oral health status.^[3] With a pronounced diversity inherent in Indian culture, variations in oral health among people from different socioeconomic and religious backgrounds may be anticipated, considering the variations in cultural values, social norms, and oral hygiene practices.^[4]

Oral healthcare data creates an actionable roadmap to provide oral preventive health services in the primary care setting and strengthen the referral process from primary care doctors to dentistry. Oral healthcare-seeking behavior/utilization is the actual involvement of members of the public at healthcare facilities that reflects the number of visits every year or the number of individuals with at least one visit in the previous year as a significant method for oral health policy decision-making.^[5]

This study is mainly focused to determine the oral healthcare-seeking behavior in Tenali Mandal, Guntur (Dist.), Andhra Pradesh among WHO recommended index age groups.

Aim and Objectives

To find out the oral healthcare-seeking behavior, profile, and pattern in Tenali Mandal among WHO recommended index age groups. To document self-reported oral health diseases that the population suffered in recent times along with the type of service received, place, avenue, and personnel involved in delivery of health services and also to determine the factors influencing pattern of oral healthcare-seeking behavior.

Material and Methods

A cross-sectional, single-visit, multilocality, pretested (Cronbach's α value of 0.7) and interviewer-administered questionnaire-based study was done to generate evidence on oral healthcare-seeking behavior.

Study population

Both males and females of WHO recommended index age groups- 5 years, 12 years, 15 years, 35–44 years, and 65–74 years.

Sample size calculation

All the five WHO index age groups were selected from each site of 4 urban, 2 peri-urban, and 9 rural sites where 20 individuals from each group i.e. a total of 100 individuals from each site were selected resulting in sample size of 1500 and for the age groups of 5 years, 12 years, and 15 years the questionnaire was administered to their parents/guardians.^[6]

Each site 5 age groups = 5×20 questionnaires = 100

15 sites = 100×15 = 1500 sample size.

Sampling technique

National Pathfinder Survey (Stratified cluster random sampling)

Inclusive criteria and exclusive criteria

Subjects with the following criteria were included in the study

- Both male and female subjects of WHO recommended age groups.
- Subjects who are available on the day of the study

Subjects with the following criteria were excluded from the study

- Uncooperative and mentally challenged
- Nonresidents of the study area.

Ethical clearance

Ethical clearance was obtained from the Institutional Ethics Committee of SIBAR Institute of Dental Sciences with IEC protocol no: Pr. 35/IEC-SIBAR/CIR/15/B and an informed consent were obtained from all the study participants prior to the start of the study while the study was done from July 2017–September 2017.

Formulation of questionnaire

A pretested, interviewer-administered questionnaire was used which consisted of 2 parts. The 1st part of the questionnaire includes sociodemographic details, 2nd part consists of 8 questions regarding the utilization of oral health services, and a single common question regarding the importance of both general and oral health.

Through pilot test internal consistency of the questionnaire was tested and yielded a result of Cronbach's α value (0.81) which was satisfactory.

Statistical analysis

Statistical package for social sciences (IBM SPSS, Chicago) version 20.0 was used for the analysis and P value of ≤ 0.05 was regarded as statistically significant. Statistical tests such as descriptives, Chi-square test, Spearman's correlation, and binomial logistic regression were used.

Results

The study sample comprised of 1500 subjects who were equally divided among WHO index age groups. Out of 1500 subjects, 52.8% were females and 47.2% were males [Table 1] with 60% of the study participants belonging to rural areas followed by urban (26.7%) and peri-urban (13.3%). Majority of them belonged to open category (O.C) caste (53.1%)

Only 35.4% of the study participants utilized dental services while most of them sought care in the last 1–6 months (23.4%) and the main reason for the last dental visit was tooth pain (20%), followed by gingival problems (7.2%) [Table 2].

When asked about nearest available facility, its distance, place of care sought, and their experience regarding treatment

Table 1: Distribution of study population according to the demographic profile

Demographic Profile	Frequency	Percent	
Age	5 years	20.0	
	12 years	20.0	
	15 years	20.0	
	35-44 years	20.0	
	65-74 years	20.0	
	Total	1500	100.0
Gender	Male	708	47.2
	Female	792	52.8
	Total	1500	100.0
Marital status	Married	600	40
	Unmarried	900	60
	Total	1500	100.0
Place	Urban	400	26.7
	Peri-urban	200	13.3
	Rural	900	60.0
	Total	1500	100.0
Caste	ST	34	2.3
	SC	201	13.4
	BC	468	31.2
	OC	797	53.1
	Total	1500	100.0
Socioeconomic Class (BG Prasad SES 2019)	Upper class	308	20.5
	Upper-middle class	451	30.1
	Middle class	602	40.1
	Lower-middle class	95	6.3
	Lower class	44	2.9

received most of them reported utilizing private dental services (85.4%) with distance from their residence less than 5 km (52.6%) and majority sought care from private dental clinics (33.3%) followed by dental college and hospital (1.5%) while the main reason for the selection of particular center/place was accessibility (18.7%) followed by doctors reputation (11.7%) and lastly they reported that they were very much relieved from suffering after they sought care. Majority of the participants has spent around 251–500 INR (21.3%) for their last dental visit and the main mode of payment was out of pocket (34.7%) [Table 3].

Decayed tooth without pain (8%) was the prominent reason among those who had not utilized the services and predominant barriers found for not seeking care was lack of time (4%), followed by high cost (2.1%), does not affect my work (1.9%), and milk teeth shed-off (1.9%). Moreover, a majority (38.1%) of the respondents had reported that oral diseases are as important [Table 4].

Oral healthcare-seeking behavior among 12 years age group was 1.611 times more when compared to 65–74 years age group which was statistically significant ($P \leq 0.010$). For dental care service utilization among gender, the males utilized 0.982 times less when compared to females which was not statistically significant ($P \leq 0.881$), while for the place of residence, urban place was 2.707 times more utilizing the

Table 2: Distribution of subjects according to dental services utilization, last time of visit, and reasons for last dental visit

		Frequency	Percent
Utilization of dental care	No	969	64.6
	Yes	531	35.4
Last dental visit	1-6months	351	23.4
	7-12 months	38	2.5
	1-2 years	104	6.9
	2-3 years	7	.5
	More than 3 years	31	2.1
Reasons for last dental visit	Decayed tooth with pain and discomfort	300	20
	General checkup	5	0.3
	Loss of tooth	27	1.8
	Gingival problems	108	7.2
	Tooth fracture/trauma	24	1.6
	Mucosal problems	2	0.1
	Retained deciduous tooth	3	0.2
	Dentinal hypersensitivity	6	0.4
	Food lodgment	25	1.7
	Malodor	31	2.1

services when compared to rural place which was statistically significant ($P \leq 0.001$). Dental care service utilization among socioeconomic status, the upper middle class was 13.135 times more when compared to lower class which was statistically significant ($P \leq 0.001$) while for utilization among pink card ration card holders it was 1.577 times more when compared to no card holders which was statistically significant ($P \leq 0.036$) [Table 5].

Discussion

Dental health professionals should recognize that oral health is a health norm that allows a person to eat, talk, and socialize without active disease, pain, or embarrassment, and contributes to general well-being. To lead a healthy and longer life, people should be made aware of all these information.^[7]

The present study was conducted on a sample of 1500 subjects who were permanent residents of Tenali Mandal. The sample comprised of 47.2% males (708) and 52.8% females (792), showing a slight female preponderance.

Dental services utilization

Oral healthcare-seeking behavior in the present study was 35.4% and it was on par with the studies done by others, namely, 21.4% in Pradeep *et al.* (2016),^[8] 31.9% in Vikram *et al.* (2016),^[3] and 36% in Priyadarshini *et al.* (2016).^[9] While the access to care was higher in the studies done by 80.01% in Neha *et al.* (2019) and^[10] 68% Deolia (2020).^[11] It may be due to the type of region covered/ attitudes and research on trends of oral healthcare-seeking behavior and barriers towards seeking treatment provide a foundation for oral health promotion strategies being formulated and executed.

Table 3: Distribution of subjects according to the availability of the dental facility, distance, reasons for care sought, the amount spent for oral healthcare, and mode of payment during their last dental visit

		Frequency	Percent
The nearest available dental facility	Dental college and hospital	118	7.9
	Private clinic	1281	85.4
	PHC	3	0.2
	Do not know	98	6.5
Distance for the dental facility from your place	Do not know	104	6.9
	<5 km	789	52.6
	>5 km	607	40.5
Center/place where care has been sought	Govt. dental clinic	8	0.5
	Private dental clinic	500	33.3
	Dental college and hospital	23	1.5
Reasons for the selection of a particular center/place	Near	281	18.7
	Doctors reputation	167	11.1
	Better care	65	4.3
	Known doctor	18	1.2
Are you relieved from suffering after you sought care?	Very much	406	27.1
	Somewhat	118	7.9
	Undecided	7	0.5
Expenditure for last dental visit In INR	1-250	24	1.6
	251-500	319	21.3
	501-1000	117	7.8
	1001-5000	60	4.0
	>5000	1	0.1
Mode of payment	Out-of-pocket (OOP)	521	34.7
	Government hospital/ schemes	10	0.7

Table 4: Distribution of subjects according to various oral health problems suffered and reasons for not seeking care to their perception on the importance of oral health in comparison with general health

		Frequency	Percent
Various Dental problems of the participants who have not utilized the services	Maligned tooth	23	1.5
	A decayed tooth without pain	120	8.0
	Loss of tooth/teeth	21	1.4
	Swollen gums	8	.5
	Fractured tooth	15	1
	Mobile tooth	7	.5
	Malodor	32	2.1
	Dental treatment is lengthy/frequent visits are required	20	1.3
	Fear of procedures and instruments	26	1.7
	Lack of time off from work (morning or evening)	60	4.0
Reasons for refraining from seeking dental care	Laidback attitude	50	2.4
	Dental Treatment is Expensive	31	2.1
	I will go to a dentist only when I no longer can bear the pain	11	.7
	Milk teeth will shed off	28	1.9
	Extremely Important	172	11.5
Do you think oral health is as important as general health	Important	399	26.6
	Moderately Important	368	24.5
	Little Important	109	7.3
	Not Important at all	311	20.7
	Do not know / Cannot say	141	9.4

Center/place where care has been sought

In the total study population, 94.16% sought care from private dental clinic, though a bit higher but similar to studies which have reported 90%, 68.25%, and 68.25% by Thomas *et al.* (2011),^[12] Vikram *et al.* (2017),^[4] and Vikram *et al.* (2014),^[13]

respectively which was explained by the fact that the majority of the study participants reported private clinic (85.4%) as the nearest center and which was less than 5 km (52.6%) from their place of residence and this was evident from the study by Devaraj and Eswar (2011)^[14] where place of residence and

Table 5: Binomial logistic regression between dental services utilization and independent variables

Variables	OR	95% CI		P
		Lower	Upper	
Age groups				
5 years	0.121	0.074	0.199	0.001*
12 years	1.611	1.119	2.319	0.010*
15 years	0.777	0.539	1.121	0.177
35-44 years	1.236	0.854	1.790	0.261
65-74 years (constant)	1.000	---	---	---
Gender				
Males	0.982	0.776	1.243	0.881
Females (constant)	1.000	---	---	---
Place				
Urban	2.707	2.041	3.591	0.001*
Peri-urban	1.497	1.050	2.133	0.026*
Rural (constant)	1.000	---	---	---
SES				
Upper class	8.539	2.489	25.593	0.001*
Upper-middle-class	13.135	4.432	38.930	0.001*
Middle class	5.948	2.035	17.383	0.001*
Lower-middle class	5.182	1.650	16.278	0.005*
Lower class(constant)	1.000	---	---	---

OR=Odds Ratio, CI=Confidence Interval, * Statistically significant

income/month were significantly associated with dental service utilization.

Reasons for selection of particular dental center/ place

Around 18.7% of the respondents reported that the main reason for selection of particular service was accessibility near, which was on par with the study done by Vikram *et al.* (2017).^[4] This could be due to the fact that the private dental clinics and most of the dental college hospitals are situated within the city limits or even in the peri-urban regions and very less or virtually no dental care services are available in the rural areas. Usually the dental treatments are complex, hence, multiple visits are needed for its completion and also appointments may take longer as dental colleges will be working only during week day with day shifts mostly, which acts as a barrier for working class because missing 1 day at work, they may lose wages for 1 day's pay. Government clinics do not provide wide variety of services and the aim of the service was to relieve dental pain of the patients and they provide service for free while the treatment includes extraction and medication.

Since oral healthcare-seeking behavior was a phenomenon, which gets affected by multitude of factors, it was almost inevitable that differences exist between the studies, similarly in the present study there was complimentary and contrasting results which were observed.

Reasons for last dental visit

Decayed tooth with pain and discomfort was the main reason for last dental visit and it was on par with the studies, 58.5% in Salunke *et al.* (2019),^[15] 46.32% in Vikram *et al.* (2017),^[4] 71% in

Priyadarshini *et al.* (2016),^[9] and 68.5% in Shailee *et al.* (2013).^[16] Besides, in the study done by the Shekhawat *et al.* (2019)^[17] the main reason for last dental visit was decayed teeth followed by the tooth pain. Pain was an important factor in the utilization of dental services, and may be caused by pulpal, periodontal, and oral lesions where etiology and treatment management require an interdisciplinary approach.^[18]

Reasons for refraining from seeking dental care

The main reason for not seeking care was lack of time off from the work where as other studies like Sarika *et al.* (2014),^[19] Ravneet *et al.* (2014),^[20] Bindu *et al.* (2016),^[21] and Pradep *et al.* (2016)^[8] mainly reported the high costs of dental treatments as the main reason for not seeking care viz. 90.03%, 57.5%, 68.6%, and 48%, respectively, where as poor attitudes towards seeking oral care was reported by Vikram *et al.* (2017)^[4] with 43.75% and finally accessibility and affordability was also mentioned as a reason Anehosur *et al.* (2016).^[22] This might be due to the fact that most of the participants were under 15 years and majority being females along with poor attitude towards dental problems due to lack of knowledge. Females who are engaged in agriculture; besides, executing the household responsibilities, may restrict to spend more time in the quest of seeking oral healthcare. According to Uma Shankaret *et al.* (2012),^[23] they are largely dependent on other family members, and decisions regarding matters such as visits to the dentists are made by others while two-thirds being 15 years and below, the responsibility towards their children depends on parents attitude and anxiety. For preadolescents and adolescent's, dental attendance and compliance with preventive advice will be influenced by their stage of psychological development as mentioned by Nandhini *et al.* (2013).^[24] Health behaviors are closely connected with ways of living while theories from sociology, education, and psychology describe learning and behavioral change in any individual as well as in mothers of young children. This was also proved in the study done by Sujlana *et al.* 2016.^[25]

Last time of visit for seeking dental care

In the present study, 23.4% have visited a dentist within last 6 months, which was in accordance with the study done by Devaraj and Eswar (2012)^[4] where 31.4% of the study participants utilized services in less than 6 months. Time since last dental visit represents dental care that was initiated by the people and therefore, could reflect personal motivation and independent decision making.

Mode of payment during their last dental visit

In the present study, 98.11% of the participants have paid in the form of out-of-pocket for their dental care expenses and mostly they spent around 251–500 INR. This was more when compared to study done Naidu *et al.* 2014 (54.5%).^[26] Since our country does not provide dental insurance for most of the common people in India, the magnitude of out-of-pocket expenses on dental care was almost always 100% (Garcha *et al.* 2010)^[27] which was not the case in countries like the United

States (Manski *et al.* 2002)^[28] and Australia (Marshall and Spencer. 2006)^[29] which have governmental or insurance support.

Various dental problems of the participants who have not utilized the services

Decayed tooth without pain was the main reason for not seeking care showing the negligent behavior and this could be due to the fact that people do not go to a dentist unless severe symptoms appear as dentistry was perceived to be a useful service only when necessary but it was not a crucial part of overall health. This reflects their compliance towards one's oral health and the low priority given to oral problems as people think dental problems were not life-threatening.^[14]

Perception on importance of oral health in comparison with general health

Around 34.06% of the study population had reported that oral health was as important as general health followed by 24.5% moderately important while 7.3% said that it was little important and 20.73% had reported that oral health was not important as general health.

Most oral diseases share common risk factors with NCDs (noncommunicable diseases) such as cardiovascular diseases, cancers, diabetes, and respiratory diseases. These risk factors include unhealthy diets (particularly those high in added sugars), tobacco, and alcohol use. They result in a very similar pattern of inequalities in oral and general disease burden between different population groups.^[30]

With the global improvement in life expectancy, a life-course approach to oral health will become more important. Different ages in life have different oral health needs, and the specific problems of older people, who are often also suffering from other diseases, are becoming more prevalent. Knowledge and awareness of the close associations between oral and general health are thus important for holistic care, as was the collaboration between oral and general health professionals.^[31]

With inadequacy to tackle social and material determinants and incorporate oral health into general health promotion, millions suffer intransigent tooth problems and poor quality of life and end up with a great suffering.^[32] Only a broader integrative strategy that takes account of the common risk factors and the root determinants of health will result in fair and equitable approaches in promoting better oral health and general health.

By validating the data on oral health provides the picture of prevalence, trends of oral disease occurrence, care sought by the populace, and also the economic impact of oral health and on overall health, by providing primary health care physicians to establish reasonable care.

Generalizability

The sampling process assured representativeness of the population and contributes to the validity of the study. On

the other, the process of training and standardization that the research went through, and also by the high concordance obtained through kappa test ensured the reproducibility of the data.

Conclusion

The study revealed inadequate utilization of oral health services due to the fact that dental problems are not considered as life-threatening situations when compared to general health problems. This unveiled the need of health education and awareness programs to remove the disparities in oral healthcare-seeking behaviors. The study revealed that lack of time, accessibility, and usage of self-care remedies are the most common reasons for not utilizing professional dental and general health services, respectively. There is a need to minimize these barriers by motivating people and making them aware about the oral and general health problems so that they develop positive attitude towards healthcare utilization.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.


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Prescription writing skills among Dental Students in East-coast India.

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ABSTRACT

Introduction:

Prescription writing is one of the core competencies expected from medical and dental fraternity. Prescribing appropriate drugs in correct doses may influence a patient's health positively.

Aim & Objective:

Due to concern growing regarding incomplete or ill eligible prescriptions, the present survey was undertaken to assess the prescription writing skills of interns and postgraduates in a dental college located in Guntur city.

Material and methods:

Questions regarding awareness are assessed for patient's details, doctor's information, and their legibility using self-administered, pretested, validated semi-structured questionnaires. The collected data was analyzed using SPSS v.20 and subjected to Chi-square, Pearson's correlation coefficient tests, where p-value ≤ 0.05 is considered as statistically significant.

Results:

84.1% of students seek the need for reinforcing classes during their third and fourth years of training for prescription writing. In prescribing a drug 50.8% of students are using the generic name and 49.2% are using trade names. 15.1% of students are not concerned about patient education and discharge counseling on their medicines. 14.3% of students don't know that legible handwriting is important for prescription writing. 32.5% of students stated that the faculties are not discussing prescription writing as a part of case discussion while 67.5% of students stated yes among which 38.1% of students opined that they rarely discuss.

Conclusion:

The awareness and perceptions regarding the prescriptions writing skills of the students were found to be substandard and it requires the use of a standardized prescription format.

Keywords: Dental students; Drug prescription; writing skills; Prescribing Pattern; Rational Prescribing.

INTRODUCTION

Prescribing drugs is an important and complex intervention, which needs to be based on meticulous information with critical thinking, results in writing a rational and safe prescription. Decision-making and proper transcribing are quintessential hallmark of writing an ideal prescription.¹

The word, 'prescribe' is a Latin word meaning to write or to designate or order the use of as a remedy. Prescription becomes the most important clinical pharmacology tool as it not only indicates names of drugs, dosage, and duration of treatment but also acts as medico-legal document evidence of the medication access, prescription errors, prescribing errors, negligence, and further litigations which also indicating instructions to patients, pharmacists and contains essential contact information of prescriber and patient.^{1,2}

The basic and regulatory knowledge of prescription writing is essential for every dental

student to evolve into a prescribing physician. Any failure in writing an appropriate prescription results in prescription errors, which in effect lead to medication error. Prescription errors are reported to have a heavy toll on health care as in the United States, prescription errors are the 3rd leading cause of death where as similar data is not available in India.^{3,4,5}

Prescription writing is taught in the second year of dental education in India, while they start prescribing during their internship, utilization and retaining of this knowledge throughout their life is a must. Most of them learn by the tedious way by error methods even though it is taught systematically well in advance during undergraduate training.

World health organization (WHO) has made a few recommendations for optimized effective prescriptions. They are intended to list the identity and medical information of the patient, method of administration, generic names of the

medication, dosage and frequency of use and course of treatment. Care must be taken to avoid any errors in prescribing as it adversely affects the treatment outcome. Therefore, there is a need for proper education-based intervention to aid improvement in prescribing competency. ⁶

AIM & OBJECTIVE

Due to concern growing regarding incomplete or ill eligible prescriptions, the present study was undertaken to assess the prescription writing skills of interns and postgraduates in a dental college in Guntur city.

MATERIAL AND METHODS

A cross-sectional study was carried out at a Dental College and Hospital, Guntur, among the Interns and postgraduates. Students who were willing to participate were included in the study where while the approval from the Institutional Ethics Committee was obtained with the protocol No: Pr.29/IEC/SIBAR/UG/2018 whilst informed consent from the students was obtained before the study was conducted.

Out of 200 questionnaires distributed, only 126 were considered as they are filled. Questions regarding awareness are assessed for patient's details, doctor's information and their legibility using a pretested questionnaire and It was assessed for face validity, content validity, and reliability. A statistically significant Cronbach's alpha values of 0.8, Flesch-Kincaid Grade Level of 12.1 and Flesh Reading Ease score of 34.7 were obtained. The data obtained were analyzed using the Statistical Package for the Social Sciences software version 20. Chi-square & Pearson's correlation tests were done where $p \leq 0.05$ was considered as statistically significant.

RESULTS

A total of 126 dentists participated into the study with a mean age of 27.9 years, most of them belonging to 23-25 years age group. Most of the participants are female (72.2%), 54.8% are doing their internship and 45.2% are post graduate students (Table 1).

Table 2 showing the responses of the participants for various questions about prescribing medications to the patients. 98% of the students said that they have learnt about prescription writing where 89% of them had opted that they had written a prescription previously which showed the experience of the students; About 93% felt that Undergraduate training had prepared them for prescribing medication while staff discussed prescription writing as a part of case discussion (85%) and 84% felt that such discussions and writing help them in increasing

the efficiency of prescribing drugs. About 84.1% of the students felt that there is a need of reinforcing classes during III and IV BDS on prescription writing.

Almost all the students showed a great knowledge on the procedure and format of writing prescription (Table 3). Compared to interns (66.7%), postgraduates felt that their undergraduate program had trained them well in writing prescriptions ($P=0.045$, $r=0.179$) and also compared to interns (78.3%), postgraduates (82.5%) felt that there is a need of reinforcing classes during III and IV BDS for prescription writing ($P=0.557$, $r=0.052$) (Table 4). About 85.5% of interns stated that they provide enough patient education and discharge counselling on their medicines when compared to post graduates (84.2%) ($P=0.840$). Internship students find it difficult to prescribe drugs to special population compared to postgraduates (36.2%) which is statistically significant ($P=0.005$) and showed a positive correlation ($r=0.251$) (Table 4).

DISCUSSION

According to DCI revised code of ethics and regulations 2014; Duties and obligation of dentists in general prescription of drugs. 3.5 states that the dentist should take care to prescribe and administer drugs in a responsible manner and ensure safe and rational use of drugs while they should as far as possible, prescribe drugs in a generic form.⁷

In the present study there were 72.2% females which shows female preponderance while it is on par with the other studies done by Astha Doshi et al (2017)⁸ 79.5% females, Cariacy Silva de Moura et al (2014)⁹ 73%, Javad Yazdani et al (2018)¹⁰ 52.2%

In the present study 50.8% of the individuals has reported that they prescribe the drugs using generic name and 49.2% preferred Trade name whereas in the studies done by the Astha Doshi et al (2017)⁸ 43.7% Generic name & 56.3% Trade name, 43.7%, Khan et al (2013)¹¹ 58.9% Generic and 41.1% Trade name, Sudha M. J et al (2016)¹² 41.6% Generic name & 58.4% Trade name.

Only 73.8% of the participants reported that legible handwriting was required for prescription writing whereas in other studies done by Sudha M. J et al (2016)¹² 97.9%, Raghu G et al (2017)¹³ 64.2%, Naveen J.V et al (2018)¹⁴ 75% reported similar response.

93.7% has reported that the symbol R_x is important for prescription writing were as in the studies done by Khan et al (2013)¹¹ 47%, Sudha M. J et al (2016)¹² 94.7%, Naveen J.V et al



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(2018)¹⁴ 90% and Pavani V et al (2011)¹⁵ reported similar responses.

The main source of drug related information was mainly sought from Both Books & Internet which accounts for 38.9% followed by Text books 23.8%, Internet 17.5%, Drug Index 9.5% while in other study done by Ankit Jain et al (2016)¹⁶ Sought information from Staff 36.7%, Pharmacology course 23.5%, Classmates 18.2%. 67.5% of the participants reported that prescription writing was discussed as a part of case discussion followed by rare during case discussion 23.8% and Regular discussion by 11.1% while in the study done by Sudha M. J et al (2016)¹² during case discussion 23.8%, Discussion more often 44%, Regular discussion 9%.

Only 89.7% of the study participants reported that date as important item in prescription writing whilst in the studies done by Akram et al¹⁷ 29%, Khan et al (2013)¹¹ 22.3% of students did not include a date on the prescription while testing their prescription writing skills. While 90.5% & only 46.8% of the study participants reported that patient address and patient name. Recording the patient's particulars on the prescriptions helps the pharmacist make sure that prescription held by the patient really belongs to him/her. Indicating the name of the patient and the date of the prescription are important in avoiding misappropriation and loss. Whereas in the Rauniar et al (2008)¹⁸ study it was noted that the most common errors were related to the prescription date and address of the patient.

89.7% of the participants have reported that While prescribing, they take time to instruct the patient regarding usage of medicines which was slightly lower when compared to the study done by Astha Doshi et al (2017)⁸ 99.8%. If the drug should be administered before or after meals, during the day or before bedtime, lapses in writing may also cause possible harm to the patient or may result in failure of medication.

CONCLUSION

In this study, several lacunae were observed in the prescription writing skills of the dental students and it can be hypothesized that there is lack of awareness regarding rational prescribing of drugs among the students. Therefore, Continuing Dental Education (CDEs) and workshops and training classes are recommended from time to time to update their Knowledge.

LIMITATIONS

Since the study has been conducted in one dental teaching institution, the results cannot be generalized. Metacentric studies on larger sample

size are further suggested to know the drug prescribing pattern prevailing in country among dentists.

RECOMMENDATIONS

- 1) Blended learning could provide a favorable transition from the pre-clinical to clinical years with enhanced flexibility, location convenience and time efficiency.¹⁹
- 2) Clinical governance, use of electronic computerized system of prescribing and continuing professional educational programs.
- 3) Usage of a standard prescribing chart, educating, and training the prescribers followed by clinical discussions will help to inculcate the habit of writing a rational prescription.²⁰

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Table 1: Demographic details according to participants age, gender, and academic year

Demographics		Frequency	Percent
Age	20-22 years	38	30.2
	23-25 years	54	42.9
	26-28 years	21	16.7
	29-30 years	6	4.8
	31-33 years	2	1.6
	34-36 years	2	1.6
	≥ 37 years	3	2.4
Gender	Male	35	27.8
	Female	91	72.2
Academic year	Internship	69	54.8
	Post graduation	57	45.2



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Table 2: Frequency distribution for various questions regarding prescription writing

Questions		Frequency	Percent
1. Have you learnt the prescription writing	No	28	22.2
	Yes	98	77.8
2. Have you written a prescription previously for any common ailments	No	37	29.4
	Yes	89	70.6
3. Do you feel that your undergraduate training has prepared you to prescribe	No	33	26.2
	Yes	93	73.8
4. Does staff discuss prescription writing as a part of case discussion?	No	41	32.5
	Yes	85	67.5
If Yes, how often	No	41	32.5
	Very rare	20	15.9
	Rare	28	22.2
	Often	23	18.3
	Regular	14	11.1
5. Do such discussions and writing help in efficient prescribing?	No	20	15.9
	Yes	106	84.1
6. Do you need any reinforcing classes during third and fourth year of training for prescription writing	No	20	15.9
	Yes	106	84.1
7. While prescribing a drug which name do you prescribe	Trade Name	62	49.2
	Generic name	64	50.8
8. Do you Provide patient education and discharge counseling on their medicines	No	19	15.1
	Yes	107	84.9
9. Do you find it difficult to prescribe medicines for special population (like-Pregnant, children, liver failure, renal failure, heart failure etc.)	No	33	26.2
	Yes	93	73.8
10. Did you have enough training about pediatric drugs	No	100	79.4
	Yes	26	20.6
11. Where do you obtain drug related data (Source of drug information)	Physicians' desk reference	11	8.7
	Drug index	12	9.5
	Indian pharmacopeia	2	1.6
	Textbooks	30	23.8
	Internet	22	17.5
	Both books and internet	49	38.9

Table 3: Participants perceptions on the importance of various options of prescription writing

Among the following which options are important for prescription writing	Yes	No	Don't know
Date	89.7 %	1.6%	0.8%
Name of doctor	89.7%	6.3%	4%
Address of the doctor	46.8%	43.7%	9.5%
Phone number of the doctor	69.8%	24.6%	5.6%
Patient name	90.5%	6.3%	3.2%
Patient address	46.8%	44.4%	8.7%
Patient phone number	61.9%	34.9%	3.2%
Patient's age	98.4%	0.8%	0.8%
Gender	95.2%	3.2%	1.6%
Weight of the patient	82.55	12.7%	4.8%
Symbol – R̄	93.7%	4.8%	1.6%
Frequency of usage of drug	98.4%	0.8%	0.8%
Duration of usage of drug	96%	2.4%	1.6%
Generic Name of the drug	70.6%	22.2%	7.1%

Brand Name of the drug	63.5%	23%	13.5%
Dose of the drug	97.6%	1.6%	0.8%
Patient instructions	89.7%	6.3%	4%
Legible Hand writing	73.8%	11.9%	14.3%
Signature and registration number	72.2%	10.3%	17.5%
Instructions to pharmacist	55.6%	25.4%	19%

Table 4: Perceptions and knowledge of dental students regarding prescription writing

QUESTIONS		INTERNS	PG's	Sig.	R value
Do you feel that your undergraduate training has prepared you to prescribe?	Yes	66.7%	82.5%	0.045*	0.179
	No	33.3%	17.5%		
Do you need any reinforcing classes during third and fourth year of training for prescription writing?	Yes	78.3%	82.5%	0.557	0.052
	No	21.7%	17.5%		
Do you Provide patient education and discharge counseling on their medicines?	Yes	85.5%	84.2%	0.840	-0.018
	No	14.5%	15.8%		
Do you find it difficult to prescribe medicines for special population?	Yes	62.8%	36.2%	0.005*	0.251
	No	86%	14%		
Did you have enough training about pediatric drugs?	Yes	15.9%	84.1%	0.123	0.024
	No	28.1%	71.9%		
Source of drug information	Physicians' desk reference	14.5%	1.8%	0.001*	0.130
	Drug index	5.8%	14.0%		
	Indian pharmacopeia	0.0%	3.5%		
	Textbooks	27.5%	19.3%		
	Internet	26.1%	7%		
	Both books and internet	26.1%	54.4%		

Chi-square test, R value= Pearson's correlation coefficient, * statistically significant



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Documentation of Oral Health Care Seeking Behavior and Influencing Factors through Community Consultations in the Field Practice Area of a Teaching Dental Institution

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Abstract

Background: Preventive dental visits help in the prior detection and treatment of oral diseases; therefore, to improve oral health outcomes, an opposite knowledge of the way the individuals use health services and the factors apocalyptic of this behavior is essential. **Aim:** To quantify oral health-care-seeking behavior and influencing factors in the field practice area. **Materials and Methods:** A cross-sectional study was done in the field practice area among WHO indexed age groups using a pretested and validated questionnaire in August 2017. A stratified random sampling technique was used resulting in a sample size of 200 to assess oral health-care-seeking behavior and influencing factors in this population. Obtained data were analyzed using SPSS version 20. Descriptive statistics, Chi-square test, and binomial logistic regression analysis were used to summarize the results, where $P \leq 0.05$ was considered as statistically significant. **Results:** The results of this study evinced a female preponderance of 64% with less proportionate (26%) of the study population seeking oral health services, out of which private dental clinic occupied the first choice in using dental services (17%). A carious tooth without pain was the most common dental problem irrespective of age (47.5%). Females were 0.392 times more likely to attend dental visits when compared to males ($P = 0.003$), while considering social class, people belonging to the upper-middle class were 0.24 times more likely to avail dental services when compared to lower social class ($P = 0.015$). **Conclusion:** This study revealed lower rates of dental services sought among the field practice area where most of them availed private clinics compared to government clinics.

Keywords: Behavior, community, consultations, dental health services, oral health

INTRODUCTION

Although the mindful of thought exists as oral health an additionally integral part of general health, when it comes about the usage of services, it is the other way around suggestive of barriers in obtaining services, unawareness or ignorance of oral health condition, and socioeconomic status (SES) which have already been documented in previous studies.^[1-5]

Community consultations are one of the effective vehicles to educate policymakers and health-care professionals regarding important areas of perceived health-care needs for developing effective programs or policies.^[6]

Generating evidence requires a complex undertaking that calls for proven procedures and protocols and cross-disciplinary, cross-cultural expertise. An exchange of versions and views as part of the review process makes it easier to deal with regional variance, idiosyncratic interpretations, and inevitable oversights. Group discussion, including input from survey fielding staff, helps to identify comprehension problems for low-literacy

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populations and ambiguities more easily missed by someone working on his or her own. Therefore, generating meticulous instruments is essential to get reliable information on oral health-seeking behavior. To improve oral health outcomes, an adequate knowledge of the way the individuals use health services and factors predictive of this behavior is required. Therefore, this study was undertaken to generate evidence on oral health-seeking behavior in the field practice area.

This study was the first in kind, owing to the importance of WHO index age groups as they reflect the overall population, while the questionnaire was constructed by observing human behavior, conversing or interaction with people about their views, actions or beliefs to access areas that are generally not amenable to conventional questionnaires. With this background, we aimed to elicit oral health-care-seeking behavior, its profile, pattern, self-reported oral health diseases, and type of service received in the field practice area through community consultations.

MATERIALS AND METHODS

A cross-sectional, single-visit, multilocality study was done using an interviewer-administered, pretested, and validated questionnaire to generate evidence on oral health-care-seeking behavior and influencing factors.

Study area

Field practice area: Area of the study and practice located within 10 km radius of the dental teaching institution.

Period of the study

Data were collected in the period of August 2017.

Sampling method

Stratified random sampling technique

The study area was divided into four sites, i.e., north, east, west, and south directions around the field practice area, where from each site, one ward is randomly chosen; further data were collected from 10 members of each WHO age group from each site resulting in the sample size of 200.

Study population

We included both males and females of WHO-recommended index age groups: 5 years, 12 years, 15 years, 35–44 years, and 65–74 years. In the present study, the data were collected using an interviewer-administered questionnaire, while for the age groups, i.e. 5 years, 12 years, and 15 years, the questionnaire was administered to their parents/guardians/caretakers.

Inclusive criteria

Individuals with the following criteria were included in the study:

1. Both males and females of WHO-recommended age groups
2. Individuals who are present on the day of the study.

Exclusion criteria

Individuals with the following criteria were excluded from the study:

1. Noncooperative and mentally challenged
2. Who are nonresidents of the field practice area.

Ethical clearance

Ethical clearance was obtained from the institutional ethical committee with protocol no: 45/IEC/SIBAR/15, and informed consent was obtained from all the study participants before the start of the study.

Developing a questionnaire

Initially, the topic was explained to the public chosen on a random basis ($n = 40$) from the same locality where the study was planned to conduct, and then, the participants were divided into groups constituting 2–3 members for each group, as with larger groups, some people may not participate.

They were asked about their comments regarding health-seeking behavior that were recorded into domains, and finally, the results of group work were integrated into the closing discussion and refer back to those comments made, when relevant, in subsequent sessions until there are no more responses stated. Finally, the comments were sorted and made into questions for further evaluation of psychometric analysis.

Evaluation of psychometric properties of the questionnaire

Initially, face validity was tested which signified the validity of the question and adequate representation of each and every aspect that totally balances the coverage of the issue.

Further content validity was checked to know whether the questionnaire covers the domains to be measured, and it was done by quantifying eight experts' degree of agreement regarding the content relevance of the questions from the department of public health dentistry.

Content validity was calculated by the formula:

Calculation of content validity ratio (CVR)^[7]

$$CVR = n - N/2/N/2$$

where n is the number of experts who gave a rating of essential for an item

N is the total number of experts

where values can range from -1 to $+1$ and those closer to $+1$ suggest that a majority of experts agree there is an association between item and domain. In the present study, the content value of the questionnaire is 0.75, that is, adequate.

A pilot study was done in the month of July 2017 on a sample of 25 participants, to check the feasibility of the study using a semi-structured, pretested, validated, interviewer-administered questionnaire. Through the pilot test, the internal consistency of the questionnaire was tested and yielded a result of Cronbach's α value of 0.81. The questionnaire consist of two parts, the first part comprises of demographic details of the study participants where the socioeconomic status (SES) was calculated using BG Prasad SES scale^[8] and the second part consists of questions which were based on the frequency of dental care visits, reasons, and barriers for dental visits.^[8]

Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS, Chicago, IL, USA) version 20.0 was used for the analysis, and data obtained were summarized using descriptive statistics, Chi-square test, and binomial logistic regression analysis, where $P \leq 0.05$ was considered as statistically significant.

RESULTS

The total study population comprises 200 participants, out of which 36% were male and 64% were female. Twenty-six percent of the participants sought dental services where the majority of them sought care from private dental clinics (16%), followed by teaching dental institutions and hospitals (8%), while least from the government dental clinic (2%).

Out of 26% of the study participants who have sought dental services, a different portion of participants were using them for different reasons under four domains. The main reasons for the selection of particular dental services were due to better and quick treatment, followed by accessibility, while the main reason for the last dental visit was carious teeth without pain (12%). Most of the participants have visited the dentist in the last 1–2 years, followed by 1–6 months. Most of the participants have spent 251–500 INR for treatment, while the mode of payment was mostly through out of pocket (86.5%), followed by usage of government hospital/schemes (13.5%) [Table 1].

According to the age groups considered, 65–74 years had suffered from most of the dental problems, and in all the age groups, the most commonly suffered dental problem by the participants was tooth decay without pain that was found to be statistically significant ($P = 0.028$) [Table 2].

According to the distribution of various barriers for seeking services with respect to age, 12 and 65–74 years of age groups have reported with a maximum number of barriers, and among all the age groups, negligence toward the dental problems was found to be statistically significant ($P = 0.001$) [Table 3].

According to BG Prasad (2017) SES scale, lower-middle class was found to be the predominant class suffered by almost all the dental problems, but the common dental problem suffered by all the classes was tooth decay without pain [Table 4], while the middle and lower-middle classes have experienced almost all the barriers compared to other classes for exercising oral health care, and the common barrier was found to be negligence toward dental treatment [Table 5].

Binomial regression analysis was done by taking oral health-seeking behavior as the dependent variable and gender, social class, and age groups as independent variables. Females were 0.392 times more likely to attend dental visits when compared to males ($P = 0.003$), while considering social class, people belonging to the upper-middle class were 0.24 times more likely to avail dental services when compared to a lower social class ($P = 0.015$). Participants of 5 years, 12 years, and 15 years were more likely to utilize dental

Table 1: Distribution of participants with reference to care

Domain	n (%)
Individuals who sought care	54 (26)
Place of care sought	
Private dental clinic	34 (16)
Dental college and hospital	16 (8)
Government dental hospital	4 (2)
Better and quick treatment	18 (8.5)
Near	15 (7.0)
Friends' advice	6 (3.0)
Dentists' reputation	5 (2.5)
Less cost	4 (2.0)
Known dentist	4 (2.0)
Free of cost	2 (1.0)
Reasons for last dental visit	
Carious teeth without pain	25 (12.0)
Tooth pain and swelling	12 (5.5)
Periodontal problems	10 (5.0)
Both periodontal and carious teeth problems	5 (2.5)
General checkup	2 (1.0)
Time of last dental visit	
1-6 months	18 (8.5)
1-2 years (12-24 months)	19 (9.0)
2-3 years (25-36 months)	8 (4.0)
More than 3 years (≥ 37 months)	9 (4.5)
Cost of dental treatment (INR)	
<250	7 (3.5)
251-500	26 (12.5)
501-750	9 (4.5)
Above 750	12 (5.5)
Mode of payment	
Out of pocket	46 (86.5)
Government hospital/schemes	8 (13.5)

services with odds 13.61, 1.78, and 3.41, respectively, when compared to participants belonging to higher age groups that are 65–74 years [Table 6].

DISCUSSION

The development of the questionnaire is one of the prime requisites to draw authoritative conclusions. To generate evidence on oral health-care-seeking behavior of the public, an instrument that reflects the inner essence of the public being studied requires meticulous construction without which comprehension of the health-seeking behavior cannot be achieved. At first, investigators need to be given support materials, for example, texts, and other information relevant for their part in producing instruments, including information on the target audience and administrative mode.

Following the methodological way, an instrument was developed to assess the oral health-care-seeking behavior of 200 participants among WHO-recommended index age groups in the field practice area. The developed schedule was then subjected to participants who revealed that 26% exercised oral health care.



Table 2: Distribution of different dental problems suffered according to age

Age group	Edentulous (%)	Deposits (%)	Dental hypersensitivity (%)	Tooth pain (%)	Mobility (%)	Tooth decay (%)	Fracture (%)	P
5	-	-	-	1 (2.5)	-	7 (17.5)	-	0.028*
12	-	1 (2.5)	-	4 (10)	-	4 (10)	4 (10)	
15	-	1 (2.5)	1 (2.5)	1 (2.5)	-	1 (2.5)	2 (5)	
35-44	-	1 (2.5)	3 (7.5)	2 (5)	2 (5)	2 (5)	-	
65-74	1 (2.5)	3 (7.5)	1 (2.5)	3 (7.5)	4 (10)	5 (12.5)	-	

*Statistically significant. Chi-square test

Table 3: Distribution of various barriers for oral health-seeking behavior according to age

Age group	Costly (%)	Doesn't affect my daily work (%)	No time (%)	Milk tooth (%)	Negligence (%)	Lack of accompanying person (%)	P
5	1 (2.5)	-	2 (5)	3 (7.5)	2 (5)	-	0.001*
12	1 (2.5)	1 (2.5)	3 (7.5)	-	6 (15)	2 (5)	
15	3 (7.5)	-	1 (2.5)	-	2 (5)	-	
35-44	1 (2.5)	5 (12.5)	1 (2.5)	-	3 (7.5)	-	
65-74	4 (10)	-	-	-	6 (15)	4 (10)	

*Statistically significant. Chi-square test

Table 4: Distribution of different dental problems suffered according to socioeconomic status

SES	Edentulous (%)	Deposits (%)	Dental hypersensitivity (%)	Tooth pain (%)	Mobility (%)	Tooth decay (%)	Fracture (%)	P
Upper class	-	-	-	-	-	-	-	0.872
Upper-middle class	-	3 (10)	-	4 (13.3)	-	3 (10)	1 (3.3)	
Middle class	-	1 (2.4)	1 (2.4)	1 (2.4)	-	4 (9.8)	3 (7.3)	
Lower-middle class	1 (1.1)	2 (2.2)	3 (3.3)	6 (6.6)	4 (4.4)	7 (7.7)	1 (1.1)	
Lower class	-	-	1 (2.9)	-	2 (5.9)	5 (14.7)	1 (2.9)	

Chi-square test

Table 5: Distribution of various barriers for health-care-seeking behavior according to socioeconomic status

SES	Costly (%)	Doesn't affect my daily work (%)	No time (%)	Milk tooth (%)	Negligence (%)	Lack of accompanying person (%)	P
Upper class	-	-	-	-	-	-	0.214
Upper-middle class	2 (6.7)	3 (10)	-	3 (10)	3 (10)	-	
Middle class	3 (7.3)	1 (2.4)	1 (2.4)	-	3 (7.3)	2 (4.9)	
Lower-middle class	3 (3.3)	5 (5.5)	3 (3.3)	-	10 (11)	3 (3.3)	
Lower class	2 (5.9)	-	3 (8.8)	-	3 (8.8)	1 (2.9)	

Chi-square test

In spite of the presence of the dental institution in the area of the study, the oral health-seeking behavior was found to be very low and the results are not conclusive with the other studies done.^[9-12]

Regardless of so many dental colleges in India and mass of graduating dentist every year, researchers have reported that heaps of barriers in seeking dental care services were confronted by consumers with the factors such as presence or absence of dental symptoms, poverty, geographical region, social status, religion, race or ethnicity, occupation, income, marital status, and community type.^[13]

Participants who sought care from private dental clinics (16%) were in agreement with the study done by Bommireddy

VS *et al.* (2017),^[14] whereas in another study done by Nirma R *et al.* (2014),^[15] most of the participants sought care from dental college. However, none of the studies documented the validity and reliability of the schedule. The reason for consulting private dental clinics as the choice could be due to mushrooming of the private dental clinics in and around the city even to the nearest villages located next to city limits in the peri-urban area resulting in the provision of accessibility and might also due to the fact that they provide quick treatments when compared to dental colleges and government clinics. Typically, dental procedures are complex, multiple visits are required to complete them, and even appointments may take longer because dental colleges only operate during the weekdays resulting in missing a day at

Table 6: Binomial logistic regression between health-care-seeking behavior and independent variables

Model	Beta	Significant	OR Exp B	95.0% CI for B	
				Lower bound	Upper bound
Gender (female)	-0.936	0.003	0.392	0.209	0.735
Dependent variable: Dental visit, reference=male					
Upper-middle class	-1.407	0.015	0.245	0.079	0.763
Middle class	-0.658	0.245	0.518	0.171	1.570
Lower-middle class	-0.514	0.313	0.598	0.221	1.622
Dependent variable: Dental visit, reference=lower class SES					
5 years	20.797	0.997	13.61	0.000	0.0000
12 years	0.325	0.486	1.785	0.554	3.458
15 years	0.693	0.155	3.417	0.769	5.198
35-44 years	-0.305	0.499	0.906	0.304	1.787
Dependent variable: Dental visit, reference=65-74 years' age group					

SES - Socioeconomic status, OR - Odds ratio, CI - Confidence interval

work and may lose one day's pay. Besides government clinics do not provide a wide variety of services limiting the utilization of dental services.

The reason for not exploiting health-care facilities by lower SES population maybe that experiencing budgetary, social and material burdens that bargain their capacity to bear the expenses of dental services and benefits to live in salubrious condition; furthermore, low financial status people have more fatalistic notions about their well-being and have a lower perceived need for care, leading to less self-care and lower usage of preventive health services.^[16] People high in dental anxiety usually stay away from consistent dental care and avoid dental care in the case of dental emergencies.^[17]

There is a lack of oral health awareness, which affects oral health perception, behavior, and practices among all age groups. Most of the participants in the present study are females (64%), who are engaged in agriculture besides executing the household responsibilities that may restrict them to spend more time in the quest of seeking oral health care. For long-term ailments, rural Indian women are three times more inclined to abandon treatment than rural Indian men, an inclination saw even among the nonpoor. Furthermore, the treatment costs are altogether lower for women than men.^[18] To enhance steady dental attendance, the barriers must be controlled by proper education and intervention.^[12]

The main barrier for oral health-seeking behavior in the present study was negligence towards oral health which was in accordance with the study done by Bommireddy VS *et al.* (2017),^[14] whereas high cost and unaffordability is the main barrier for dental health-seeking resulted in the studies done by Pradeep Y *et al.* (2016),^[19] Bhaskar BV *et al.* (2016),^[20] Gupta S *et al.* (2014),^[9] Malhi R *et al.* (2015).^[21] The negligence towards care seeking in this study could be because of poor attitude and lack of knowledge toward dental problems suggesting awareness programmes to the parents as responsibility of child's oral health depends on parents attitude.

The present study emphasizes the importance of oral health care seeking behavior, which indicates the general population's

need for community involvement and coordination to improve their oral health.

Despite the fact that the major proportion of studies conducted in dental research are questionnaire studies where development of inventory was often neglected which may results in erroneous conclusion. Therefore, this study was done to attenuate the specious results in generating evidence through community consultations regarding oral health-seeking behavior in the field practice area.

CONCLUSION

Community consultations conducted have revealed inadequate use of dental services among the field practice area where private clinics were used more relatively compared to teaching dental college and government clinics. The main reason for the last dental visit was carious teeth without pain, while negligence toward dental problems was found to be the common barrier for oral health care. Development of inventory in this study has culminated the significance of qualitative research methods such as observing human behavior, conversing with people about their beliefs or actions, and views to access areas that are generally not amenable to conventional questionnaires.

Public health significance

This study bestowed sufficient evidence on significant barriers in accessing health-care services and highlights the importance of inventory development and also the prerequisite of effective awareness programs to increase health-seeking behavior in the field practice area.

RECOMMENDATIONS

- Changing the perception toward oral health, undertaking an effective program to build science transfer, increasing the workforce by strengthening the safety net system, and successfully partnering in all levels of society can bring healthy life
- Dental institutions should train their workforce and increase their competencies in such a way that they could

provide better and faster treatments on par with private establishments

- Insight should be given to inventory framing as most of the studies done in the research are questionnaire based.

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Conflicts of interest

There are no conflicts of interest.

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Comparative study of hydroxyapatite prepared from eggshells and synthetic precursors by microwave irradiation method for medical applications

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Abstract

Recently research on biogenic derived hydroxyapatite (HAp) is being focused. In the present study it is proposed to make a comparative analysis of microwave synthesised HAp derived from eggshell based calcium precursor with two commercial calcium sources calcium nitrate tetrahydrate and calcium carbonate using EDTA as the organic modifier. The as-synthesized samples were characterized by XRD, FT-IR, SEM and EDAX. Comparative studies were carried out with eggshell derived HAp and synthetic precursors. Eggshell derived HAp exhibited lesser decomposition into β -TCP and EDAX confirmed the presence of magnesium element which plays a key role in bone metabolism. Eggshell derived hydroxyapatite also revealed somewhat better changes in the cytocompatibility, antimicrobial activity and drug release.

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1. Introduction

The global market for biomaterials is expanding greatly year by year and there is an increasing demand for dental application materials [1]. Dental bone graft substitutes aims at the development of natural bone to repair and restore the natural tooth. Clinically dental bone grafts are utilized in different forms such as powder, paste, cement etc depending upon the nature of the repair [2]. Biocompatibility, biofunctionality, availability, multifunctionality etc are some of the major criteria for selecting materials [3]. The economic aspects that surround this social and health issue are also of paramount importance, hence any action aimed at cost reduction needs to be seriously considered [4].

Hydroxyapatite (HAp) is an important inorganic biomaterial which has attracted the attention of researchers for more than four decades [5,6]. It is one of the most effective bioceramics in the clinical repair of hard tissues due to its chemical and structural similarity with the mineral phase of bone and teeth which is composed of calcium, phosphate and hydroxyl ions with Ca/P ratio within the range known to promote bone regeneration (1.50 - 1.67) [2,7,8]. It possesses excellent biocompatibility, both in vitro and in vivo and it is also degradable in body environment [9,10]. Huge volume of reports exist in synthetic HAp derived from calcium and phosphate precursors [11,12].

The advantages of natural biomaterials are that they mostly come from an in vivo source and also constantly available in large quantities as a biowaste at a reasonable price. Natural materials such as corals, fish bone, snail shell, eggshells etc have been selected as the source for the synthesis of HAp as a mean of recycling the biowaste into a value added product for biomedical applications [13–16] Eggshell is one of the biowastes available in plenty and there are few reports on eggshell derived hydroxyapatite nanostructure for biomedical applications [17–19]. It is essential to compare the characteristic of the eggshell derived HAp with analar grade commercial precursors derived HAp. In the present study it is proposed to make a comparative analysis of HAp derived from eggshell based calcium precursor to that obtained using commercial calcium and phosphate precursors. Synthetic HAp was prepared through two different commercial calcium sources viz calcium nitrate tetrahydrate and calcium carbonate.

2. Materials and methods

2.1. Materials

The reagents ethylenediaminetetraacetic acid (EDTA), di-sodium hydrogen phosphate (Na_2HPO_4), calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$], calcium carbonate (CaCO_3), sodium hypochlorite (NaOCl) and sodium hydroxide (NaOH) were used as the starting materials which are analar grade obtained from Merck and are utilized for the experiments without any further purification. Ciprofloxacin hydrochloride monohydrate ($\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_3 \cdot \text{HCl} \cdot \text{H}_2\text{O}$) was purchased from Himedia, India. Double distilled water was employed throughout the experiments.

2.2. Methods

The raw eggshells collected were washed several times with tap water followed by distilled water and was then boiled for half an hour and dried at 110°C in an oven. Later, they were ground into powder in an agate mortar and immersed in sodium hypochlorite solution for removing the organic components present. Then, they were extensively washed with double distilled water and dried in vacuum oven for 5 h at 110°C . 14 g of eggshell powder was then mixed with 0.14M of EDTA solution to form Ca-EDTA complex. Subsequently, 0.08M of Na_2HPO_4 solution was slowly added with the obtained Ca-EDTA complex while maintaining the pH at 13 using NaOH solution and stirred for 30 min. Then, the prepared reaction mixture was kept in a microwave oven (2.45 GHz, 600 W, LG, India) and irradiated with microwave for 3 h. The white product formed was washed three times using distilled water and dried at 110°C in hot air oven for 5 h. The obtained sample was designated as EHA. For comparison instead of eggshell powder CaCO_3 and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were used as the calcium precursors in the above procedure and the powders obtained were named as HA-1 and HA-2.



2.3. Characterization

The powder X-ray diffraction (PXRD) patterns of the as-synthesized samples were recorded using a Rigaku MiniFlex II powder X-ray diffractometer in the range between $20^\circ \leq 2\theta \leq 60^\circ$. The average crystallite size was calculated from PXRD data using the Scherrer approximation [20].

$$D_{hkl} = \frac{k\lambda}{\beta \cos\theta}$$

where D_{hkl} is the average crystallite size, as calculated for the (hkl) reflection, λ is the wavelength of Cu K α radiation (1.5406 Å), $\beta/2$ is the full width at half maximum for the diffraction peak under consideration (in radian), θ is the diffraction angle (degree) and K is the broadening constant chosen as 0.9. The diffraction peak at $2\theta = 25.8$ degree was chosen for the calculation of the crystallite size because it is sharper and isolated from others which is (002) Miller's plane of the HAp crystal. The phase composition of synthesized samples was determined by following equation [21].

$$RC_i = \frac{I(hkl)_i}{\sum I(hkl)_i}$$

Where i and $I(hkl)_i$ refer to the phase of interest and intensity of the characteristic peaks of the corresponding phase, respectively, in the XRD pattern. $\sum I(hkl)_i$ is the total intensity of the characteristic peaks of the phases appeared in the XRD pattern. The functional group identification of EHA, HA-1 and HA-2 was done by recording the FTIR spectra in a Perkin Elmer FTIR spectrometer by the KBr pellet technique in the wave number range from 4000 to 400 cm^{-1} . The morphology of the as-synthesized samples was examined using a Zeiss ultra plus scanning electron microscope (SEM). The specific surface area of samples was determined by the Brunauer–Emmett–Teller (BET) nitrogen adsorption method in a porosimeter ASAP 2020.

2.4. Bioactivity test

The compacted samples (under a pressure of 24 MPa, 13 mm) were immersed in 30 ml of simulated body fluid (SBF) and incubated at 37 °C for 21 days. The SBF solution was prepared by dissolving reagents like NaCl, NaHCO_3 , KCl, Na_2HPO_4 , $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, Na_2SO_4 , $(\text{CH}_2\text{OH})_3\text{CNH}_2$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ one by one in deionized water and the pH was adjusted to 7.4 using 1 M HCl to mimic the human blood plasma. The SBF solution was refreshed once in 3 days for a period of 3 weeks [22]. Finally, the surface of the disk was analyzed using scanning electron microscope (SEM).

2.5. In vitro drug release

In vitro release of ciprofloxacin was carried out by mixing 75 mg of as-synthesized sample with 25 mg of ciprofloxacin hydrochloride and compacting it into a disk and soaking in 200 ml of PBS contained in a conical flask, which was placed in a shaker incubator maintained at a temperature 37 °C (close to the body temperature) and the shaking speed was 100 rpm. 1 ml of PBS solution was withdrawn and replaced by fresh one at predetermined time intervals. The concentration of drug released in the buffer solution was estimated spectrophotometrically using Perkin Elmer UV/Vis spectrometer. The maximum absorbance wavelength (λ_{max}) for ciprofloxacin hydrochloride was found to be at 272 nm. The concentration of ciprofloxacin hydrochloride was calculated by employing a calibration curve. The experiments were repeated in triplicate to get the mean and the standard deviation.

2.6. Antibacterial activity

The antibacterial activity of ground drug loaded samples was studied by the standard disc diffusion method. Discs of 8 mm diameter and 1 mm thickness were made by pressing the ground sample ~ at 100 MPa. The microorganism *S. aureus* were cultured on agar plates. After the culture, sample discs were placed on this plate and incubated for 24 h at 37 ± 0.5 °C. The microbial inhibition zone including the disc was measured after the incubation period and its images were documented.

2.7. Cytocompatibility

The cytotoxicity test was performed for samples EHA, HA-1 and HA-2 on MG 63 cells (human fibroblast like cells) by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium) reduction assay. The cells were grown in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS) with 100 U/ml penicillin-streptomycin at 37 °C under a humidified atmosphere of 95% air and 5% CO₂ incubator. Cells were seeded at a density of 1 X 10⁵ in 96 well plate and cultured for 24 h. After 24 h incubation sample EHA, HA-1 and HA-2 was added to the well at various concentrations 25,50,100,250 and 500 µg/ml, and incubated the cultured for up to 48 h. At the end of the 48 h, 50 µl of MTT reagent (5 mg/ml in phosphate buffered saline) was added to each well and incubated for 4 h at 37 °C. The water insoluble formazan product was dissolved in 200 µl dimethyl sulfoxide (DMSO) and optical density was measured at 570 nm. The untreated sample was used as a control, mean and standard deviation were calculated. Cell viability was calculated using the following equation [23].

$$\text{Cell viability (\%)} = (\text{OD of treated sample} / \text{OD of untreated sample}) \times 100$$

Where OD_{sample} and OD_{control} represent the optical density (OD) values of cells cultured with the sample and without the sample respectively.

3. Results and Discussions

3.1. XRD & FT-IR

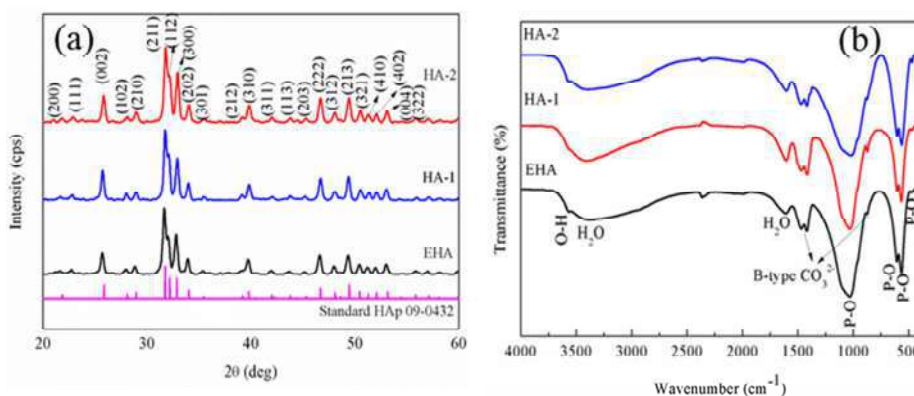


Fig.1. (a) XRD pattern of EHA, HA-1 and HA-2 samples (b) FTIR spectra of EHA, HA-1 and HA-2 samples

XRD patterns recorded for as-synthesized samples are shown in Fig.1 (a). Comparison of the patterns with JCPDS files revealed that the crystalline phase present in the samples to be hexagonal phase HAP (JCPDS File No: 09-0432). The patterns did not exhibit any significant differences though different precursors were utilized. The peaks in the region 30-35° can be ascribed to (211), (112), (300) and (202) reflections of HAp. The calculated average crystallite size and the degree of crystallinity of the samples from the XRD pattern are given in Table 1. Of the three samples, HA-2 exhibited lower degree of crystallinity and crystallite size whereas HA-1 showed higher values. The increased lattice parameter of sample EHA may be due to the impurity in eggshell.

The FT-IR spectra of the as-synthesized samples are shown in the Fig. 1 (b). The characteristic PO₄³⁻ (ν₄) vibrations of HAP appeared at 563 and 601 cm⁻¹ along with other ν₁, ν₂ phosphate peaks at 468 and 1029 cm⁻¹ respectively. The band at 1608 cm⁻¹ is attributed to the ν₂ bending mode of the H₂O molecules. The band at 3566 cm⁻¹ is the characteristic O–H stretching and librational mode of HAp and the broad band extending from 2363 to 3566 cm⁻¹ is attributed to adsorbed the H₂O molecules. Besides, it showed additional peaks at 876 cm⁻¹ (ν₂), 1419 cm⁻¹ (ν₃) and 1467 cm⁻¹ (ν₃) that indicates the formation of B-type carbonated apatite.

Table.1 The crystalline parameter of synthesized HAp samples

Sample code	Lattice parameters (Å)		Average crystallite size (nm)	Lattice distortion c/a
	a=b	c		
JCPDS	9.4180	6.8840		
EHA	9.4455	6.9224	59	0.7328
HA-1	9.3627	6.9116	62	0.7382
HA-2	9.4123	6.8900	55	0.7320

3.2. Thermal stability

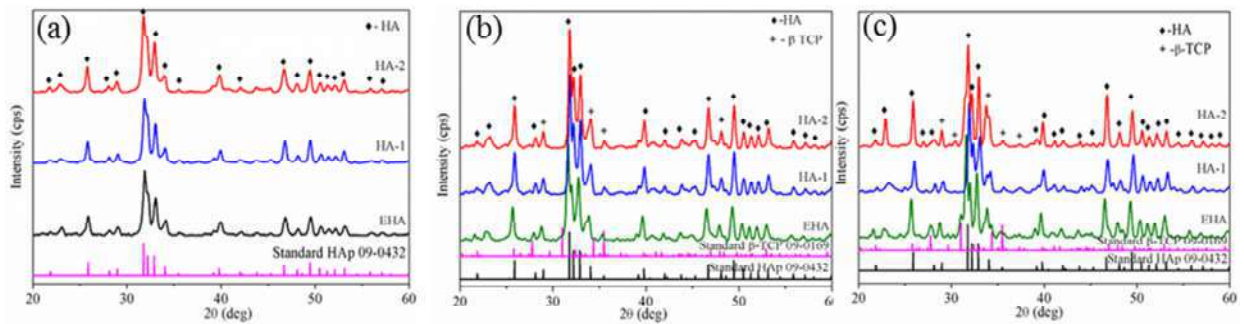


Fig.2 XRD pattern of samples heat treated at (a) 700 °C, (b) 900 °C, (c) 1100 °C

Fig. 2 (a), (b) and (c) showed the XRD patterns of the samples EHA, HA-1 and HA-2 after heat treatment at 700 °C, 900 °C and 1100 °C. There is no other crystalline species detected except HAp in all the three samples when heated to 700 °C [JCPDS file no.09-0432]. The XRD pattern of all the three samples heat treated at 900 °C and 1100 °C (Fig. 2. b & c) revealed the existence of β -TCP [JCPDS file No 77-2376] along with HAp. It suggested that heat treatment on higher temperature HAp transformed partially into β -TCP. The phase composition of the three samples after heat treatment are given in Table 2. Thermal stability of the EHA is higher when compared to the other two samples.

Table.2 Phase composition of the samples heat treated at different temperatures

Sample code	Phase composition (%)				
	700 °C		900 °C		1100 °C
	HAp	HAp	β -TCP	HAp	β -TCP
EHA	100	71.8	28.2	66.5	30.0
HA-1	100	63.2	36.8	59.4	40.6
HA-2	100	65.8	34.2	62.7	37.3

3.3. SEM

Fig.3 shows the morphology of the as-synthesized samples EHA, HA-1 and HA-2. All the three samples show the typical three dimensional flower like HAp which is formed by self-assembled hierarchical nanostructure under microwave condition. It is observed that EHA is composed of leaf-like flakes having 200–500 nm width and 1-2 μ m length which are extending radially from center. The flakes has sharp edges and mostly flakes are fused together. In HA-1 the flakes are around 400–800 nm width and 0.3-0.5 μ m length. The edges of the flakes is not

sharp like EHA. In HA-2 each flower is composed of rod like petals and each petal is a hexagonal rod with smooth rounded edge having length and width of 0.6 to 0.9 μm and 200-300 nm.

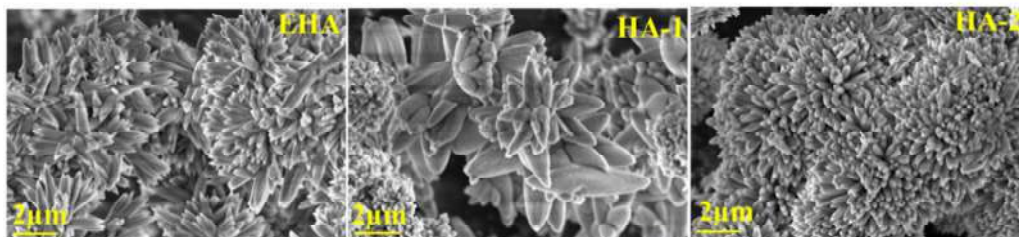


Fig.3. SEM images of the samples EHA, HA-1 and HA-2

3.4. EDAX

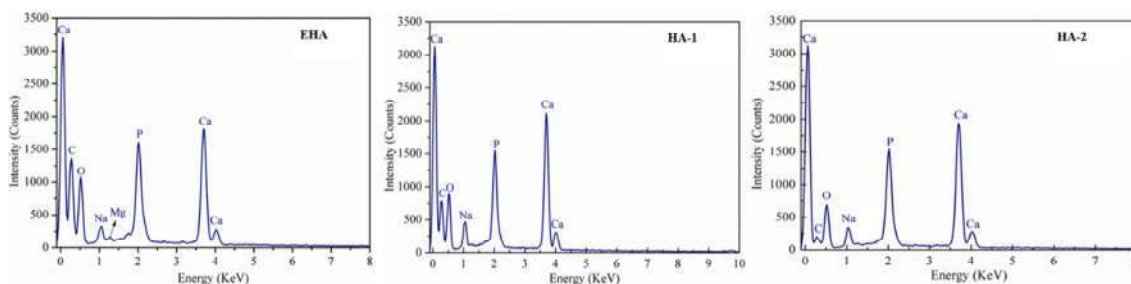


Fig.4 EDAX spectrum of the samples EHA, HA-1 and HA-2

The EDAX spectrum is used to identify the elemental composition. The EDAX spectrum of samples EHA, HA-1 and HA-2 are shown in figure 4. The EDAX spectrum confirms the presence of oxygen (O), calcium (Ca), phosphorus (P), carbon (C), sodium (Na) and small amount of magnesium (Mg) in the samples. Mg is present in the EHA sample only. Mg is one of the important trace elements present in calcified tissues and it plays a key role in bone metabolism. The atomic percentage and weight percentage of the elemental composition is given in table 3.

Table.3 Elemental composition of the samples EHA, HA-1 and HA-2

Sample	Elemental composition (At.%)					
	Ca	P	O	Na	Mg	C
EHA	13.47	8.13	60.05	6.07	0.65	11.63
HA-1	13.08	8.17	67.28	5.39	-	6.08
HA-2	15.13	9.89	64.67	5.28	-	5.03

3.5. Specific surface area

The specific surface area of the samples obtained from BET studies are given in table 4. Variations are observed in specific surface area, sample HA-2 had larger specific surface area compared to the others, whereas EHA showed the larger specific surface area than HA-1.

Table.4 Specific surface area of the samples EHA, HA-1 and HA-2

S.No	Sample code	Specific surface area m^2/g
1	EHA	2.0944
2	HA-1	1.9313
3	HA-2	3.3744

3.6. Bioactivity

Bioactivity is the vital ability of the material to induce the formation of an interfacial bone bonding between the implant and living tissues. Fig. 5 shows the SEM image of the surfaces of sample discs EHA, HA-1 and HA-2 before (a, b, c) and after (d, e and f) soaking in SBF for 21 days. All the samples exhibited a plain surface before soaking whereas after soaking in SBF the surface of all the samples revealed the formation of deposits of almost same amount. Apatite deposits formed due to availability of large amount of calcium ions that was released from the matrix during dissolution. These calcium ions further interact with $(\text{PO}_4)_3^-$ present in SBF and owing to its supersaturation with respect to calcium phosphate results in the formation of decisive size nuclei and subsequent growth as HAp particles on the surfaces of the samples [24].

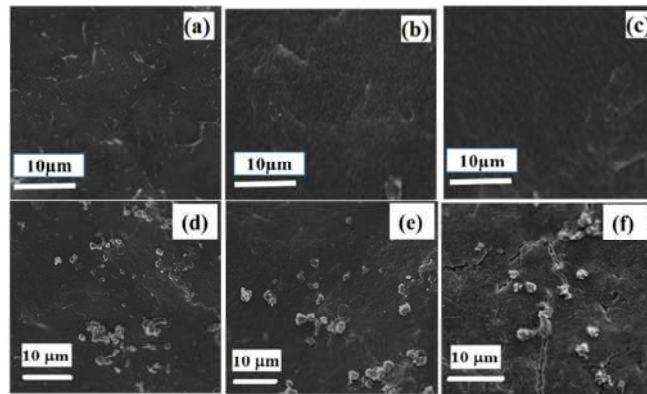


Fig.5. SEM micrographs of the surfaces of samples before and after exposed to SBF

3.7. Drug release

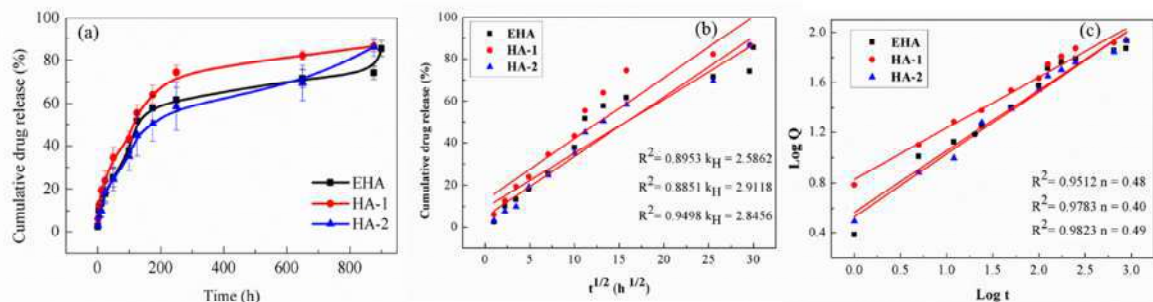


Fig.6.(a) In vitro drug release profile of samples EHA, HA-1 and HA-2, (b) Higuchi plots of ciprofloxacin release, (c) Korsmeyer peppas model of ciprofloxacin release

The amount of ciprofloxacin released from the as-synthesized HAp nanoparticles with time are shown in Fig. 6(a). All the samples exhibited almost similar release pattern throughout the release period. Release of ciprofloxacin followed almost the same trend and releasing about 55% of ciprofloxacin in HA-1 and 45 % in HA-2 within 24 h. Then the release slowed down and prolonged to 876 h (about 86%) in HA-1 and HA-2. In the case of EHA, about 17% of ciprofloxacin released in 24 h and the release sustained up to 50% for 120 h then prolonged to 85 % in 900 h. The rate of release at the initial stage is higher up to 24 h after that the release was sustained and released slowly. Desorption of ciprofloxacin molecules that are located at the surface of the HAp is responsible for higher initial release of ciprofloxacin whereas the release of ciprofloxacin molecules from the interior of the HAp nanoparticles by diffusion as well as dissolution processes is responsible for later release.

The drug release data of the samples studied was fitted to Higuchi models. The best fit with higher correlation value (R^2) was found with the Higuchi model and the drug release from all the three samples was governed by diffusion process Fig.6(b). In order to understand the type of diffusion mechanism, the release data

were further confirmed using Korsmeyer-peppas model that showed fair linearity Fig.6(c), with the slope values less than 0.5, indicating that drug release mechanism from the samples was Fickian diffusion controlled.

3.8. Antibacterial activity against S.aureus

S. aureus is the most common micro-organism found in patients of all kinds of osteomyelitis. Antimicrobial activities of the as-synthesized samples against S. aureus are shown in Fig. 7. There is no inhibition zone around pure HA. Ciprofloxacin drug loaded samples CEHA, CHA-1 and CHA-2 showed inhibition zones around the pellet in which bacterial growth was inhibited. The diameter of the inhibition zone of the samples against S. aureus are given in Table 6. The observed antimicrobial activity of CEHA, CHA-1 and CHA-2 can be due to the presence of ciprofloxacin molecules in the samples.

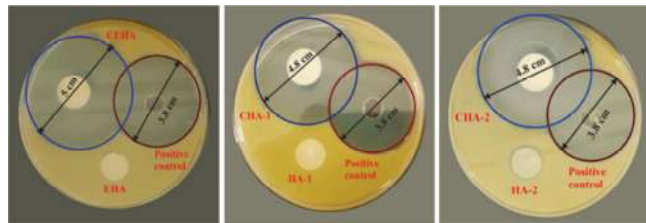


Fig. 7. Inhibition zone formed around the sample discs against S. aureus

Table 6. Zone inhibition of the samples against S.aureus

Sample code	Diameter of the zone inhibition (cm)
	S. aureus
EHA	5
HA-1	4.8
HA-2	4.8

3.9. Cytocompatibility

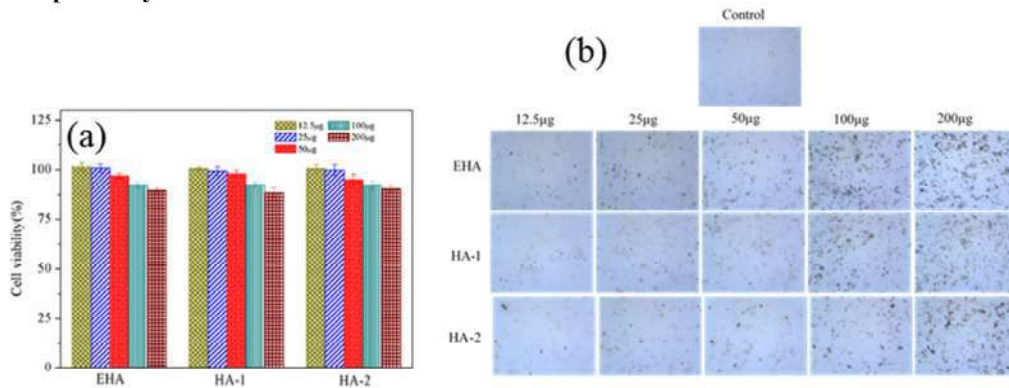


Fig.8. (a) Cell viability of EHA, HA-1 and HA-2 with human osteoblast MG-63 cells, (b) Optical microscope images of EHA, HA-1 and HA-2 with MG-63 using a MTT assay.

In vitro models based on cell cultures provide useful information regarding material biocompatibility. Fig.8(a) shows the cell viability of human osteoblast like MG- 63 cells cultured with EHA, HA-1 and HA-2 samples. Optical images of human osteoblast like MG-63 cells and cells cultured with different dosages of EHA, HA-1 and HA-2 are shown in Fig.8(b). The cell viability of the samples decreased with increasing dosage of the samples. According to ISO 10993 – 5: 2009, cell viability greater than 70% indicating that the samples are non-toxic. All the three samples EHA, HA-1 and HA-2 exhibits cell viability greater than 90% demonstrating that all samples are biocompatible with human osteoblast like MG-63 cells.

4. Conclusions

In this work physico-chemical properties of HAp derived from eggshell biowaste was compared with that of HAp derived from two different commercial calcium sources. The results of eggshell derived HAp exhibited almost similar physico-chemical properties to that of HAp derived from commercial calcium precursors, eggshell derived HAp contained magnesium which was not found in the other samples. All the studied samples exhibited controlled and prolonged release of ciprofloxacin over 38 days. All the three samples showed the excellent cytocompatibility with the human osteoblast like MG-63 cells. Both types of HAp can be considered to be potential bone graft materials.

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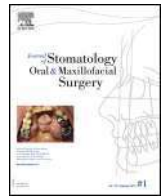


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Original Article

A three dimensional (3D) musculoskeletal finite element analysis of DARSN temporomandibular joint (TMJ) prosthesis for total unilateral alloplastic joint replacement

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ABSTRACT

Purpose: The purpose of this study was to evaluate the strain and stress distribution for DARSN alloplastic unilateral temporomandibular joint (TMJ) prosthesis and the effects on contralateral natural joint using a finite element analysis (FEA).

Methods: The replacement of the TMJ may have complications like infection, failure of hardware, facial paralysis and perforation. The understanding of the mechanical forces exerted by muscles of mastication and jaw movement on the joint helps in identifying the regions on alloplastic TMJ with various maximum forces, which makes that area more prone for failure of hardware. A three dimensional structural FEA was applied using a validated finite element model (FEM) where the areas of stress and strain were evaluated in the alloplastic joint and the contralateral natural joint. As the pattern of stress and strain can be influenced by the materials used for alloplastic joint and geometry of the design, mechanical property of bone and the attached musculature were also considered while construction the FEM analysis.

Results: The forces of the muscles of mastication has a vital role on the amount of stress and strain present across the alloplastic joint. Masseter and temporalis exhibited the greatest resultant force on the alloplastic as well as the natural condyle with a magnitude of 272 N and 329 N. This study assessed the maximum stress and strain on the condyle-ramus unit and fossa.

Conclusion: FEA shows that alloplastic DARSN TMJ prosthesis distributes stress and strain equally between the alloplastic joint site and the contralateral natural joint causing minimal adverse effects to the natural joint. FEA also evaluated the stress and strain on alloplastic component and resulted in drawing clinical implications for operating surgical team.

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1. Introduction

The temporomandibular joint (TMJ) aids in speech, mastication, swallowing is an essential component of the craniofacial complex

as it involves complex inter-related components producing jaw joint movements which is a combination of translation and rotation. The TMJ is affected by a wide spectrum of diseases but very few require surgical intervention. Literature evidence opines that more than 25% of the population is affected by TMJ disease, of which 5% patients require invasive surgical management, which includes degenerative joint disease, tumors, developmental anomalies, bony and fibrous ankylosis and trauma. It is essential to have an in-depth knowledge of the biological and mechanical

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Dassault System' following which virtual condylectomy was performed and the alloplastic joint was incorporated. The meshwork used in the construction of the model was solid mesh with curvature based mesh. The meshing section was parabolic tetrahedral solid elements with 90011 nodes and 58435 number of elements in it.

4. Construction of Joint Fossa eminence

The custom made fossa eminence of the alloplastic joint was constructed using the CT scan images of the patient followed by a 3D model construction. The fossa eminence was constructed using an ultra high molecular weight polyethylene (UHMWP) and the properties of the same was used in the simulation of the model. The resultant forces on the condylar head was applied at the center of the fossa eminence. The stress and strain within the fossa eminence was obtained and studied. The total number of nodes used in construction of fossa eminence were 15905 with 9728 elements and 1.549 mm was the size of the element. Then the constructive 3D model of TMJ integrated with the condyle was imported into the simulation platform powered by SolidWorks 2012 3D CAD Simulation (Dassault Systèmes, Version SP0.0).

The mechanical properties of alloplastic joint and fossa eminence were used for more accurate analysis of forces over the joint. Assigning of materials to the different components of the model was done and their respective mechanical properties were imported from the SolidWorks Database Catalogue, and other external resources where required. Eight screws of Ti alloy of Grade 5 was utilized, with 2.5 mm diameters and 8 mm length. The relative effect on the screws were assessed according to Von Mises stress and strain over the alloplastic joint.

A force vector was assigned to the model. The assignment of the direction of the forces was done on the basis of custom made plane method. The direction planes were created with the help of reference plane and respective geometry, which allowed to assign the forces to the respective direction and angles. The magnitudes of the forces, which was assigned to the model where as per the literature [4]. The forces of the muscles of mastication has a vital role on the amount of stress and strain present across the alloplastic joint. All the available forces exerted by the muscles of mastication were used during the simulation of the 3D Model. All the muscles of mastication were included except lateral pterygoid muscle of the side with alloplastic TMJ. The maximum forces exerted by muscles of mastication were applied in the direction of its resultant force motion and the amount of force was assumed equal on both right and left sides of the jaw. Total 10 muscle forces were taken into account to assess the magnitude of maximum muscle forces where the magnitude was summed up for a muscle having two parts (Table 1). Initially, a connection was established between the condyle – screw interface and between the screw-mandibular interfaces, which was a sliding contact with coefficient of friction as 0.1.

5. Results

The properties of the mandible, DARSN TM alloplastic condyle, UHMWPE alloplastic fossa have been summarized (Table 2). The reaction forces in the region of screw post-application of forces exerted by the muscles of mastication were 746.463 N, 225.449 N, 79.475 N for x-, y- and z-axis respectively with a resultant force magnitude of 783.805 N. The same reactional forces after exertions of masticatory muscles in the alloplastic condylar head region corresponded to 827.984 N, 416.056 N, 865.878 N for x-, y- and z-axis with 1268.23 N as the resultant force. After applying all the forces exerted by muscles of mastication, the maximum stress of 5296.04 N/mm² was concentrated in the lower most screw, screw 1. There was a maximum displacement of 45.6994 mm in the anterior mandibular region. There was a maximum strain of 3.067 × 10⁻² observed in the region of lower most screws, screws 1 and 2.

The resultant force applied in the center of the fossa by the alloplastic condyle was 1135.73. The reaction force on the entire body was 0.00120062 N for x-axis, 1135.73 N for y-axis and - 0.003184 N for z-axis with a resultant force of 1135.73 N. A maximum stress of 3.0704 × 10⁶ N/m² was on the inferior border of the fossa which corresponds to the slot for mandibular condyle with a minimum stress value of 0.000353384 N/m². The maximum strain was observed in the inferior border of the fossa, which is the slot for the condyle with a value of 0.00173763 and a minimum strain of 3.78734 × 10⁻⁸. There was a maximum displacement of 0.00438809v mm in the center of the fossa with a 0 mm (minimum) displacement.

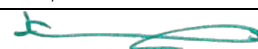
6. Discussion

The objectives of this study was to develop a musculoskeletal model of the human mandible and to simulate the model with placement of DARSN TM Joint unilateral alloplastic prosthesis to assess the stress distribution, strain and displacement when the prosthetic joint was subjected to function. The FEA comprised of analysis of following mechanical aspects of the alloplastic joint: The value and node with maximum stress on condyle-ramus unit, the value and node with maximum strain in the condyle-ramus unit, value and nodes with maximum stress on fossa eminence and, values and nodes with maximum and minimum strain on fossa eminence. After the simulation of the 3D model all the aspects were thoroughly analyzed.

The results from the present study opines that an increased stress and strain was experienced in the inferior most screws with an increased displacement factor (Fig. 2a and b). To minimize displacement of the prosthesis and loosening of the screws, use of long bi-cortical screws for the inferior most screw slots is recommended. The ramal plate of the prosthesis was oriented and fixed parallel to the posterior border of the ramus (Fig. 3). Fixation of the plate was done inferiorly and posteriorly to mimic a pseudo translation of the condyle

Table 2
The properties of the mandible, DARSN TM alloplastic condyle (Ti-6Al-4V), UHMWPE alloplastic fossa.

Properties	Mandible	Alloplastic condyle	Alloplastic fossa
Mass	0.0679409 kg	0.00584497 kg	0.0033 kg
Volume	3.67248 × 10 ⁻⁵ m ³	1.31977 × 10 ⁻⁶ m ³	3.633 × 10 ⁻⁶ m ³
Density	1850.78 kg/m ³	4428.78 kg/m ³	934 kg/m ³
Weight	0.665821 N	0.0572807 N	0.0332 N
Yield strength	2.5 × 10 ⁸ N/m ²	1.05 × 10 ⁹ N/m ²	2.3 × 10 ⁷ N/m ²
Tensile strength	-	8.2737 × 10 ⁸ N/m ²	4 × 10 ⁷ N/m ²
Elastic modulus	1.47 × 10 ⁻¹⁰ N/m ²	1.048 × 10 ¹¹ N/m ²	1.5 × 10 ⁹ N/m ²
Poison's ratio	0.3	0.31	0.46
Shear modulus	-	4.102 × 10 ⁻¹⁰ N/m ²	-
Thermal expansion	-	9 × 10 ⁻⁶ /Kelvin	-



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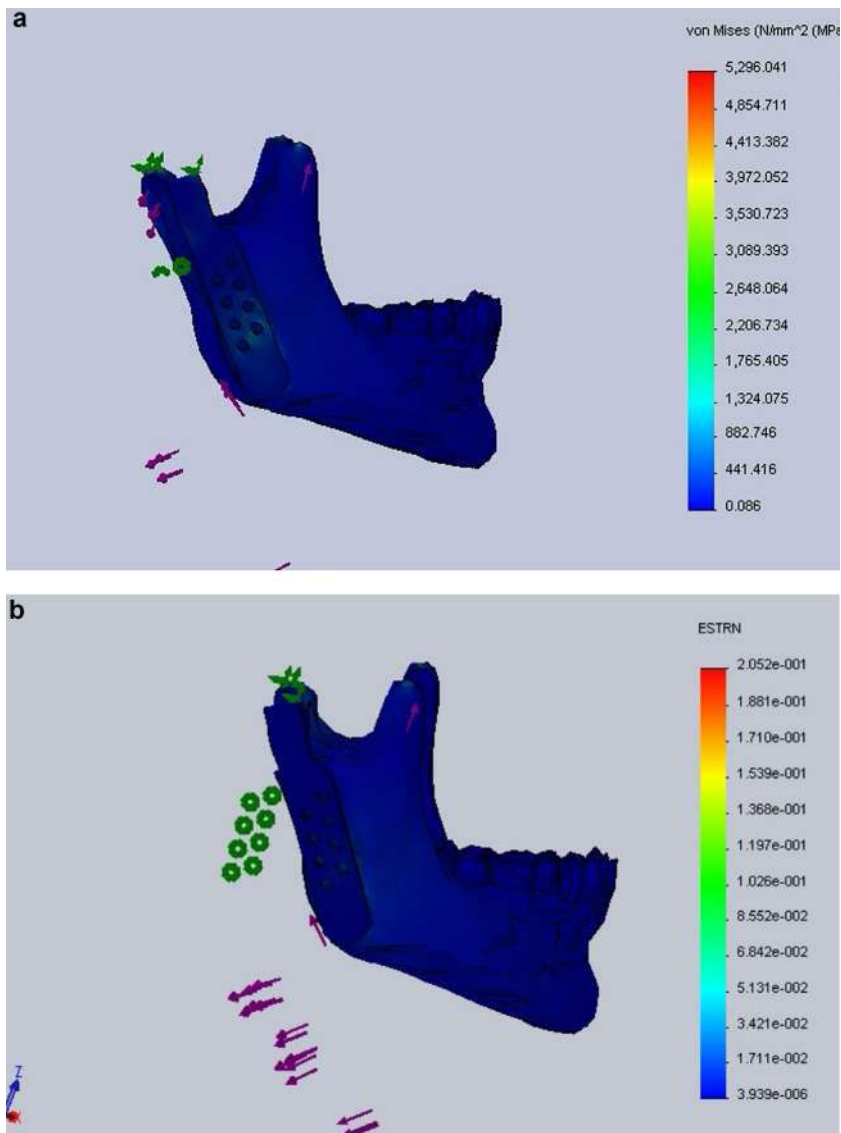



Fig. 2. a: lateral view of stress distribution in the mandible after placement of DARSN TM Joint prosthesis during closing of the jaw; b: lateral view of stress distribution in the mandible after placement of DARSN TM Joint prosthesis during opening of the jaw.

(Fig. 4). The principle emphasized is tripod fixation of screws in the lateral ramal plate of the prosthesis that can provide adequate stability, which is supported by the literature evidence that even three screws can provide sufficient stability. The stress and strain level in the cortical and cancellous bone was highly influenced by the screw position [4]. Another important factor was the implant geometry and the material used for prosthesis construction that influences the biomechanical behavior of the mandible. The geometry of the implant plays a major role in distributing the stress and strain in the joint replacement side as well as on the mandible. These factors can be studied and the prosthesis can be designed according to the FEM accurately as the number and position of the screws have a significant influence on mandibular behavior and stress pattern of the prosthesis to avoid stress concentration and implant fracture [1].

Various studies in the literature have focused on the clinical and kinematic outcomes of alloplastic prosthetic designs with various screw configurations which needs a special consideration in TJR [6–9]. Each TMJ joint functions in harmony with the contralateral side and any undesirable function on either side can affect the other joint as well. Authors have observed changes in the

masticatory pattern and ranges of motion (ROM) in patients who underwent unilateral and bilateral TMJ replacements with an alloplastic prosthesis. A 3D kinematic model study revealed that with unilateral TMJ prosthesis, the load on the contralateral joint was significantly changes suggesting that when an unilateral TMJ alloplastic prosthesis is placed, the lack of translational movements has major effects on the mandibular movements and also on the movements of the contralateral natural joint [10]. The variation in the translational capacity between the prosthetic and the natural joint changes the natural situation leading to an abnormal loading on the natural contralateral TMJ along with the alterations observed in masticatory muscles [9]. To overcome the adverse effects on the contralateral natural joint and, the design of the joint placement in the present study was planned and fixed inferiorly and slightly posterior so as to achieve a pseudo-translatory motion during jaw opening and closing thus minimizing the stress on the contralateral natural TMJ.

Perez et al. evaluated patients who required unilateral TMJ TJR and the associated risk for developing post-surgical contralateral TMJ pain and dysfunction who may require subsequent contralateral TJR.


Dr. L. KRISHNA PRASAD

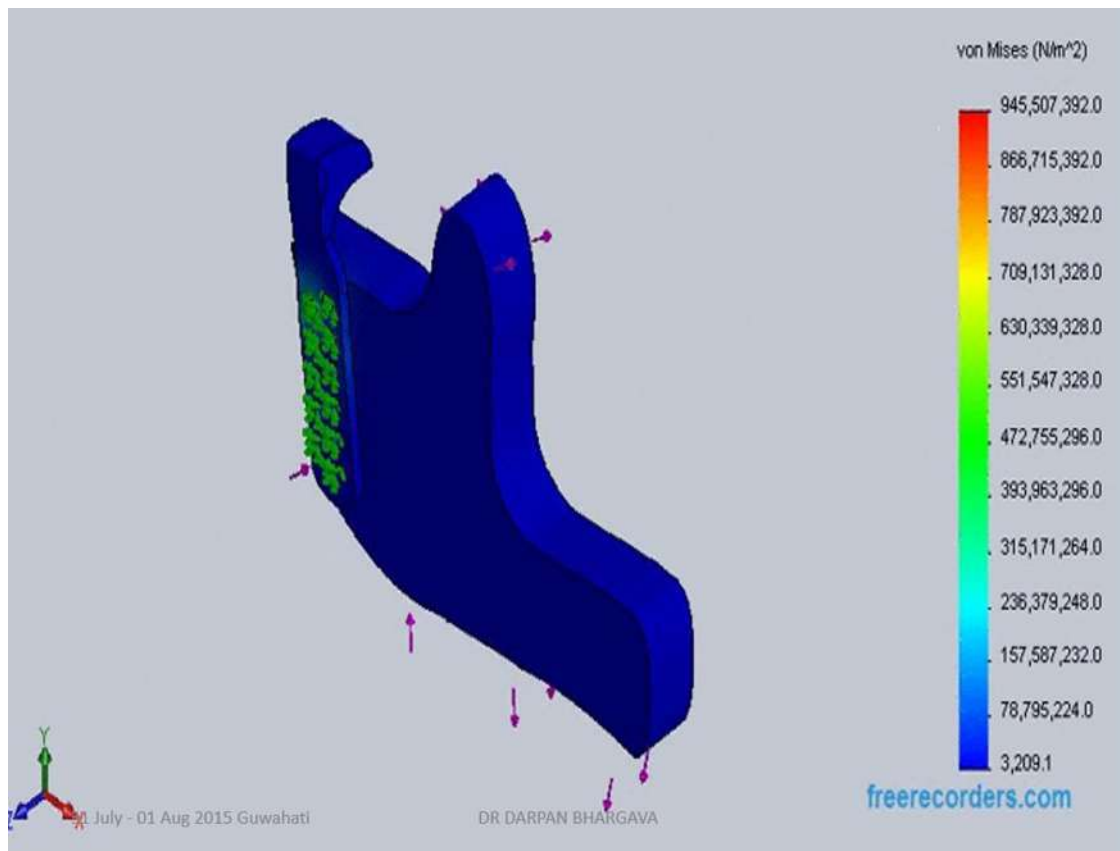


Fig. 3. Finite element model (FEM) of DARSN TM joint prosthesis placement showing orientation parallel to the ramus of the mandible.

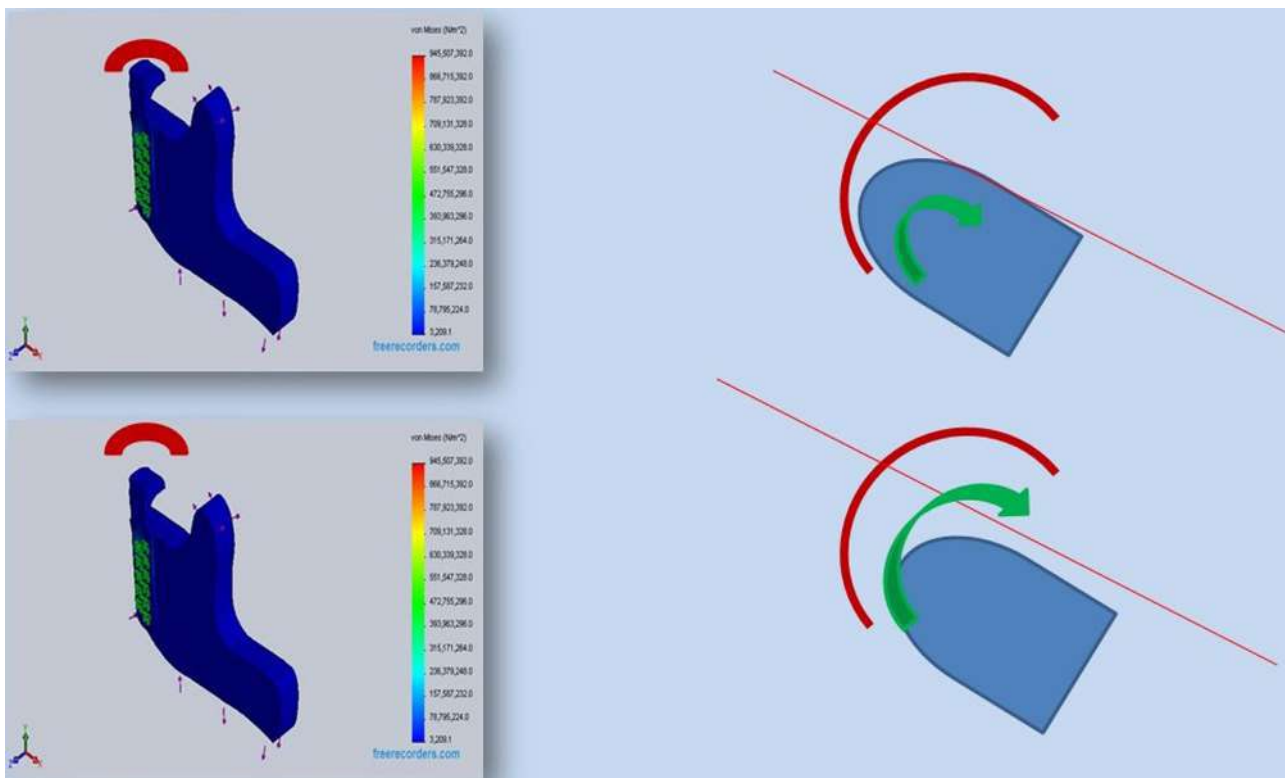


Fig. 4. Finite element model (FEM) showing the anterior placement of the prosthesis where the translatory motion of the mandibular condyle is hampered but when it is placed inferiorly and posteriorly the prosthesis mimics a pseudo-translatory motion of the mandibular condyle.

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Subjective and objective outcomes of unilateral TMJ reconstruction were also evaluated for a long-term follow-up. They observed that patients who underwent unilateral TMJ reconstruction have increased probability for improved clinical condition and does not require contralateral TJR if the joint is healthy. But there is 30% chance that the contralateral joint may require TJR later if there was history of previous surgery to the joint [11]. In the present study, we did not observe any stress or strain on the contralateral healthy TMJ thus we hypothesize that the need for TJR on the contralateral joint may not be required, provided TMJ TJR are done following sound biomechanical principles.

Auckland DC et al. in their FEM analysis observed that the average resultant joint force on their prosthetic TMJ and contralateral natural joint was 102.4 N and 83.9 N respectively. For maximum bite force, average resultant joint force at prosthetic TMJ and contralateral natural joint was 409.8 N and 335.9 N. They observed that lateral force of the TMJ component was more in prosthetic joint as compared to the contralateral natural joint, which had larger force in the posterior component. The results supported their hypothesis that alloplastic prosthesis orientation had influenced the effect of screw stress and loading at the joint. They observed no stress and strain in the condylar component when an anterior rotation of up to 15 degrees were given and a decrease in stress concentration when more number of screws were placed to achieve stability of the prosthesis and the superior screws experienced maximum stress concentration. The maximum contact stress was more when the condylar component was moved anteriorly. Contrary to the above mentioned study, in the present study the prosthesis was oriented parallel to the ramus with tripod fixation of screws where the number of screws can be minimized still maintaining a reduced overall stress concentration with adequate stability of the prosthesis as supported by Hsu et al. [12].

The joint and its associated structures involves a complicated combination of masticatory muscles and a mandible supported by two harmoniously functioning TMJ [13,14]. Relations between these complex inter-linked structures are often difficult to obtain by conducting human experiments. To understand the mechanical behavior of the TMJ and attached artificial devices and for improvising the design of the prosthetic devices, FEA is a feasible option to anatomically represent and study the mandible, the TMJ and its associated masticatory muscles. The FEA and the alloplastic joint design in the present study will provide basis for improvising outcomes for patients requiring TMJ TJRs and will contribute in understanding biomechanical principles of alloplastic materials used in TJRs.

Funding

Self-funded.

Ethical approval

Obtained. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from the patients involved in this study.

Declaration

DARSN TM Joint Prosthesis is developed as a 'Make in India' initiative, undertaken as Post-Doctoral research at People's University, Bhopal and Meenakshi Academy of Higher Education and Research (MAHER), Chennai, INDIA.

Design Patent ref

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Disclosure of interest

The authors declare that they have no competing interest.

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Vertically unstable fractured mandibular segment with attached genial tubercles as a parameter for difficulty during intubation for general anaesthesia—substantiation with computed tomographic (CT) scan evidence

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Abstract

Purpose To study and evaluate the anatomic alterations in the suprahyoid musculature, the hyoid bone, and the laryngeal inlet in patients with vertically unstable fractured mandibular segment with attached genial tubercles using computer tomography for substantiation of the clinical evidence and hypothesis of difficulty during intubation for general anaesthesia.

Materials and method Random sampling methodology was used to enrol patients with mandibular bilateral parasymphysis fracture qualifying for the classification of vertically unstable fractured mandibular segment with attached genial tubercles for group A patients. Patients with unilateral parasymphysis fracture with vertically stable mandibular segment were included in group B. Forty patients with parasymphysis fracture and no other associated facial fracture/injury were evaluated prospectively by comparing their pre-operative computer tomography (CT) images with post-operative CT images taken after the reduction of the fracture. Parameters evaluated were variation in the radiologic anatomy of the laryngeal inlet shape and alteration in the suprahyoid musculature after open reduction and internal fixation of the fracture when compared with pre-operative CT images.

Results The following were the results/observations from this study among group A patients: (1) The distance between the genial tubercles and the hyoid was found to be reduced. (2) Dorsal bodily movement of the hyoid was observed suggesting loss of anterior hyoid support. (3) The posttraumatic changes in the shape of the laryngeal inlet were observed in cases with vertically unstable bilateral parasymphysis fracture. (4) Restoration of morphology of the laryngeal inlet and anterior-posterior distance between genium and hyoid after reduction.

Conclusion Computer tomographic findings confirm that the displacement of fractured mandible and resultant displacement of the genial musculature have their effect on the laryngeal morphology. These posttraumatic changes in cases with dorsally displaced vertically unstable fractured mandibular segment with attached genial tubercles should be considered as a vital parameter for assessing difficulty during intubation.

Keywords Mandibular fracture · General anaesthesia · Difficult intubation · Computed tomography (CT) · Laryngeal inlet · Hyoid bone

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Introduction

Genial attachment to the suprahyoid musculature in addition to its contribution towards the muscle control for the mandibular dynamics also provides support to the hyoid for the maintenance of its spatial position in the inframandibular region. The genial muscular attachments add to the anterior support to the hyoid and thereby providing positional stability to the various anatomic structures in the region. Disruption of the genial support may have its effects on the spatial orientation of the hyoid and associated anatomic structures including the laryngeal inlet.

Vertically unstable fractured mandibular segment with attached genial tubercles and its associated musculature has a tendency to displace in the inferior and backward direction as genial tubercles of the mandible give attachment to genioglossus and the geniohyoid muscles and in turn aid in supporting the tongue and hyoid bone in its anatomical position [1]. Tissue prolapse adds to the intubation difficulty in patients with postero-inferiorly displaced vertically unstable fractured mandibular segment as there will be disruption of the hyoid and laryngeal inlet along with the associated anatomic structures [2–4].

This study critically evaluates the anatomic alterations in the suprahyoid musculature, hyoid bone, and laryngeal inlet in patients with bilateral mandibular parasymphysis fracture with attached genial tubercles using computed tomography (CT) scan for the substantiation with clinical evidence and hypothesis of difficulty during intubation for general anaesthesia in such patients as discussed in the previous study [3]. The consequences of dorsal and inferior displacement of the genial apparatus due to unfavourable mandibular fracture on the spatial orientation of hyoid and its effects on the laryngeal inlet using a comparative pre-operative and post-operative CT analysis have been discussed in this study.

Material and methods

A prospective randomised study was conducted after obtaining an ethical clearance from our institutional ethical committee (IEC/RAC). To prevent bias in the sampling among our study cohort, random sampling methodology was used to enrol patients with mandibular bilateral parasymphysis fracture qualifying for the classification of vertically unstable fractured mandibular segment with attached genial tubercles in group A and group B patients diagnosed with unilateral parasymphysis fracture with favourable fractured mandibular segment. Forty systemically healthy patients with parasymphysis fracture and no other associated mandibular/facial fracture and head injury were evaluated prospectively by comparing their pre-operative CT images with



Fig. 1 Pre-operative computed tomographic (CT) image, axial section showing bilateral parasymphysis fracture of the mandible with displaced hyoid apparatus and reduction in the size of laryngeal inlet

post-operative CT images taken after open reduction and internal fixation (ORIF) of the fracture.

Parameters evaluated were variations in the radiologic anatomy of the laryngeal inlet shape and the alteration in the suprahyoid musculature after ORIF of the fracture comparing with pre-operative and post-operative CT images (Figs. 1, 2, 3, and 4). Sectional data of CT scan can illustrate all the regions of the mandible in three planes—axial, coronal, and sagittal planes. Besides identifying the fracture, it is more accurate to determine the degree of fragment displacement with CT scan than with a plain radiography. Multi-section CT scan (Siemens, India) was obtained with following exposure parameter of 400 mAS, 120 kV with scan time of 15.35 s and 4-s delay to procedure, and the slice thickness of 1 mm. ORIF



Fig. 2 Post-operative computed tomographic (CT) image, axial section showing reduced parasymphysis fracture of the mandible with restoration in the size of laryngeal inlet and hyoid anatomy

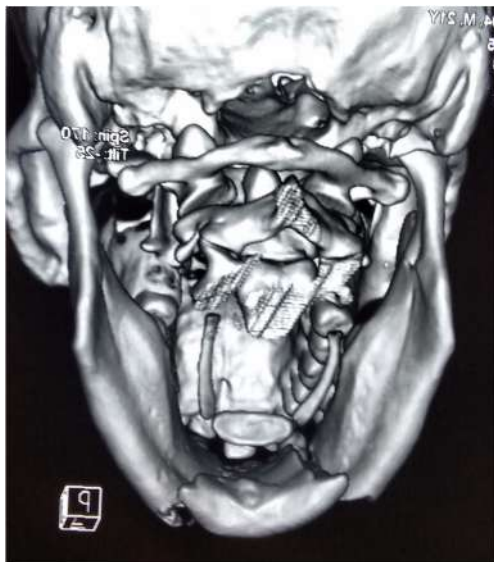


Fig. 3 Reconstructed three dimensional (3D) image showing decrease in the distance between the genium and the hyoid bone with bilateral mandibular parasymphysis fracture

was performed by a single qualified operator and intubation was performed by a single qualified blinded anaesthesiologist for all the study population. The anaesthesiologist routinely performed intubation for maxillofacial trauma patients in our maxillofacial trauma unit. The anaesthetist graded the degree of intubation difficulty using Intubation Difficulty Scale (IDS) (Table 1) based on parameters like number of intubation attempts and operators for assistance, number of alternative techniques used, Cormack’s glottis exposure score, lifting



Fig. 4 Reconstructed three dimensional (3D) image showing restoration in the distance between the genium and the hyoid bone after open reduction and internal fixation (ORIF)

Table 1 Intubation Difficulty Scale (IDS) to assess the degree of difficulty

Intubation Difficulty Scale (IDS) score	Degree of difficulty
0	Easy
$0 < \text{IDS} \leq 5$	Slight difficulty
$\text{IDS} < 5$	Moderate To major difficulty
$\text{IDS} = \infty$	Impossible intubation

force required during laryngoscopy, and necessity for laryngeal pressure for all the 40 patients from both the groups after induction of general anaesthesia (GA) drugs for muscle relaxation. Clinical parameters like obesity and short neck which adds to intubation difficulty were ruled out.

Results

The results obtained which were evaluated by two different radiologists who were blinded showed that there was an alteration in the distance between the hyoid and the genial tubercle with loss of hyoid apparatus support due to the disruption of musculature and a significant alteration in the laryngeal inlet shape which was restored after ORIF which was evident in the pre-operative and post-operative CT images.

Both the radiologists reported that out of 20 patients, 18 patients had significant alteration in the laryngeal inlet shape due to the dorsal displacement of hyoid apparatus and suprahyoid musculature pre-operatively in group A (Table 2). Chi-square test revealed a value of 0.00 and the probability of variance among the radiologists’ opinion was 1.000. The observational findings by the radiologists have been summarised (Table 3).

The anaesthetist graded the difficulty of nasoendotracheal according to the IDS score. He observed that in group A, $n = 18$ (90%) patients had “moderate to major difficulty” and $n = 2$ (10%) patients had “slight difficulty” during intubation. Among group B patients, $n = 19$ (95%) had “easy” intubation whereas $n = 1$ (5%) patient, “slightly difficult” intubation which was encountered due to decreased hyoid-mental distance and soft tissue oedema.

Discussion

Trauma patients in maxillofacial surgery have peculiar necessities for general anaesthesia as the work field is shared by both the surgeon and anaesthetist which demands an understanding for each other’s needs for the operative procedure. In most instances, nasotracheal intubation is the most suitable method of administering general anaesthesia for surgical

Table 2 Data observed by two radiologists on the change in the laryngeal inlet shape and dorsal displacement of the hyoid apparatus with loss of anterior support in the pre-operative and post-operative computed tomography (CT) image

Anatomic changes in the CT scan	Group A		Group B	
	Pre-operative CT	Post-operative CT	Pre-operative CT	Post-operative CT
Radiologist 1	18	0	0	0
Radiologist 2	18	0	0	0

procedures involving the oral cavity as the dental occlusion needs to be evaluated intraoperatively.

A bilateral parasymphysis fracture with genial attachments is displaced in an inferior and backward direction along with the tongue via its musculature anteriorly. The muscular instability causes a loss of control over tongue and the hyoid bone which can cause oropharyngeal obstruction in supine position. Genioglossus and the geniohyoid muscles attached on the genial tubercle help in supporting the anatomic position of the tongue and hyoid bone. Muscle attachments which act to place dynamic vectors of force on the mandible when in continuity allow for proper mandibular function but when there is discontinuity such as unfavourable mandible fractures can potentially disrupt appropriate fracture healing [2].

In the previous study conducted by Bhargava D et al. to assess the difficulty in nasoendotracheal intubation in patients with inferior and backward displacement of unfavourable fractured mandible using genial tubercle and genial muscles as reference landmarks, they have observed that there is alteration in the anteroposterior airway due to collapse of suprahyoid musculature. They assessed the difficulty encountered during intubation in favourable and unfavourable mandibular fracture and concluded that vertically unstable mandibular fracture with genial attachments increases the degree of difficulty during intubation. The patients had higher Cormack grade in which additional force is required for glottis exposure during laryngoscopy. The study concluded that displaced mandibular fracture with genial attachment is a vital parameter for assessing difficulty during intubation [3]. In this study, 2 patients with favourable bilateral mandibular fracture did not show much alterations in the laryngeal inlet shape and associated anatomic structures. The CT findings in the present

study shows the variation in radiologic anatomy of the laryngeal inlet shape and alteration in the suprahyoid musculature after ORIF correlates with the difficulty during intubation for general anaesthesia.

Kellman RM et al. have observed that airway obstruction in maxillofacial injuries is caused by tongue base or maxillary prolapse, pharyngeal oedema, or haematoma. Tongue retraction should be done using a suture or towel clamp followed by securing a definitive airway in bilateral mandibular body fracture as there is high risk for tongue prolapse [4]. Functional and structural disruption of the oral cavity, larynx, and pharynx can cause not only airway difficulty but also dysphagia as the individual musculature performs its function to maintain the normal anatomy and physiology [5].

A retrospective review on airway management in maxillofacial trauma patients by Saraswat V observed that mandible was commonly involved in maxillofacial injury. Nasoendotracheal intubation is commonly preferred by maxillofacial surgeons as they can use occlusion as key to reduce facial fractures. He had also mentioned that nasal or oral intubation does not pose problem if there is absence of gross disruption in the normal anatomic structures [6]. There may be varying degree of difficulty during the intubation procedure in different fracture patterns as discussed in the presented study.

Hutchinson et al. have put forward six situations associated with maxillofacial trauma which can affect the airway which includes bilateral fracture of mandible causing backward displacement of the tongue and soft tissue oedema of the head and neck [7–9]. Similar observations were made by Kellman et al. in their study [5]. Walls RM has reviewed on management of the difficult airway in a trauma patient with short

Table 3 Observational changes in the computed tomography (CT) scan summarised by the radiologist

S. No.	Observational changes observed by the radiologist
1	The distance between the genial tubercles and the hyoid was found to be reduced
2	Dorsal bodily movement of the hyoid was observed suggesting loss of anterior hyoid support
3	The posttraumatic changes in the shape of the laryngeal inlet were observed in cases with vertically unstable bilateral parasymphysis fracture
4	Restoration of morphology of the laryngeal inlet and anterior-posterior distance between the genium and hyoid after reduction.

neck; retruded mandible will pose difficulty for intubation as the tongue has to be pushed out of line. They further mentioned that intubation in patient with upper airway compromise is a rule rather than an exception [10] which has been observed from our previous study [3] and the present CT correlation.

Conclusion

Vertically unstable bilateral fractured mandibular segment with attached genial tubercles with genial musculature is a vital parameter for surgery under general anaesthesia as it poses difficulty during intubation even in the presence of adequate muscle relaxation. The reason for this difficulty in intubation has been substantiated with CT study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Obtained. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from the patients involved in this study.

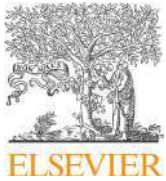
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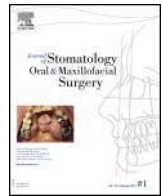


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Original article

Comparison of latency and efficacy of twin mix and modified twin mix in impacted mandibular third molar surgery – A Preliminary Randomized Triple Blind Split Mouth Clinical Study

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ABSTRACT

Introduction: Intra-space drug administration have recently gained popularity in the clinical practice posing several advantages over the conventional routes of drug administration. A preliminary prospective randomized triple blind clinical study was conducted to compare the latency and duration of anesthesia with twin mix (1.8 ml 2% lignocaine with 1:200,000 epinephrine and 1 ml/4 mg dexamethasone) and modified twin mix (1.7 ml of 4% articaine with 1:100,000 epinephrine and 1 ml/4 mg dexamethasone) to two conventional local anesthesia solutions along with co-relation of clinical effects in the postoperative phase in patients undergoing extraction of impacted mandibular third molars in terms of patients comfort post-surgery.

Materials and Methods: The study was conducted among 20 patients with bilateral impacted mandibular third molars who were randomly allotted to two groups, Group A and B. Each patients in both the groups was allotted with study and control site. Among Group A, patients were further divided into Sub-group L (Control) and Sub-group TM (Twin Mix). Group B patients were divided as Sub-group A (Control) and sub-group MTM (Modified Twin Mix). Sub-group L patients received 1.8 ml of 2% lignocaine with 1:200,000 adrenaline and sub-group TM received twin mix. Sub-group A received 1.7 ml of 4% articaine with 1:100,000 adrenaline and sub-group MTM received modified twin mix solution. All the procedure was performed by a single operator with a gap of 1 month between the two interventions among both the groups. Various subjective and objective parameters were measured pre-operatively and postoperatively to assess the latency and efficacy of various anesthesia solutions used in this study for third molar removal.

Results: Mean (\pm SD) VAS scores for sting on injection and pain were found to be less in TM and MTM sub-group with a score of 2.3 (\pm 0.768) and 2.7 (\pm 0.065) respectively. The anesthetic latency was significantly less in sub-group TM, with a mean (\pm SD) of 52.4 (\pm 28.3) seconds. Sub-groups A and MTM had longer latency of anesthesia when compared with L and TM sub-groups. The duration of soft tissue anesthesia was maximum in sub-group MTM as compared to the other sub-groups. Patients from control sub-groups among both the groups had increased swelling, post-surgical pain and trismus postoperatively.

Discussion: Intra-space administration of twin mix and modified twin mix is clinically efficacious in impacted mandibular third molars surgery with better clinical outcomes postoperatively. We observed one significant difference between TM and MTM that the latter solution provided a prolonged duration of anesthesia increasing the patient's comfort postoperatively after surgical removal of mandibular third molars.

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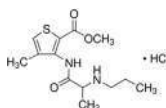


Fig. 1. Structure of articaine hydrochloride, chemically 3-N-Propylamino-propionylamino-2-carbomethoxy-4-methylthiophene hydrochloride.

1. Introduction

Literature evidence supports the technique of intra-space administration of local anesthesia (LA) admixed with dexamethasone, twin mix (TM) solution which reduces the latency and prolongation of soft tissue anesthesia duration with better clinical outcomes postoperatively after mandibular third molar extraction which is equally effective as compared to the conventional methods of steroid administration. There are various studies which emphasizes the postoperative period that is uneventful after administration of steroids through various routes such as intravenous, intra-muscular, sub-mucosal and intra-space. Literature signifies the effectiveness of intra-space administration of TM was effective in reducing adverse events postoperatively after mandibular third molar removal [1,2].

Articaine hydrochloride is a hybrid molecule belonging to the amide class of LA containing a thiophene ring as it lipophilic moiety, possessing both amide and ester characteristics. Chemically, 3-N-Propylamino-propionylamino-2-carbomethoxy-4-methylthiophene hydrochloride was known as articaine initially in 1969, was marketed by Germans in 1976. (Fig. 1) The pharmacological character of this anesthetic solution is responsible for its main advantages such as enhanced liposolubility of the drug and increased potency (1.5 times greater than that of lidocaine) [3] There are several studies which opines that 4% articaine is a safe alternative to 2% lignocaine for extraction of lower third molars and other dental procedures in terms of latency and duration of anesthesia [4–6].

This study compares the effectiveness of TM to that of modified twin mix (MTM, 1.7 ml of 4% articaine with 1:100,000 epinephrine and 1 ml/4 mg dexamethasone) (Table 1) along with conventional LA solutions. (1.8 ml of 2% lignocaine with 1:200,000 adrenaline and 1.7 ml of 4% articaine with 1:100,000 adrenaline). The methodology for administering the MTM solution was similar to the previous studies to administer LA for standard inferior alveolar nerve block.

2. Materials and Methods

A randomized prospective triple blind split mouth clinical study was conducted after obtaining approval from the institutional ethical committee, IRB number: OMFS/17/2866. A total of 20 patients requiring extraction of bilateral impacted mandibular third molar with similar difficulty index were included for the

Table 1

Composition of Modified Twin Mix (MTM) solution (2.7 ml), single use vial of articaine hydrochloride (Hcl).

Local anesthesia (1.7 ml)	Corticosteroid (1 ml/4 mg)
Articaine hydrochloride 68 mg/1.7 ml	Dexamethasone sodium phosphate IP 4 mg/ml
Adrenaline (as tartrate) 0.017 mg	Sodium Methylparaben IP 0.15%
	Sodium Propylparaben IP 0.02%
	Water for injection IP q.s

study after obtaining a signed written informed consent. The inclusion criteria were ASA Class I subjects aged 18 years and above with bilateral impacted mandibular third molar of difficulty index, Class II and position B according to Pell and Gregory's classification [7]. The exclusion criteria were the presence of acute infection/swelling at the time of surgery, history of systemic illness, previous history of allergy to LA solution. The patients were blinded regarding the type of LA used and single qualified blinded operator performed all the surgical removal of mandibular third molar for all the sites and a blinded research assistant assessed the clinical outcome variables.

All the 20 subjects were randomly allocated into two sub-groups ($n = 10$ sites, in each sub-group) under Group A and B ($n = 10$ subjects, in each group) based on a number given in a sealed envelope. The patients were not informed about the type of anesthesia solution used. Patient's orthomopantomogram (OPG), Bleeding time (BT), Clotting time (CT), Complete Blood count (CBC), HIV, HbsAg, HbcAg screening were done as a part of pre-surgical work-up.

Under Group A:

- Sub-group TM: 1.8 ml of 2% lignocaine with 1:200,000 epinephrine + 1 ml/4 mg dexamethasone;
- Sub-group L: (Control site) 1.8 ml of 2% lignocaine with 1:100,000 epinephrine.

Under Group B:

- Sub-group MTM: 1.7 ml of 4% articaine with 1:100,000 epinephrine + 1 ml/4 mg dexamethasone;
- Sub-group A: (Control site) 1.7 ml of 4% articaine with 1:100,000 epinephrine.

Each patient was administered the inferior alveolar nerve block with the LA dispensed by a trained staff nurse according to the allotted sub-group. Freshly prepared TM is an admixture of 1.8 ml of 2% lignocaine with 1:200,000 epinephrine (Lox, Neon, India) and 1 ml/4 mg dexamethasone (Decdon, Wockhardt, India). MTM is an admixture of 1.7 ml of 4% articaine with 1:100,000 epinephrine (Septanest, Septodont Heathcare) and 1 ml/4 mg dexamethasone (Decdon, Wockhardt, India). The anesthesia selection was allotted randomly that on either of the one sides, patients received TM or MTM with the respective conventional LA solution on the contralateral side among both the groups. The LA solution was injected using an Unolok aspirating leur-lock syringe (HMD, India) fitted with a 26 gauge needle (Hindustan Syringes and Medical Devices Ltd, India) at the rate of 1 ml/min by the same blinded surgeon depending upon the sub-groups. The latency was measured from the time elapsed after full needle withdrawal until the onset of subjective signs of anesthesia and duration of anesthesia was recorded as the time from initial patient perception of the anesthetic effect to the moment in which the effect began to fade, sting on injection using a 10-point Visual Analog Scale and the need to re-anesthetize the site was recorded. The research assistant evaluated the various study parameters on 1st, 3rd and 7th postoperative day.

Surgical procedure and clinical study parameters record

Surgical access to the site was acquired using a modified ward's mucoperiosteal flap, bone removal was done using a 702 surgical carbide fissure bur, (SS White, Lakewood, NJ, USA) on a straight surgical hand piece and micro-motor. The impacted third molar was extracted in toto or after odontectomy, if required. Copious irrigation was done intra and postoperatively followed by simple

interrupted wound closure using 3-0 silk sutures (Ethicon Mersilk, NW5028). Time required for the surgical procedure was recorded from incision placement to wound closure using a digital stopwatch. Post-extraction measures were instructed followed by prescribing 500 mg amoxicillin and 400 mg ibuprofen thrice a day for 5 days. Postoperative pain was measured using a 10-point VAS score. Facial swelling was measured from tragus to angle of the mouth. Mouth Opening (MO) was recorded for all the patients' pre and postoperatively using a calibrated ruler on a standard Performa on the 1st, 3rd and 7th postoperative day. Data was assessed using College of Saint Benedict and Saint John's University statistical calculators; student's paired t test, Analysis of variance (ANOVA) to analyze the significance in the collected data with a significant $P < 0.05$.

Table 2
Mean Visual Analog Scale (VAS) score for sting/pain on Local anesthesia (LA) injection.

Study Group	LA Solution	Mean VAS score for pain/sting on local anesthesia injection
Group A	Sub-group L, 2% Lignocaine with 1:200,000 epinephrine	3.45 ± 0.415
	Sub-group TM, Twin Mix	2.31 ± 0.768
Group B	Sub-group A, 4% Articaine with 1:100,000 epinephrine	3.0 ± 1.247
	Sub-group MTM, Modified Twin Mix	2.7 ± 0.065

Table 3
Onset and duration of soft tissue anesthesia among the study groups.

Study Group	Mean latency in seconds (±SD)	Mean duration of anesthesia in minutes (±SD)
Sub-group L	75.12 ± 30.1	178 ± 15.2
Sub-group TM	52.4 ± 28.3	310 ± 60.2
t Value	-6.02	9.48
p Value	< 0.0001	< 0.0001
Sub-group A	67.45 ± 38.3	360 ± 2.25
Sub-group MTM	60.6 ± 24.1	420 ± 38.11
t Value	-1.107	3.512
p Value	0.141(NS) ^a	< 0.0012

^a NS: non-significant.

3. Results

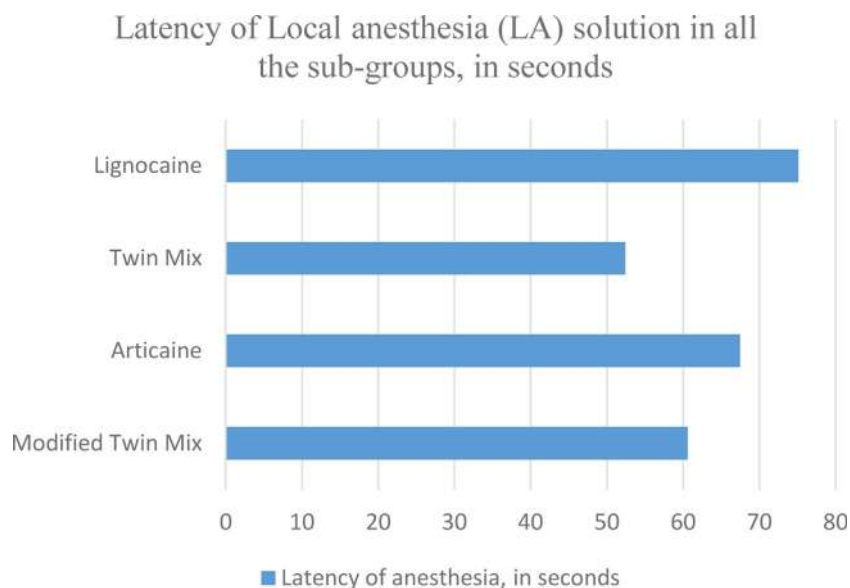
A total of 20 patients (40 sites) were included in this study with a mean age of 31.42 years where $n = 9$ (45%) were females and $n = 11$ (55%) were males. The power of the study using ANOVA test was 0.5 which is considered as medium as this preliminary study involved a limited number of study population. The mean procedural time for patients in sub-group L and TM in Group A was 25.67 ± 5.10 min and 24.89 ± 6.6 min respectively. In Group B, mean procedural time for sub-groups A and MTM was 24.88 ± 4.50 min and 24.1.66 min respectively with no statistical difference. All the 40 inferior alveolar nerve blocks were successful without the need for re-anesthesia.

Mean VAS score for sting on injection for sub-group TM and MTM was less as compared to sub-groups L and A (Table 2). There was no significant difference in the VAS score intra-operatively. The onset of anesthesia was significantly less in sub-group TM followed by MTM with a mean value of 52 ± 28.3 s and 60.6 ± 24.1 s respectively as compared to the control sub-groups. (Table 3, Graph 1) The duration of soft anesthesia was significantly higher in sub-group MTM patients with a mean of 420 ± 38.11 min followed by 360 ± 2.20 min in sub-group A, 310 ± 60.2 min in sub-group TM and 178 ± 15.2 min in L sub-group. (Table 3, Graph 2). An inter-group comparison was done between all the sub-groups. (Tables 4 and 5) Postoperative VAS was higher in patients from sub-group L and A as compared to TM and MTM sub-groups. (Table 6) In the study sub-groups, facial swelling was on peak on 1st postoperative day and gradually decreased till 7th postoperative day contrast to the control sub-groups where there was gradual increase in swelling up to 3rd postoperative day followed by reduction on 7th postoperative day. (Table 7) There was marked reduction in MO among patients in sub-groups L and A when compared to the study sub-groups TM and MTM. (Table 8).

All the patients had complete recovery from anesthesia without any complications in all the sub-groups.

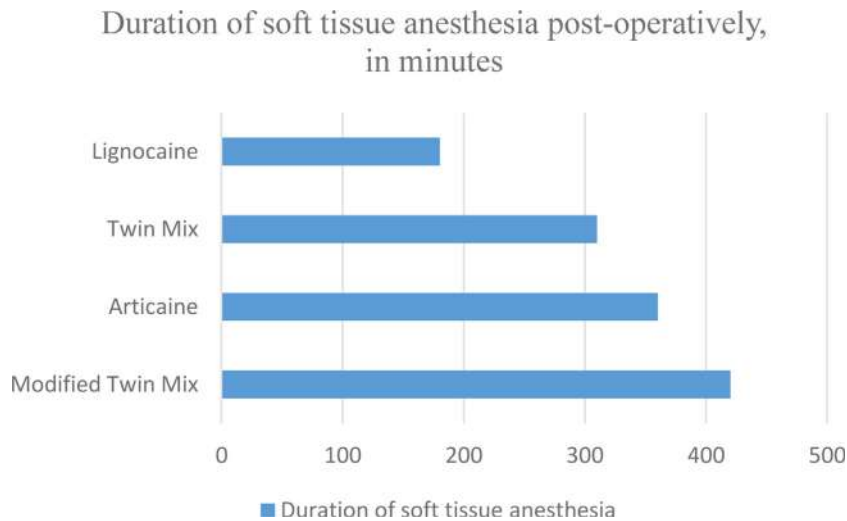
4. Discussion

From this preliminary study, it is evident that admixing dexamethasone to the conventional lignocaine and articaine proved to be beneficial to the patients especially in terms of



Graph 1. Graph showing latency of the local anesthesia (LA) solution among the study sub-groups, in seconds.


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Graph 2. Graph showing the duration of soft tissue anesthesia among the study sub-groups, in minutes.

Table 4
Inter-group comparison to assess the latency of anesthesia using Analysis of variance (ANOVA) between the sub-groups.

Inter-group comparison for latency of anesthesia	Sum of squares	Degrees of freedom	Mean square	Fs	P	Variance component (%)
ANOVA among groups	3115.62	3	1038.54	16.62	6.09	60.96
ANOVA within groups	2249.9	36	62.496			39.04
Total	5365.48	39				

Table 5
Inter-group comparison to assess the duration of anesthesia using Analysis of variance (ANOVA) between the sub-groups.

Inter-group comparison for duration of anesthesia	Sum of squares	Degrees of freedom	Mean square	Fs	P	Variance component (%)
ANOVA among groups	280637.02	3	93545.7	85.36	1.98	89.40
ANOVA within groups	39453.78	36	1095.84			10.60
Total	320090.81	39				

Table 6
Mean postoperative Visual Analog Scale (VAS) score among the study population.

Study sub-groups	Mean Visual Analog Scale (VAS) score (\pm SD)		
	1st Postoperative day	3rd Postoperative day	7th Postoperative day
Sub-group L	3.45 \pm 0.823	2.24 \pm 0.416	1.21 \pm 0.489
Sub-group TM	2.01 \pm 0.616	1.80 \pm 0.451	1.41 \pm 0.453
Sub-group A	3.01 \pm 1.197	2.10 \pm 1.449	0.90 \pm 0.567
Sub-group MTM	2.50 \pm 1.260	2.00 \pm 1.640	0.7 \pm 0.820


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Table 7
Facial swelling assessment among the study population.

Study sub-groups	Mean increase in distance to assess facial swelling (mm) (=Baseline facial measurement-Postoperative facial measurement)		
	Tragus to the angle of mandible		
	1st Postoperative day	3rd Postoperative day	7th Postoperative day
Sub-group L	5.69 \pm 2.20	6.45 \pm 3.68	0.62 \pm 1.12
Sub-group TM	3.00 \pm 1.61	2.44 \pm 1.67	0.21 \pm 1.00
Sub-group A	3.89 \pm 0.73	4.00 \pm 0.61	0.72 \pm 0.40
Sub-group MTM	2.48 \pm 0.64	1.86 \pm 0.60	0.24 \pm 0.56

Table 8

Mean reduction in Maximum inter-incisal opening (MO) postoperatively in the study population, (in millimeters).

Study sub-groups	Mean reduction in MO postoperatively (\pm SD)		
	1st Postoperative day	3rd Postoperative day	7th Postoperative day
Sub-group L	4.45 \pm 1.30	2.52 \pm 0.567	0.89 \pm 578
Sub-group TM	2.65 \pm 1.341	1.67 \pm 1.67	0.32 \pm 0.231
Sub-group A	3.68 \pm 882	2.38 \pm 4.48	0.85 \pm 158
Sub-group MTM	2.72 \pm 4.47	1.20 \pm 4.695	0.16 \pm 3.34

reduced discomfort during injection, longer duration of anesthesia and the other parameters assessed. The novel concept is that we have dexamethasonized 4% articaine hydrochloride and christened it as MTM which is considered to be a safe alternative to 2% lignocaine as articaine has longer duration of anesthesia which in turn increases postoperative comfort of the patient. There was negligible difference between the TM and MTM sub-groups in terms in pain measured using a VAS scale, and facial swelling. MO was assessed pre-operatively and then the subsequent MO was measured on the mentioned postoperative days. The discrepancy in the reduced MO postoperatively have been tabulated and mentioned in the results. But there was a significant difference in the onset and prolonged duration of anesthesia in patients who received MTM as the latency was significantly reduced and the duration of anesthesia was longest in this sub-group. The severity of all the clinical study parameters were increased in sub-groups L and A which were the control sites. This study strongly advocates the admixture of 4% articaine with dexamethasone when longer duration of anesthesia is required without any adverse effects in any of the patients.

Dexamethonized LA solution is a novel method of intra-space drug administration to achieve anesthesia and analgesia was popularized by Bhargava D et al. is known to have several advantages over the conventional routes. Administration of steroids following third molar surgery has beneficial effects postoperatively as corticosteroids are involved in suppressing the inflammatory mediators at the injury site suppressing acute inflammation. [1] Literature evidence highlights the effectiveness of dexamethasone after third molar surgery [2,8,9]. In the present study, it is observed that patients who received TM and MTM showed improved quality of life postoperatively demonstrated by the decreased VAS score and facial swelling as compared to the control site.

LA solutions with vasopressor has a pH of 3.5 approximately. Malammed SF emphasized that the LA must be deposited near to the nerve membrane through a proper injection technique and it must cross the nerve membrane to block sodium channels to obtain adequate anesthesia. The solution should exist in RN, the uncharged form that is lipid soluble to penetrate neural sheath and membrane. Alkalinisation or buffering of LA solution alters its pH to a more basic solution which will increase the RN as compared to the charged RNH⁺ which is water soluble resulting in various advantages such as lesser sting on injection, enhanced onset of anesthesia [10]. The present study showed similar results were the patients who received TM and MTM had lesser sting on injection, faster onset and longer duration of anesthesia with improved quality of life postoperatively. The chemical stability of dexamethonized LA has been validated using a double beam ultraviolet (UV) spectrometry study. It was observed that physical compatibility and pH between LA and dexamethasone solution were similar along with no change in the property and stability of the active molecules – LA component, epinephrine and dexamethasone. [11]. The author also opined that intra-space administration of dexamethasone can achieve plasma concentration similar to the plasma concentration achieved when the drug is administered intramuscularly [12,13].

The mechanism by which dexamethasonized LA improves postoperative outcomes has been proposed by Bhargava D et al. Dexamethasone blocks superoxide production and lysosomal enzyme release in human neutrophils inhibiting functional degranulation response. Admixing it with LA causes the solution to become basic (pH = 6) as compared to the conventional LA with vasoconstrictor, which is more acidic (pH range = 3.5–4.5) enhancing the onset and duration of anesthesia. It was also proposed that there is increase in activity of inhibitory potassium channels through glucocorticoid receptors on nociceptive C-fibres and vasoconstriction property of dexamethasone [14]. To support this evidence, Williams et al. have concluded that lower doses of dexamethasone administered perineurally can cause significant clinical outcomes and larger doses did not exhibit additional benefits and the adverse effects to adverse effect neural conduction was on a dose-dependent manner [15–17]. No known incidence of neurotoxicity, complications or adverse effects in humans has been documented in the literature for the use of peri-neural dexamethasone [18].

Further studies needs to be conducted involving larger study population to establish a definitive results along with chemical stability of articaine when mixed with dexamethasone which is beyond the scope of this study.

5. Conclusion

From this preliminary study, we observed that MTM has the advantage of providing longer duration of soft tissue anesthesia and conclude that it can be used as a safe and alternative option in patients requiring prolonged anesthesia postoperatively resulting in better overall clinical outcomes after mandibular third molar surgery. Future trends for improved clinical outcomes should aim at admixing drugs with LA that can provide maximum benefit to the patient in every aspects.

Ethical approval

Obtained. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.


Informed consent

Informed consent was obtained from the patients involved in this study.

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Disclosure of interest

The authors declare that they have no competing interest.

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A Simplified Single-Penetration Technique of IANB (MK Technique) for Mandibular Anesthesia

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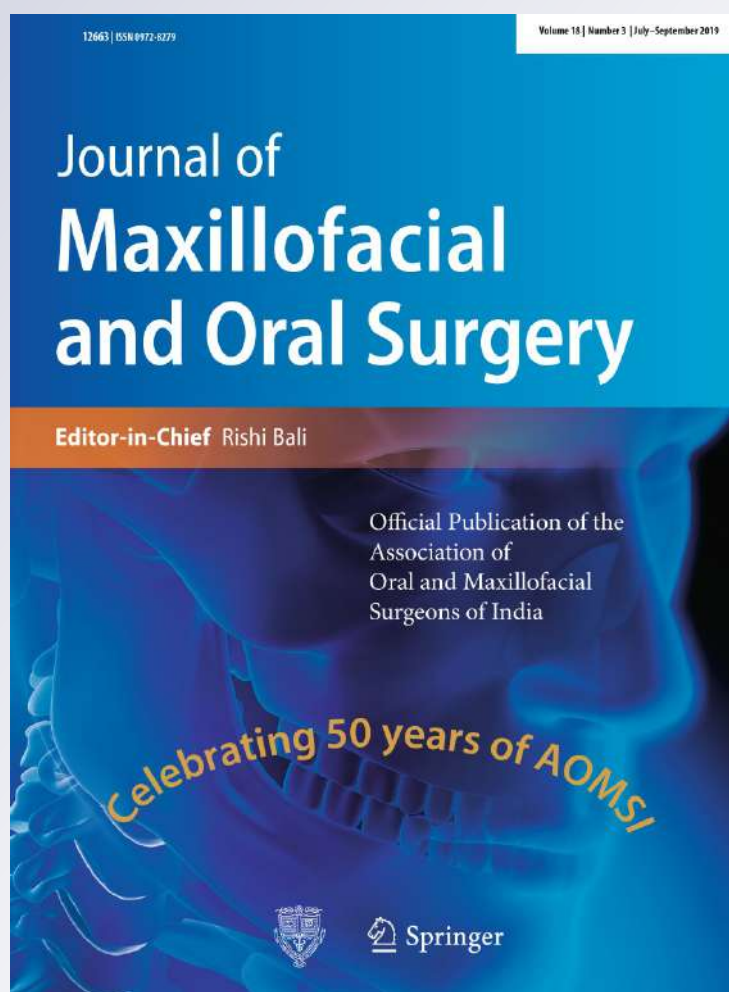
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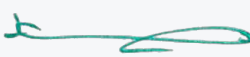
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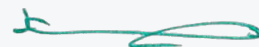
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A Simplified Single-Penetration Technique of IANB (MK Technique) for Mandibular Anesthesia

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Abstract

Introduction Owing to the anatomical basis established on the detailed course, distribution and innervations of buccal nerve in the literature, we believe that an effective and extensive buccal nerve block could be achieved when the nerve is anesthetized proximal to its branching point which relates to anteromedial aspect of ramus (retromolar fossa). Though several techniques of inferior alveolar nerve block (IANB) including few single-penetration approaches were already well reviewed and practiced, pitfalls remained in terms of achieving adequate retromolar soft tissue anesthesia, as well as undermining the very importance of contacting the bone and the orientation of the bevel. We propose a simplified single-penetration technique aligning almost similar to conventional technique and its well-adopted landmarks, but still obviating the need for a separate penetration for long buccal nerve anesthesia.

Aim To study the efficacy of a simplified single-penetration technique for mandibular anesthesia.

Objectives To study the adequacy of the simplified IANB technique in minimizing the number of penetrations required to achieve buccal nerve anesthesia decreasing patient's pain and discomfort when IANB is performed and the ease of adaptability across the operators.

Materials A 25-gauge 42-mm needle, local anesthesia with adrenaline—2% lignocaine HCl (1:80,000), 2.5-ml syringe, observation form.

Method Study 1—A sample size of 120 cases, in oral surgery department of our college, with 60 patients in Group I (control) where patients received anesthesia through conventional IANB and 60 patients in Group II (experimental) where patients received anesthesia through simplified IANB. Both the groups are again subdivided into group A (single operator) and group B (multiple operators). Study 2—20 bilateral mandibular impacted third molar in oral surgery department of our college, with right side (control—conventional technique) and left side (experimental—simplified single-penetration technique). VAS data are recorded in the excel sheet.

Results The success rate of the simplified technique is quite comparable to the conventional groups. The mean pain and discomfort score in simplified technique was 3.08 which is relatively lower when compared to mean value in conventional technique which was 6.88. The difference was statistically significant.

Conclusion Simplified technique minimizes the number of penetrations, which substantially decreases patient's pain and discomfort levels not only during injection, but also during surgical procedures on mandibular posterior region because of extensive and profound buccal nerve territorial anesthesia.

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Keywords Simplified technique · MK technique · Long buccal nerve distribution · Buccal nerve block · Anatomical considerations for inferior alveolar nerve block · Intra oral technique for inferior alveolar nerve block

Introduction

Extraction of teeth from mandibular second premolar to third molar requires long buccal anesthesia along with inferior alveolar nerve block and lingual nerve blocks. Long buccal nerve is anesthetized in same penetration in few techniques like Fischer 1, 2, 3 technique, Gow-Gates technique and Vazirani–Akinosi technique described for mandibular dental anesthesia [1].

Most commonly used mandibular anesthesia includes open mouth techniques like inferior alveolar nerve block: alternative technique [2], classical inferior alveolar nerve block (direct and indirect techniques), Halsted block (conventional IANB), method of Clarke and Holmes, technique of Angelo Sargenti, extra oral mandibular nerve block, Fischer 1, 2, 3 technique while the least adopted techniques are the “A. R. T.” (anterior ramus technique) mandibular block [3], Gow-Gates and closed mouth technique by Akinosi–Vazirani [4]. Except in Gow-Gates, Fischer 1, 2, 3 and Akinosi–Vazirani, in all the other techniques, a separate injection for buccal nerve anesthesia is needed. The separate second penetration for long buccal is observed to provoke pain and discomfort to patient [1].

We proposed a modification of conventional IANB, similar to Fischer 1, 2, 3 technique, which obviates the need for a separate injection for buccal nerve anesthesia. After trying various techniques clinically and studying the reasons for either delayed onset or failure of anesthesia, we narrowed at Fischer 1, 2, 3 for being far more simpler and accurate single-penetration technique retaining the conventional landmarks, with problem being the needle deflection as the technique per se is anterograde. We modified this into a retrograde deposition pattern targeting IANB first with BRIT followed by lingual and long buccal on the way withdrawing and swinging barrel and simultaneously nullifying needle deflection to a greater extent. The current study was conducted to evaluate the efficacy of the technique and the ease of adaptation between various groups of operators (beginners and experienced operators).

Basis of the Study

Anatomical Course and Distribution of Buccal Nerve

The knowledge of the course and distribution of buccal nerve is of a great importance in this technique. After releasing the anterior deep temporal nerve, the long buccal nerve descends behind the superior head of the lateral pterygoid muscle and then turns laterally between two heads of this muscle. At this point, nerve releases the fibers that enter the lateral pterygoid muscle. The buccal nerve turns sharply downward to descend on the outer surface of the inferior head of lateral pterygoid and temporal muscles and often closely attached to the fascia of temporalis muscle. The nerve may even pass through the substance of temporal muscle near its anterior border. At the anterior border of the tendons of the temporal muscle, long buccal nerve here exchanges fibers with branches of facial nerve. Singly, the branches of buccal nerve perforate the buccinator muscle and reach the mucous membrane of the cheek. Almost the entire mucosa of the cheek is supplied by the buccal nerve, with the exception of a poster superior area, which may receive sensory fibers from the gingival branch of the superior alveolar nerves. The buccal branches of the superior alveolar nerve and the buccal nerve itself are in reciprocal relation. Often the buccal nerve participates in the nerve supply of a small area of buccal gingival in the distal part of the upper jaw. Branches of the posterior superior alveolar nerves, on the other hand, may supply a larger area of the cheek and may, in rare cases, even replace the buccal nerve [5].

An anatomical study on buccal nerve distribution reveals that in some cases some twigs from the buccal nerve were given to the posterior part of the buccal mucosa. These twigs branched from the main trunk of the nerve before it perforated the insertion of the tendon of the temporalis. On superficial examination, the buccal nerve appeared to give branches only to the buccal mucosa. However, detailed fine dissection, after removal of the mandibular bone and reflection of the periosteum and gingival flap downwards, revealed that these twigs ran to and supplied the lower buccal gingivae above the mandibular insertion of the buccinator. In contrast, no twigs were seen traveling beyond the insertion of the buccinator toward the gingivae in the maxilla. Anteriorly, in all cases, the main trunk of the buccal nerve is divided into two branches (toward the upper and lower lips) and, along its course, gave fine twigs to the buccal mucosa after passing through the layer of facial musculature proximal to the modiolus. At the angle of mouth, the buccal nerve formed a plexus with the facial nerve, the inferior orbital

nerve and the mental nerve. Buccal nerve terminal branches thereafter ran deep to these three nerves (Fig. 1). The furthest distribution of the buccal nerve branches to the lips extended to approximately half of lateral side of the lips in two cases and, in the other 10 cases, it is extended.

These findings indicate that the posterior buccal mucosa is innervated by the buccal nerve, and the mucosa of the fauces is innervated by the buccal nerve and other adjacent nerves, for example, the glossopharyngeal nerve. It is therefore important clinically, when giving a buccal nerve block, to anaesthetize the nerve proximal to the branching point of the twigs to the posterior buccal mucosa and gingivae. If the buccal nerve is blocked distal to its passage through tendon of the temporalis, the posterior buccal gingivae, buccal mucosa and part of the mucosa of the fauces may not be anaesthetized [6].

The Technique

Landmarks:

1. Anterior border of ramus—coronoid notch
2. Deepest point of pterygomandibular raphe
3. Occlusal plane
4. Opposite premolars of mandible

Target areas:

1. Mandibular foramen on medial side of ramus
2. Pterygomandibular space
3. Retromolar fossa

The technique used in this study is a simplified inferior alveolar nerve block technique. The simplified IANB used in this study includes the following steps:



Fig. 1 Buccal nerve distribution

1. A 25-gauge-long needle (42 mm) is penetrated at deepest point of pterygomandibular raphe from the opposite mandibular premolar, where bone is contacted at 3/4th of its length above the mandibular foramen and where inferior alveolar nerve traverses in the pterygomandibular space before entering the mandibular canal. The needle is retrieved slightly to avoid subperiosteal injection. At this position, 1.2 ml of local anesthetic solution is deposited after confirming for negative aspiration to avoid intravascular injection (Fig. 2).
2. Then, the barrel is redirected to same side withdrawing half of the needle. This minimizes deflection, ensuring that half the length of the long needle still inside the pterygomandibular fossa 0.8 ml of local anesthetic solution is injected for lingual nerve block (Fig. 3).
3. Finally, the needle is swung to original position (as in IANB) withdrawing the needle to just one-fourth of its length; 0.5 ml of local anesthetic solution is deposited between buccinator muscle and anteromedial aspect of ramus posteromedial to the tendinous insertion of temporalis. Just before the needle is withdrawn completely from the site of penetration, we targeted long buccal nerve in retromolar fossa area (Fig. 4).

Aims and Objectives

- Aim is to study the efficiency of a simplified single-penetration technique for mandibular anesthesia.
- The objective of the study is to prove the adequacy of the simplified IANB technique in minimizing the patient's pain and discomfort while studying the adaptability of the technique (mean variation between beginners and experienced).



Fig. 2 Demonstration of targeting inferior alveolar nerve in pterygomandibular space



Fig. 3 Demonstration of targeting lingual nerve by withdrawing half the length of long needle and swinging barrel of the syringe onto same side simultaneously



Fig. 4 Demonstration of long buccal nerve block as it is targeted in retromolar fossa

Materials

The materials used are 25-gauge 42-mm needle, 2% local anesthetic solution (2% lignocaine HCL) with adrenaline of 1:80,000 dilution, 2.5-ml luer lock syringe, diagnostic instruments (mouth mirror and straight probe), observation form for recording symptoms.

Method

Ethical approval for the study was obtained from institutional ethical committee. We included the patients deemed fit for procedures under local anesthesia and patients requiring mandibular procedures under inferior alveolar nerve block anesthesia including extractions, impactions

and alveoloplasties. We excluded the patients deemed not fit for procedures under local anesthesia (with or without adrenaline).

The study 1 consists a total of 120 patients with 60 patients in each group of Group I (control) and Group II (experimental). Consent was obtained from each patient involved in the study. Both control group and experimental group are subdivided into group A and group B. Group A, 30 patients, received local anesthesia by a single operator, and the 30 patients in group B received local anesthesia through multiple operators (i.e., trained group of doctors). The efficiency of the block was checked in all the groups by evaluating subjective symptom which is numbness of cheek and objective signs which are the presence/absence of pain on probing in buccal vestibule/periodontal pockets of molar/retromolar area. The pain and discomfort levels of the patients who received the blocks were recorded using visual analogue scale (VAS).

The study 2 consists a total of 20 patients who have underwent procedures requiring bilateral mandibular blocks, where 20 right side blocks are Group I (control) and 20 left side blocks Group II (experimental). Consent was obtained from each patient involved in the study. Both control group and experimental group patients received local anesthesia by multiple operators (i.e., trained group of doctors). The efficiency of the block was checked in all the groups by evaluating subjective symptom which is numbness of cheek and objective signs which are the presence/absence of pain on probing in buccal vestibule/periodontal pockets of molar/retromolar area. The pain and discomfort levels of the patients who received the blocks were recorded using visual analogue scale (VAS).

Results

Study 1

Table 1 represents symptoms indicative of onset of local anesthesia. Results are compared between males and females, anesthetized by single operator or multiple operators, following conventional technique and simplified technique. In single-operator category, all male and female subjects, who were anesthetized following conventional technique, reported satisfactory symptoms, whereas in simplified technique, 81% males and 93% of females reported satisfactory symptoms of onset of anesthesia. However, difference in the proportion of respondents who reported satisfactory symptoms with conventional and simplified techniques is not statistically significant. Multiple-operator category, all the subjects, irrespective of gender and technique, reported satisfactory onset of symptoms.

Table 1 Symptoms of onset of local anesthesia

Administrator	Gender of subjects	Technique		Symptoms		Total	Chi-square test <i>p</i>
				Satisfactory	Not satisfactory		
Single operator	Male	Conventional	Count	15	0	15	0.12
			%	100.0%	0.0%	100.0%	
	Simplified	Count	13	3	16		
		%	81.2%	18.8%	100.0%		
Female	Conventional	Count	15	0	15	0.48	
		%	100.0%	0.0%	100.0%		
	Simplified	Count	13	1	14		
		%	92.9%	7.1%	100.0%		
Multiple operators	Male	Conventional	Count	18	0	18	*
			%	100.0%	0	100.0%	
		Simplified	Count	10	0	10	
			%	100.0%	0	100.0%	
	Female	Conventional	Count	12	0	12	
			%	100.0%	0	100.0%	
		Simplified	Count	20	0	20	
			%	100.0%	0	100.0%	

*No statistics are computed because combined is a constant

Table 2 shows patient pain and discomfort level, as measured on a visual analogue scale, in patients anesthetized by using simplified and conventional techniques. The number of patients is the same in both the techniques. The mean patient pain and discomfort level score in simplified technique was 3.08 which was relatively lower when compared to mean value in conventional technique which was 6.88. The difference was statistically significant. The median value is higher in conventional technique when compared to simplified technique.

Study 2

Table 3 shows distribution of respondents according to pain and discomfort level category on visual analogue scale. More than half of the subjects in the simplified technique group reported pain score of 3 on VAS, and none reported beyond 3, whereas 40% each of the respondents reported pain scores 6 and 7. The difference in the proportion of respondents among different categories of VAS

is highly significant as analyzed by Chi-square test ($p = 0.0001$).

Table 4 shows certain descriptive statistics of patient pain and discomfort scores. While the maximum pain and discomfort levels reported by the subjects in modified group is 3, the subjects in conventional group reported a minimum score of 6. The statistical difference in the range of pain and discomfort level scores is very highly significant between the groups, as indicated by Moses test ($p = 0.0001$). The median scores of pain and discomfort level reported by subjects in simplified and conventional groups were 3 and 7, respectively. The difference in median score is highly significant, as analyzed by independent sample median test ($p = 0.0001$). Mean pain and discomfort level score in modified group was 2.65 (SD = 0.49), whereas that in conventional group was 6.80 (SD = 0.77). The difference in mean scores is highly significant as analyzed by Mann–Whitney U test. Thus, subjects in simplified technique group reported remarkably

Table 2 Patient pain and discomfort levels of patients in simplified and conventional groups

	Technique	N	Mean*	SD	Median
Patient pain and discomfort level	Simplified	60	3.08	0.645	3
	Conventional	60	6.88	0.825	7

*Mann–Whitney U test: $p = 0.001$ (statistically significant); VAS 1–4: mild or very low pain; VAS 5–8: moderate pain

Table 3 Patient pain and discomfort levels on VAS scale in conventional and modified technique groups

Technique		Patient pain and discomfort levels on VAS*					
		2	3	6	7	8	Total
Simplified	Count	7	13	0	0	0	20
	% Within technique	35.0%	65.0%	0.0%	0.0%	0.0%	100.0%
Conventional	Count	0	0	8	8	4	20
	% Within technique	0.0%	0.0%	40.0%	40.0%	20.0%	100.0%
Total	Count	7	13	8	8	4	40
	% Within technique	17.5%	32.5%	20.0%	20.0%	10.0%	100.0%

*Chi-square test, $p = 0.0001$

Table 4 Patient pain and discomfort levels on VAS scale in conventional and simplified technique groups

Technique	N	Patient pain and discomfort levels on VAS				
		Minimum*	Maximum*	Median**	Mean***	SD
Simplified	20	2	3	3.00	2.65	0.489
Conventional	20	6	8	7.00	6.80	0.768

*Independent samples Moses test, $p = 0.0001$

** Independent samples median test, $p = 0.0001$

***Mann–Whitney U test, $p = 0.0001$

lesser pain and discomfort levels compared to those in conventional group.

Discussion

Ever since the inception, various modifications of IANB have been proposed to address the limitations of each technique in terms of accuracy and ease of reproducibility of the technique, complex anatomy, number of penetrations and other local complications. One common objective of all the endeavors in simplifying the technique of IANB has been to minimize patient's pain and discomfort during the procedure while achieving the maximum anesthetic effect. The most commonly practiced open mouth techniques for IANB are conventional IANB and Gow-Gates. Despite documented evidence of high success rate of Gow-Gates technique and 15–20% of failure rate of conventional technique [7], Gow-Gates technique remains as the second choice while conventional technique is adopted as the first choice [8]. Few significant disadvantages of the Gow-Gates technique like slower onset of anesthesia, which can take from 5 to 7 min and lack of definitive anatomical landmarks, make it a difficult choice for IANB [9]. Though the literature suggests that there are no significant differences in pain on injection among conventional and Gow-Gates techniques [10, 11], the latter technique minimizes patient pain and discomfort as the need for a second penetration for buccal nerve anesthesia is obviated. However, few

authors fervently oppose the widespread use of Gow-Gates Technique for a variety of reasons [12].

The considerations for any nerve block technique are definitive landmarks. Targeting long buccal nerve within retromolar fossa in fact delivered profound buccal anesthesia all the way posteriorly till anterior faucial pillar (blocking even the very first few twigs of long buccal nerve [6] aiding in utmost comfort of soft tissue manipulation during transalveolar extractions, especially in distoangular impaction with reasonable time of onset of anesthesia and no or minimal complications. So, in the current study a modification to the conventional IANB is similar to Fischer 1 2 3, but differs being retrograde minimizing needle deflection, and therefore, reducing onset time is made to achieve regional mandibular anesthesia in a single penetration. The technique adopted in this study has been described under methodology. Irrespective of gender, the simplified technique used in this study was well performed by multiple operators, i.e., trained group of interns and postgraduates, but there was a slight decrease in the proportion of respondents who reported satisfactory symptoms when the technique was performed by single operator who was just a beginner. In fact, bulky cheeked patients with ptotic buccal fat pad accounted for misappropriating depth of needle penetration into retromolar fossa lead to failure of IANB, but gave a profound and extensive buccal nerve anesthesia which formed the very basis of targeting buccal nerve more proximal in its course as a single-penetration technique. Overall performance of simplified technique by skilled persons was significantly effective, and mastering is

quite easy (Table 1). Subjects in this simplified technique reported significantly lower levels of pain and discomfort compared to conventional group, as indicated by mean and median scores of pain on a visual analogue scale. Lower pain and discomfort levels improve the ability of the patients to cooperate with the operator aiding in effective execution of surgical procedure (Table 4).

Conclusion

Simplified technique achieved better onset and extent of long buccal nerve anesthesia than the conventional technique while following the same landmarks as + that in conventional technique. It also significantly reduced patient's pain and discomfort levels compared to conventional technique not just during injection, but also during surgical procedure. This single-penetration technique (retrograde) can thus be a modified Fischer 1 2 3 (anterograde) with minimal needle deflection; thus, there are rapid onset and very minimal failures of achieving anesthesia.

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Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique

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Objectives: Socket grafting is vital to prevent bone resorption after tooth extraction. Several techniques to prevent resorption have been described, and various bone graft substitutes have been developed and used with varying success. We conducted this pilot study to evaluate the performance of nanohydroxyapatite (nHA) derived from chicken eggshells in socket preservation.

Materials and Methods: This was a prospective, single center, outcome assessor-blinded evaluation of 23 sockets (11 patients) grafted with nHA and covered with platelet-rich fibrin (PRF) membrane as a barrier. Bone width and radiographic bone density were measured using digital radiographs at 1, 12, and 24 weeks post-procedure. Postoperative histomorphometric and micro-computed tomography (CT) evaluation were performed. The study protocol was approved by the Institutional Ethics Committee.

Results: All patients had uneventful wound healing without graft material displacement or leaching despite partial exposure of the grafted socket. Tissue re-epithelialized with thick gingival biotype (>3 mm). Width of the bone was maintained and radiographic density increased significantly with a trabecular pattern (73.91% of sockets) within 12 weeks. Histomorphometric analysis showed 56.52% Grade 3 bone formation and micro-CT analysis revealed newly formed bone with interconnecting trabeculae.

Conclusion: Use of a PRF membrane with nHA resulted in good bone regeneration in sockets. Use of a PRF membrane prevents periosteal-releasing incisions for primary closure, thereby facilitating the preservation of keratinized mucosa and gingival architecture. This technique, which uses eggshell-derived nHA and PRF membrane from the patient's own blood, is innovative and is free of disease transfer risks. nHA is a promising economic bone graft substitute for bone regeneration and reconstruction because of the abundant availability of eggshell waste as a raw material.

Key words: Bone, Tooth extraction, Socket grafting, Wound healing, Bone regeneration

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I. Introduction

Socket grafting is vital to prevent bone resorption¹. Remodeling of alveolar bone is a continuous process in physiology and pathology, but alveolar bone is essential for the retention of teeth. Extraction of teeth leads to an edentulous space and loss of alveolar bone, function, and aesthetics²⁻⁶. A systemic

review of dimensional changes of the alveolar bone following extraction showed a mean reduction of 3.8 mm in width and 1.24 mm of height in the first six months after extraction⁷. Replacement of missing teeth is essential, but without the support of adequate healthy alveolar bone, teeth replacement is difficult⁸. Socket preservation attempts are therefore made to maintain bone to enable patients to have implant supported prosthesis in the future without the requirement for additional surgical procedures for augmentation¹.

Socket preservation has been performed using different materials with varying success rates^{1,9-12}. Materials used for grafting include autogenous bone, allograft, xenograft, and alloplasts, among others^{1,9}. Various membranes have been used for wound closure, including polytetrafluoroethylene and bovine and porcine collagen matrices¹. Graft materials enhance bone formation by promoting osteoconduction, os-

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teoinduction, and osteogenesis¹³⁻¹⁶. However, no graft material to date is considered ideal. Recently, nanohydroxyapatite derived from chicken eggshell (nHA) was developed¹⁷⁻¹⁹ and used as an innovative graft material for bone regeneration purposes²⁰⁻²², but none of these previous studies evaluated the performance of nHA and a platelet-rich fibrin (PRF) membrane in socket preservation²⁰⁻²³.

We therefore performed this pilot study to evaluate the histomorphometric and radiographic characteristics of bone regeneration when nHA and a PRF membrane were used for alveolar bone preservation and enhancement in the maxilla and mandible of humans to enable implant-supported restoration.

II. Materials and Methods

1. Study design

A total of 11 (eight male and three female) patients between the ages of 15 to 45 years who presented to the outpatient Department of Oral and Maxillofacial Surgery, Sibar Institute of Dental Sciences (Guntur, India) for extraction and willing to participate in the study protocol were enrolled during the period from June 2017 to June 2018. The Institutional Ethics Committee of Sibar Institute of Dental Sciences approved the study protocol (IEC-16/09/2014) and this study was registered with the Clinical Trials Registry-India (CTRI). Patients and patients' relatives were informed about the study

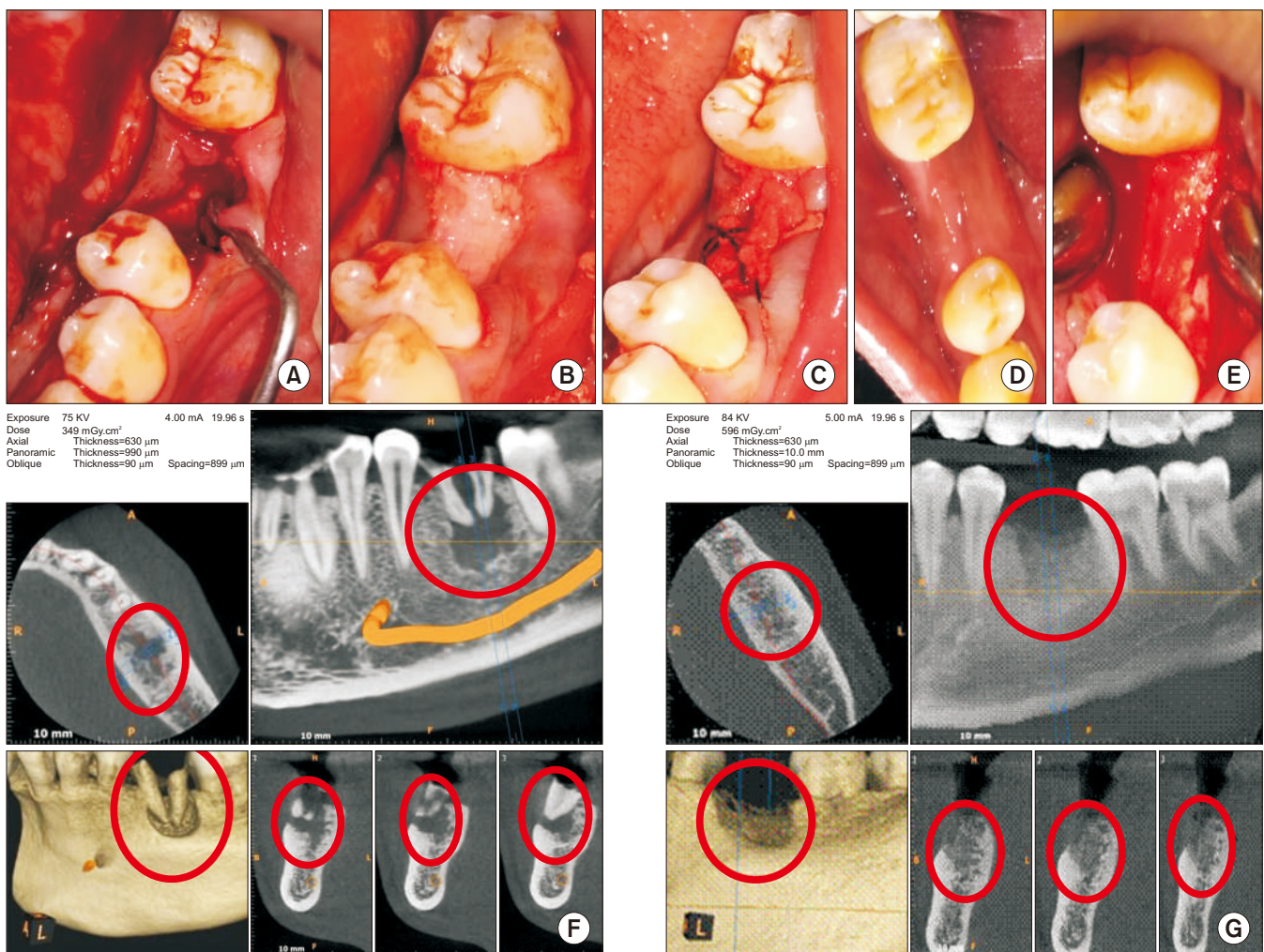


Fig. 1. A. Bone defect immediately after extraction with curette in place. B. Bone graft material placed till crestal level in the mandibular left first molar socket. C. Wound closure using platelet-rich fibrin membrane and figure of eight suture. D. Well healed wound after two weeks. E. Well-formed bone observed during exposure for implant bed preparation. F. Cone-beam computed tomography pre-extraction view with roots and bone defect marked in red circles. G. Bone formation (red circles) assessed before implant placement.

Vivekmand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. *J Korean Assoc Oral Maxillofac Surg* 2019

protocol before enrollment and written informed consent was obtained. This was a prospective, single center, outcome assessor-blinded pilot study.

1) Sample size calculation

The sample size required for this pilot study was 10 patients but we recruited 11 patients involving one or more sockets per patient depending on the clinical scenario. Sample size was calculated with 80% power and a 5% alpha error based on a two-sided test. Total of 23 extraction sockets were assessed among the 11 patients.

2) Inclusion and exclusion criteria for patient selection

Patients with grossly decayed teeth, unrestorable teeth, those willing to undergo implant restoration, and those readily available for periodic recall were enrolled. Pregnant patients, patients with gross tooth mobility due to complete bone loss (more than 3/4 of root length), those in a vulnerable category or with a systemic illness (diabetes, thyroid, hypertension, etc.), and medically compromised patients were excluded from the study protocol to eliminate bias.

3) Materials

(1) Nanohydroxyapatite

nHA granules used for grafting were synthesized from natural calcium precursors obtained from chicken eggshell waste and produced using a rapid microwave processing technique^{17,18}. The nHA obtained in this manner is typical flower-like nanostructured HA¹⁹. It is composed of leaf-like flakes extending radially from the center with a width of 100 to 200 nm and length of 0.5 to 1 μ m. nHA contains Ca, P, O, C, and Mg^{18,19}. Incorporation of Mg ions into the HA structure is important for the development of artificial bones¹⁹.

(2) Platelet-rich fibrin membrane

PRF membrane was prepared using autologous blood under standard aseptic precautions. Intravenous blood (5 mL) was drawn from the antecubital region using a sterile disposable syringe. Blood was centrifuged using a tabletop centrifuge for 10 minutes at 3,000 rpm immediately after collection. The resultant product comprised acellular plasma as the top layer, a PRF clot in the middle, and red blood cells at the bottom. Successful preparation of PRF depends on speedy blood collection and immediate centrifugation before clotting begins. PRF component was separated and molded into a thin sheet of approximately 1×1 cm using a sterile, wet piece of gauze.

2. Operative procedure

Extraction of the tooth or tooth root was performed atraumatically under local anesthesia. If necessary, curettage was performed (Fig. 1. A) and irrigation was done with Betadine and saline. Socket compression was not performed to prevent the collapse of bony walls. After hemostasis, nHA grafting was performed to the crestal level in all 23 sockets.(Fig. 1. B) PRF membrane was transferred over the grafted socket and tucked between buccal and lingual/palatal gingiva and stabilized using 3-0 black silk suture (figure of eight). The suture passed through the membrane to prevent slippage. PRF membrane prevented leaching of materials and periosteal-releasing incisions for primary closure.(Fig. 1. C) Postoperatively, all patients were administered antibiotics and analgesics for five days as a standard of care. Patients were recalled after 8 days for suture removal.

Implant bed was prepared using a trephine bur (3×10 mm) followed by a standard sequential drilling protocol for implant placement 24 weeks after grafting. Depending on initial stability, implants were loaded immediately or in a delayed manner using NobelParallel conical connection root form implants made by Nobel Biocare (Kloten, Sweden).

3. Clinical and radiographic evaluation

Wound healing was assessed prospectively.(Fig. 1. D) Patients were followed-up for 24 weeks until implant insertion. (Fig. 1. E) Preoperative cone-beam computed tomography (CBCT) revealed bony defects with roots.(Fig. 1. F) Bone width was measured using bone calipers and radiographic bone density was measured using digital intraoral peri-apical radiograph at week 1, 12, and 24 postoperatively. CBCT was obtained for planning and assessment before implant placement.(Fig. 1. G) Digora software (Digora for Windows 2.8.107.458 Network Client, Soredex Orion Corporation, Finland) was used to assess follow-up density changes and bone formation characteristics.(Fig. 2) Radiographic evaluation criteria were modified and adapted for assessment^{24,25}. Gridlines in digital IOPA were used as reference lines to assess bone height postoperatively for available bone for implant size selection. The assessor was blinded for the time duration of follow-up during radiographic evaluation.

1) Micro-CT evaluation

During implant placement, a trephine biopsy (taken using a 3×10-mm trephine bur) harvested from the grafted site was

subjected to histomorphometric and micro-CT evaluation. (Fig. 3) Micro-CT analysis was carried out using a PhoenixV/tome/Xs machine (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). (Fig. 4. A-C) A series of microfocus CT images were acquired as x-ray images while progressively rotating the sample step by step through a 360° rotation at increments less than 1° per step. (Fig. 4. D) These projections were used to reconstruct volumetric data. The multiline configuration of the flat panel covered with a movable shield reduced scattering artifacts. A temperature-

stabilized DXR500L detector (General Electric, Boston, MA, USA) with 3,072 pixels provided a voxel size of 1.6 µm for assessment and allowed scanning of objects with voxel sizes <2 µm.

2) Histomorphometric evaluation

After micro-CT analysis, sections were decalcified using 5% EDTA. After processing, specimens were embedded in a paraffin wax block and sliced with a microtome to a thickness of 5 µm for H&E staining. (Fig. 5) The core was cut trans-

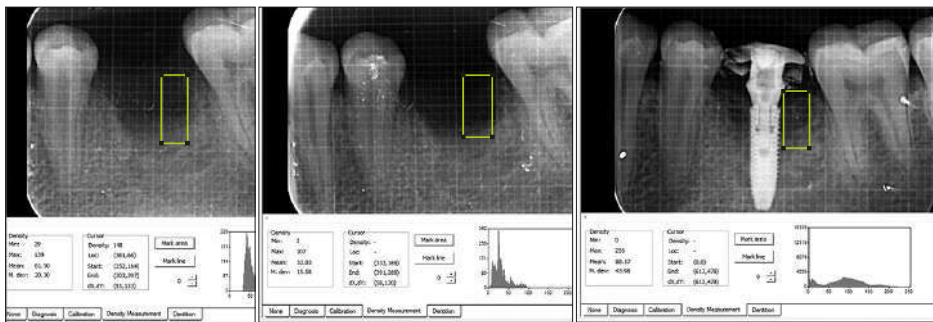


Fig. 2. Intra-oral periapical radiographic images for assessment of bone formation and density using Digora software. Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

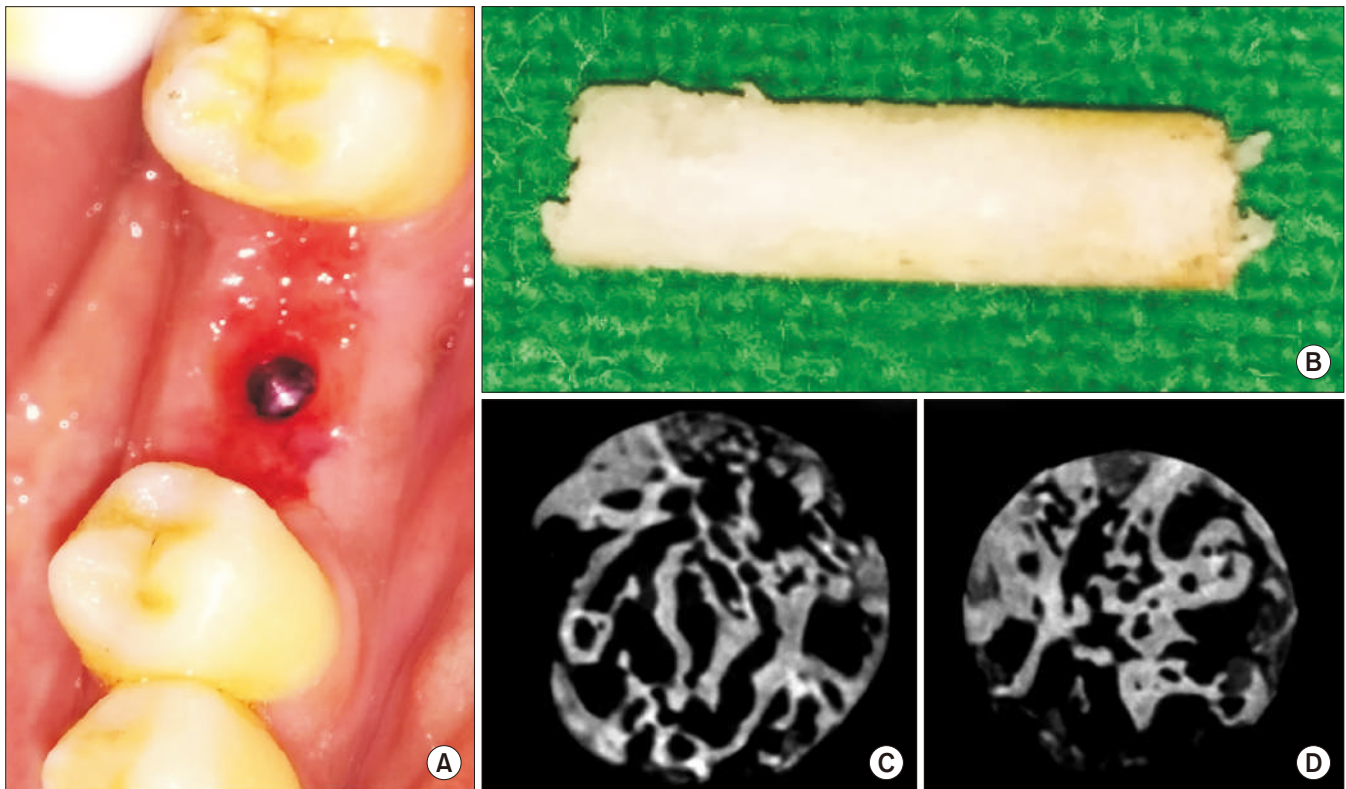


Fig. 3. A. Thick tissue biotype observed after removal of the screw-retained abutment of an immediate-loaded implant following suture removal after 1 week. B. Trephined bone (3x10 mm size) harvested from the left mandibular first molar site 24 weeks after grafting (during implant bed preparation) for micro-computed tomography (CT) and histomorphometric analysis. C, D. Micro-CT sections of trephine bone showing intervening trabeculae.

Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

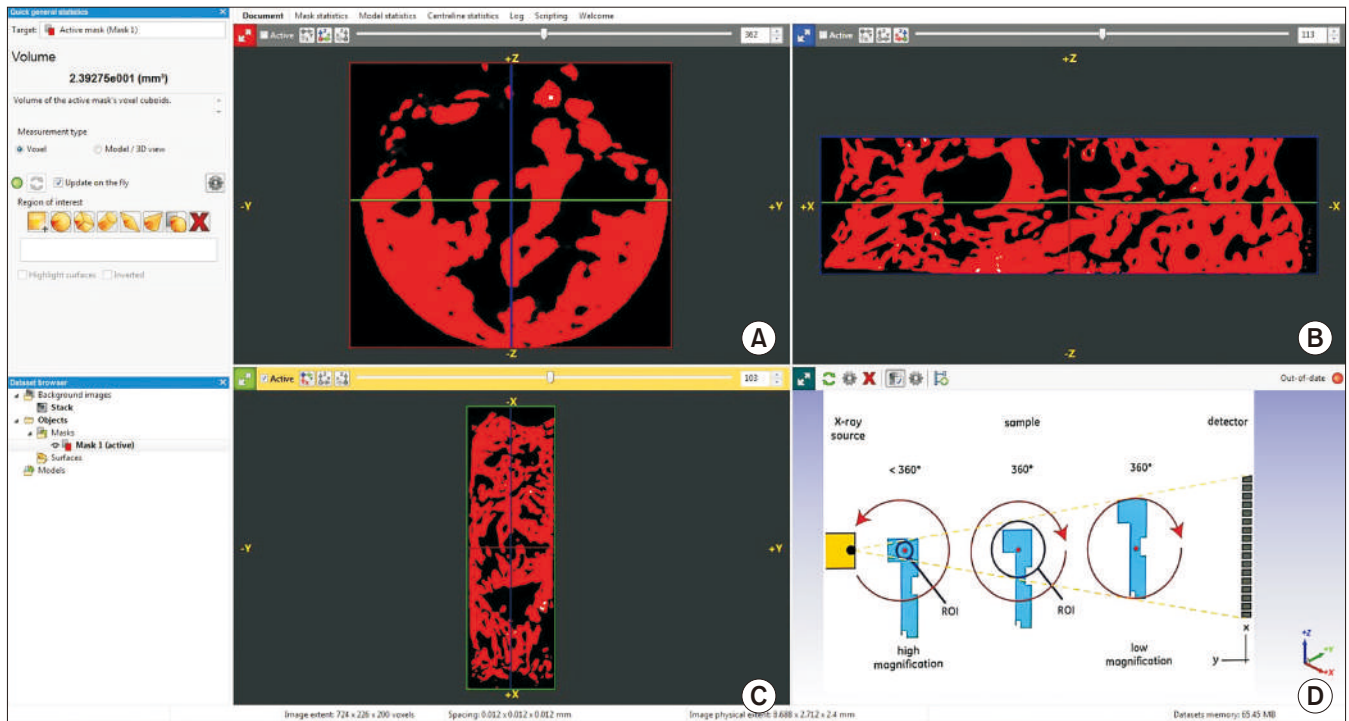


Fig. 4. A-C. Micro-computed tomography (CT) assessment of trephined bone using PhoenixV/tome/Xs machine image analyzing software. D. Schematic presentation of micro-CT image acquisition process for analysis using the PhoenixV/tome/Xs machine. Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

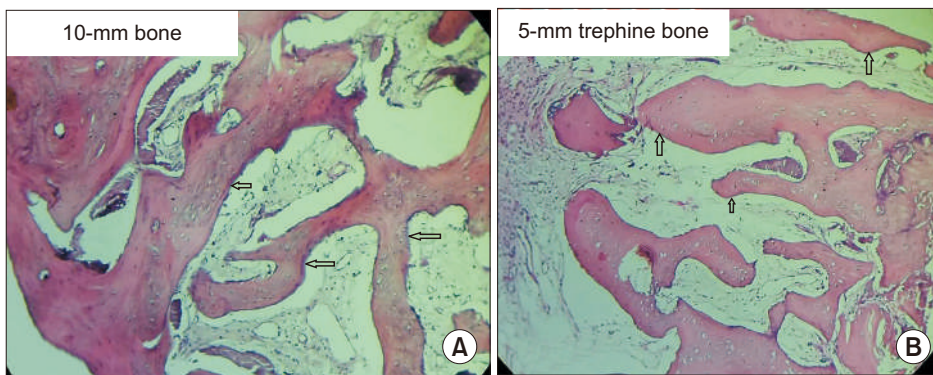


Fig. 5. A. Microphotograph of two (10 mm and 5 mm) trephine bone specimens showing intervening trabeculae with a rim of osteoblasts (arrows). B. Osteoid and fibrous connective tissue with plump lacunae with prominent osteocytes was visible (arrows). A, B. H&E staining ($\times 10$). Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

versely and interpreted using image analysis software by two oral and maxillofacial pathologists blinded to the procedure. Histology analysis criteria were modified and adopted for evaluation as follows: Grade 0, no bone formation; Grade 1, minimal bone formation; Grade 2, moderate bone formation; Grade 3, abundant bone formation; and Grade 4, exuberant bone formation at the grafted site²⁶.

4. Statistical analysis

Results were tabulated using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Data were summarized as means

and percentages and analyzed by dependent t-tests using IBM SPSS Statistics software (ver. 20.0; IBM, Armonk, NY, USA). Kappa correlation was calculated to assess the degree of observer agreement for radiological assessment.

III. Results

1. Clinical observations

All patients (23 sockets) had uneventful wound healing. No graft material displacement or leaching was observed, even though the graft material was partially exposed. Tissue

re-epithelialized entirely within 2 weeks with a thick gingival biotype (>3 mm). Radiographic analysis showed increased density with a trabecular pattern in 73.91% of sockets while in the remaining 26.09% of sockets, a ground glass appearance was observed.(Fig. 6) After 24 weeks, bone fill with well-preserved ridges was observed. Socket wall width was measured at the crestal bone level and was consistent from

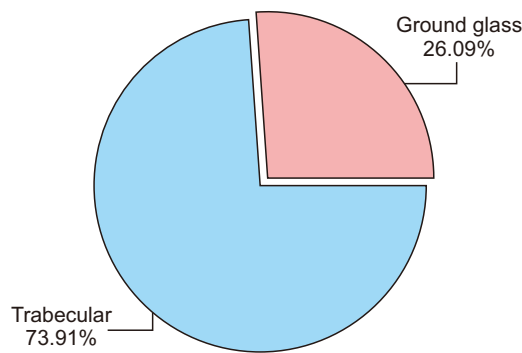


Fig. 6. Pattern of bone regeneration and distribution of these patterns.

Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

week 4 to week 24.(Tables 1-3) Graft particles were incorporated into the generated bony tissue and the augmented site was clinically indistinguishable from the original neighboring bony tissue. Many of these sites had successfully placed implants.

2. Histomorphometric and micro-CT observations

Micro-CT analysis revealed newly formed bone with interconnecting trabeculae and H&E staining revealed thin trabeculae of woven bone. Plenty of cellular osteoid in the form of trabeculae with intervening loose relatively dense cellular fibrous tissue was observed. Trabeculae showed a rich anastomosing pattern with numerous enlarged lacunae containing prominent osteocytes. Numerous bundles of collagen fibers were also observed. Osteoid showed eosinophilic areas surrounded by a rim of osteoblasts.(Fig. 5) The graft material was in apposition with the osteoid. No osteoclasts or inflammatory cells were observed. Grade 2 bone formation was observed in 13.04% of grafted sockets, Grade 3 bone formation in 56.52% of grafted sockets, and Grade 4 bone formation in 8.70% of grafted sockets.(Fig. 7)

Table 1. Socket preservation, changes in alveolar width at different time intervals, pattern of bone regeneration, and histopathology grading 24 weeks after bone grafting

No.	Tooth No. (FDI) of socket preservation	Alveolar width (mm)		Mean density after			Pattern of bone regeneration	Grade of bone formation based on histopathology ¹
		Initial	Final	1 wk	12 wk	24 wk		
1	37	13	12	60.60	40.32	76.34	Trabecular	3
2	36	14	13	63.27	55.50	88.80	Trabecular	3
3	11	14	14	54.34	34.43	70.46	Ground glass	3
4	12	13	13	50.12	45.10	65.27	Ground glass	4
5	13	15	14	53.24	40.23	58.89	Ground glass	2
6	14	14	13	50.32	43.12	65.13	Ground glass	4
7	15	13	12	44.45	34.10	67.73	Trabecular	3
8	23	15	14	54.43	45.20	73.49	Trabecular	2
9	24	14	14	50.32	40.12	75.21	Trabecular	3
10	33	13	13	45.56	34.34	58.12	Ground glass	3
11	35	13	12.5	56.75	45.65	69.15	Ground glass	2
12	44	13	12.5	45.43	34.43	72.14	Trabecular	3
13	45	14	13	45.21	32.54	73.16	Trabecular	3
14	36	14.5	14.5	50.68	45.00	69.76	Trabecular	3
15	21	14	13.5	54.45	34.80	68.23	Trabecular	3
16	36	14.5	14	50.89	29.01	75.20	Trabecular	3
17	36	15	14	59.43	36.03	76.08	Trabecular	3
18	36	14	14	66.34	35.04	79.05	Trabecular	-
19	38	15	14.5	63.12	60.06	80.03	Trabecular	-
20	14	14	13.5	64.34	58.09	76.83	Trabecular	-
21	15	13	13	44.65	35.07	69.01	Trabecular	3
22	47	15	14	56.87	43.05	70.01	Trabecular	-
23	48	16	15	68.54	46.03	74.01	Trabecular	-

(FDI: Fédération Dentaire Internationale; FDI World Dental Federation)

¹Histologic analysis graded on the following 5-point scale: 1) Grade 0, no bone formation; 2) Grade 1, minimal bone formation; 3) Grade 2, moderate bone formation; 4) Grade 3, abundant bone formation; 5) Grade 4, exuberant bone formation.

Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

Table 2. Comparison of initial and final alveolar width (mm) scores by dependent t-test

Time points	Mean	SD	Mean Diff.	SD Diff.	% of change	Paired t	P-value
Initial	14.04	0.86					
Final	13.48	0.80	0.57	0.43	4.02	6.2395	0.0001*

(SD: standard deviation, Diff.: difference)

*P<0.05.

Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

Table 3. Comparison of density scores 1 week, 12 weeks, and 24 weeks by dependent t-test

Time points	Mean	SD	Mean Diff.	SD Diff.	% of change	Paired t	P-value
1st wk	54.49	7.35					
12th wk	41.19	8.26	13.31	7.17	24.42	8.9052	0.0001*
1st wk	54.49	7.35					
24th wk	71.83	6.74	-17.34	6.32	-31.81	-13.1497	0.0001*
12th wk	41.19	8.26					
24th wk	71.83	6.74	-30.65	8.29	-74.41	-17.7222	0.0001*

(SD: standard deviation, Diff.: difference)

*P<0.05.

Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

IV. Discussion

1. General aspects

Socket preservation is essential for maintaining adequate alveolar bone height and width and enhancing bone regeneration. Alveolar bone is required to support the growth of thick soft tissue. Various materials and techniques have been used for socket preservation^{1,27}. Selection of a socket preservation technique and graft material remains the choice of the clinician. Many factors such as the pattern of resorption (resorbable or non-resorbable), graft material origin (bovine, synthetic, coral, eggshell), material availability, economic factors, and understanding of the evidence for regeneration influence the choice of graft material¹⁴⁻¹⁶.

The disadvantages associated with harvesting autogenous grafts have prompted research into novel materials for grafting¹⁴. An increasing number of synthetic alternatives are becoming available¹⁶. Improvements in technology have facilitated the synthesis of hydroxyapatite graft particles that are osteoinductive²⁸⁻³⁰.

Synthetic HA is a calcium phosphate material that can have different densities, structures, and surface chemistries depending on its exact composition. These characteristics are responsible for the bone bonding properties, turnover rate, and longevity of the material *in situ*¹⁸. The efficacy of various HA materials in long-term ridge preservation has been proven. However, HA used in socket preservation should

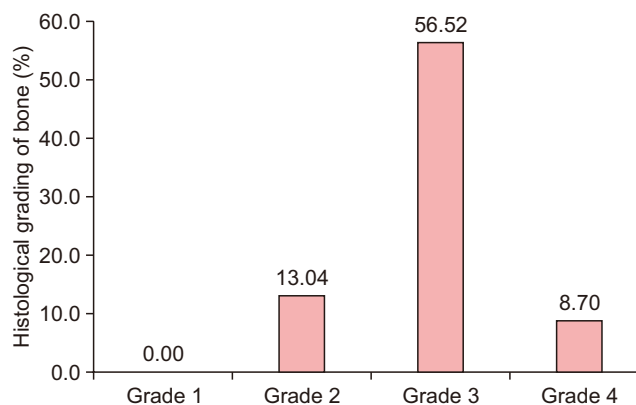


Fig. 7. Histological grade of bone regeneration and distribution of these grades.

Vivekanand Sabanna Kattimani et al: Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin as a barrier membrane: a new technique. J Korean Assoc Oral Maxillofac Surg 2019

preserve existing bone and modulate early bone regeneration to facilitate implant osseointegration^{31,32}. Synthetic nHA does not require a donor site, and raw material is available in unlimited quantities^{26,33}. Control of particle size and interparticulate spaces to mimic those of natural bone is possible during manufacturing¹⁷⁻¹⁹.

2. Nanohydroxyapatite derived from chicken eggshell

We used nHA derived from chicken eggshell in the present study. Nanosynthetic HA has shown promising results for socket preservation, and our findings further support the util-

ity of nanosynthetic HA for this purpose³⁴. Histology sections from superficial to deeper cuts showed a consistent increase in newly formed bone with a decrease in the connective tissue area fraction. Histomorphometric findings indicated moderate to abundant bone formation within 24 weeks. Moreover, lower amounts of residual graft particles were observed, similar to the study of Goetz et al.³⁵. Goetz et al.³⁵ used NanoBone synthetic material for socket filling and reported nearly complete ossification with small residues of material after 4 months follow-up. NanoBone was osteoconductive based on its ability to promote bony ingrowth and initiate integration with newly formed bone. Thus total incorporation of generated bony tissue and mineral particles was achieved^{34,35}. New tissue formation in the healed socket was observed in approximately 75% of all examined specimens. Ultimately, the graft material should induce new bone formation³⁴. New bone formation was evident in all samples and augmented sockets had an adequate morphology and density.

Preliminary experimental studies have also evaluated nano-sized ceramics as a promising class of graft substitute materials because of their osteo-integrative properties³⁶. In these studies, nHA paste was used with or without an additional barrier membrane³⁶. This paste promoted wound healing in the extraction sockets. Nanoparticles can hypothetically reduce the inflammatory response and support active osseous organization^{36,37}.

The nHA used in this study meets the characteristics of an ideal graft material noted by Gross³¹ in 1997. The material is available in granular/nanopowder form, which is easy to handle, and is not prone to infection. In this study, the grafted area was exposed to the oral cavity because we did not use primary closure or a barrier membrane other than a PRF membrane to prevent initial leaching of the material until it became cohesive with a blood clot. nHA is hydrophilic and sticks to the socket wall and settles down in the blood soon after filling. There is no possibility of disease transfer because the material is derived from chicken eggshell. Histology clearly showed the development of bone reminiscent of native bone. Mobility of the tooth has been shown to be maintained when this material is grafted into large cystic defects²³, and it also supports implant prostheses successfully. Similar findings have been reported for synthetic grafts³⁸ with clinically successful implants observed after five years³⁸. Furthermore, bone density and volume have been shown to increase over time^{38,39}. As noted by Boyne⁴⁰, synthetics act as a natural barrier for epithelial downgrowth into the socket. The ability to maintain the height and width of alveolar bone and soft

tissue anatomy has distinct functional and esthetic advantages^{33,40}. We observed a significant increase in density from the week 1 to week 12 ($P < 0.05$). Minimal bone loss (0.57 ± 0.43 mm) was observed over 24 weeks compared to baseline, consistent with the literature¹. Alveolar bone preservation and replacement therapy can be achieved using nHA without a collagen barrier membrane. nHA is therefore a promising graft material that can be produced in an environmentally-friendly manner^{17,19}.

3. Socket preservation technique and barrier membrane

We used nHA for socket preservation²³. No cases of leaching or displacement of the graft material were observed and no periosteal-releasing incisions occurred. Use of an invasive method is recommended against at this point as any procedure that requires primary intention healing with the advancement of flaps can result in an increased inflammatory response and a decrease in vestibular depth, creating an unaesthetic scar⁴¹. The study by Fickl et al.⁴² showed that flap elevation results in the loss of more bone. This is because rupture of the periosteum and connective tissue insertion consequently reduces blood flow, causing lysis of osteocytes and necrosis of mineralized tissue⁴². This necrotic bone will gradually be removed through resorption by osteoclasts present in the periosteum^{43,44}. We overcame this problem of bone loss by not elevating the flap. Elevation also negatively affects the esthetics of the ridge and papilla by altering the mucogingival line position². We used PRF membrane to cover the graft material during the initial period; we consider this an essential step for successful grafting with the existing attached gingiva and future success with implant restoration.

Many current socket preservation techniques use a barrier membrane for primary closure, but there are often difficulties in adoption of the membrane, and a second surgical procedure is required to remove the membrane⁴⁵. In general, membrane use is time-consuming and expensive⁴⁵⁻⁴⁷. Murphy^{45,46} reported exposure of non-resorbable membrane in 87% of cases, leading to infection, swelling, sloughing, and recession. We did not encounter these complications using PRF membrane to cover the graft material. Synthetic grafts do not require a second surgical procedure, nor do they elicit an inflammatory reaction, which is usually seen with a barrier membrane⁴⁷. Formation of dense lamellar bone at the grafted area has been observed²⁰. Socket grafting eliminates or reduces the incidence of dry socket³³; consistent with this, no cases of dry socket were encountered in our study. Moreover, inva-

sive procedures like guided bone regeneration and sinus floor elevation are not needed when socket grafting is adopted and planned properly before extraction⁴¹.

The PRF membrane in our study acted as a cover for graft material and as a barrier membrane for guided bone regeneration. The effect of PRF as a barrier membrane for bone formation and soft tissue healing may be limited, but PRF and nHA may interact additively or synergistically. The usefulness of PRF in the maxillofacial area is expanding⁴⁸. Our study design did not allow us to distinguish between the effects of nHA and PRF, although histomorphometry analysis at the bottom of the socket and middle of the socket showed similar bone formation characteristics as that in the crestal area. In previous studies, nHA alone enhanced bone formation in cystic defects^{23,49}. Even after disintegration of the PRF membrane, material leaching was not observed. Alveolar bone loss occurs if socket preservation techniques are not employed¹. We therefore did not include a control group in this preliminary study as this study is part of an ongoing study protocol to delineate the histomorphometric and micro-CT characteristics of bone formation in relation to radiography; previous studies have used only radiography (IOPA and CBCT/CT scan) for assessment of cystic defect grafting^{23,49}.

Grafting prevents hematoma and infection. Manipulation of graft material is easy because of the hydrophilic nature of nHA. It can be used as putty when mixed with a drop of saline or native blood or serum from the PRF test-tube. Its ease of use, absence of disease transfer risk, ready availability in large quantities, and environment-friendly production make nHA an economic graft substitute material for enhancement of bone regeneration and reconstruction. Application of nHA in grafted sockets supports implant restoration, indicating osseointegration.

4. Future research work

Advances in tissue engineering techniques are likely to provide novel biomaterials for everyday clinical use⁴¹. Long-term follow-up data are mandatory to evaluate the presence of grafted particles that could eventually interfere with the longevity of the implant. We are currently performing a study to further elucidate the histochemical and morphometric nature of the bone formed, as well as assess the resorption kinetics of nHA. The results of the current pilot study suggest that multicenter randomized controlled clinical trials with long-term implant restoration follow-up are warranted. Various grafting materials are available for purchase. Cost varies

according to the manufacturer and depends on many factors such as the raw material source and processing technique involved^{14,16,31,32}. Comparison of the costs of grafting is beyond the scope of this study.

Limitations of this pilot study are our small sample size and the fact that socket morphology, width, and presence of buccal/lingual cortical walls were not taken into account in our analyses.

V. Conclusion

Sockets preserved with nHA showed good bone regeneration. Use of nHA and a PRF membrane as a barrier can assist the healing process and provide better bone quality while preserving alveolar bone. Micro-CT provides good insight into bone healing and bone quality in three dimensions compared to conventional histopathology. The technique described here does not require primary closure, thereby facilitating the preservation of keratinized mucosa and gingival architecture. nHA derived from chicken eggshell is a promising novel bone graft substitute.

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Authors' Contributions

V.S.K. designed the study and K.P.L. mentored the study. V.S.K. collected data and wrote the manuscript. K.K.K. and G.E.K. performed the review and assisted for the study. K.K.K. participated in the study assessment and helped to draft the histological assessment part of the manuscript. All authors read and approved the final manuscript.

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Ethics Approval and Consent to Participate

The study protocol was approved by the Institutional Ethics Committee of Sibar Institute of Dental Sciences (IEC-16/09/2014), and the written informed consent was obtained from all patients and patients' relatives.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Role of Synthetic Hydroxyapatite—In Socket Preservation: A Systematic Review and Meta-analysis

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ABSTRACT

Since a long time, the preservation of the socket is emphasized for various reasons. Many studies have suggested the ridge preservation through socket grafting using various bone graft substitute materials (GSMs). But none of the studies suggested the material of choice for the grafting. So, the systematic review was planned to analyze the outcomes of synthetic hydroxyapatite (SHA) graft material for socket preservation. The review was aimed to determine the existing evidence for the use of SHA GSM for grafting and its usefulness.

Materials and methods: The literature search was performed for the studies published in the English language independently by all four authors (search team) in the Medline database through the PubMed search engine for the past 5 years. The study involved predetermined inclusion and exclusion criteria for the search. The final lists of clinical trials were analyzed to determine the existing evidence and suggested the mechanism of action.

Review results: The search resulted in 117 titles. After application of inclusion and exclusion criteria, a total of seven studies were found eligible for this systematic review. Out of seven, two studies were found eligible for meta-analysis whereas remaining included for the systematic review.

Conclusion: The meta-analysis favors socket grafting compared to control in terms of preservation of existing bone height and width. The SHA grafting showed successful bone regeneration with less connective tissue component. The histomorphometric evaluation showed a good bone regeneration associated with SHA than xenograft. Within the limitations of this meta-analysis, the synthetic GSM can be used for socket grafting.

Clinical significance: In the wake of increasing graft materials in the market and different origin raw material sources for the preparation of graft materials, clinicians are in dilemma for selection and its use. The success of grafting depends on the selection of appropriate material with a suitable calcium/phosphate (Ca/P) ratio. The review provided available evidence for the use of SHA.

Keywords: Bone, Extraction, Healing, Implant, Regeneration, Restoration.

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INTRODUCTION

Extraction is the most common procedure performed in routine dental practice because of caries, periodontal disease, and so on. Bony defects secondary to extraction if left untreated may lead to further bone loss.¹⁻³ Eventually, the fixed restoration of the missing tooth may be a nightmare for the patient and difficult for the clinician to restore without the sound alveolar bone using either implant-supported restoration or fixed partial denture using natural tooth as an abutment.⁴⁻⁷ The bone loss followed by extraction requires socket grafting to prevent bone resorption or enhance earlier bone formation.⁸⁻¹¹ Various techniques have been demonstrated, developed, and published as successful means for the preservation of the alveolar bone.^{4,9,12-25} But none of the techniques claimed the superiority over the other.^{5,20,26-29}

Many GSMs are available commercially for grafting and showed varying success rates. The published literature has shown the use of different origin synthetic substitute materials for grafting but none of them have advised single material as an ideal substitute.^{9-11,30} Many systematic reviews performed till date concluded with the uncertainty of recommendation because of heterogeneous study material, use of different origin GSMs, etc.^{5,26-29,31-44} Changing trends in the mechanism of action^{7,45} fascinated researchers to develop newer materials with the advent of production technology, and the clinicians to use it for enhancement of bone regeneration.⁴⁶⁻⁴⁹

In the light of changing the mechanism of action, synthetic GSMs are becoming more versatile, as these reduce the morbidity of the second surgery, time, and the skill required for harvesting

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autogenous graft.^{45,50-53} Because of the disadvantages associated with autogenous grafting, the paradigm shift happened toward processed graft substitutes. The processed graft substitute of different origins requires bone-banking facilities which are not economical and have a chance of disease transfer risk.⁵⁴ This led to the pursuit of synthetic materials.

To the best of our knowledge, no systematic reviews are available till date exclusively assessed the use of synthetic graft

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substitute for socket preservation. So, this systematic review was planned to delineate the suggested mechanism of action and analyze critically the existing literature to discuss the level of evidence for the use of SHAGSM for socket grafting.

MATERIAL AND METHODS

The literature search was performed for the studies published in the English language independently by all four authors (VSK, PSR, KR, and AAK) in the Medline database through the PubMed search engine for the past 5 years (January 2014–December 2018). The search for cross-reference articles was performed. The study involved predetermined inclusion and exclusion criteria for the search. The final lists of clinical trials were analyzed to determine the evidence and suggested the mechanism of action.

Search Strategy

The search was performed using MeSH keywords. Various Boolean operators were used and the search string was formed to focus the research question. The search words included “extraction” and “graft”, “extraction” and “synthetic graft”, “socket preservation”, “ridge augmentation” and “extraction”, “tooth extraction” and “hydroxyapatite” or “tooth extraction” and “bioceramic material” or “tooth extraction” and “post-extraction.” The search included title, abstract, and keywords fields. Various filters like year of publication, human, and clinical trials were applied as appropriate to derive the desired output. To broaden the understanding of the subject, the review articles were thoroughly screened for cross-reference studies. The review articles gave insights for the future directions and the lacunae noted by previous researchers were considered to deepen the understanding of the present review.

Inclusion and Exclusion Criteria

The randomized clinical trials (RCTs) which used asynthetic graft material for socket grafting have been considered with a minimum of 10 patients assessed for the nature of bone regeneration using histomorphometry and available in the Medline database (searched through PubMed). Case reports and case series of fewer than 10 patients and non-English language publications were excluded.

The Type of Patients

The patients requiring grafting after extraction for socket preservation followed by microscopic examination for bone characterization during implant placement were considered for review.

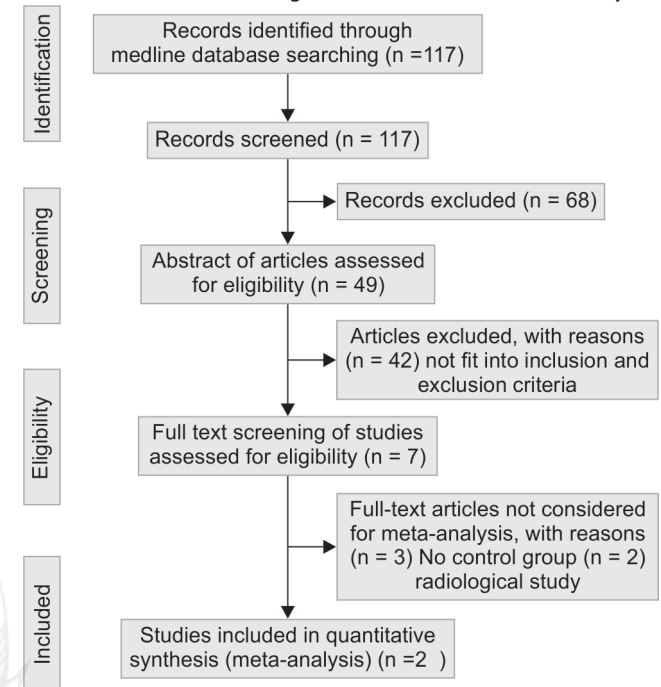
Type of Intervention and Outcome Measures

Synthetic hydroxyapatite from different origins compared with any of other graft substitutes or control (no intervention/natural healing) groups. The outcome variable considered was the bone level and the nature of bone formed irrespective of various methods opted for assessment.

Data Extraction and Analysis

The screening was performed individually by all authors (team 1—VSK and RSP; team 2—KR and AAK) along with cross-reference resources. Both groups of authors prepared the PRISMA flow diagram that was used for final screening (Flowchart 1). The bibliography was created using the Mendeley desktop app (version 1.19.3) and was used to check for duplicates. The Review Manager 5.3 (Version: 5.3.5) used to extract data from eligible studies (Tables 1 to 3). If there is any confusion in the inclusion and the exclusion of the studies, it was

Flowchart 1: PRISMA flow diagram for the review and meta-analysis



sorted out through discussion. The data extracted were validated by another team for accuracy and for any missing data from the studies (AJ and MAK). The corresponding authors of selected studies were consulted through e-mail for further clarification and the necessary data required for meta-analysis in case of missing observations in the published article. The studies included for meta-analysis were assessed for the quality using Maurits van Tulder et al.’s criteria for risk of bias⁵⁵ (Tables 4 and 5).

RESULTS

The search resulted in a total of 117 titles. After the application of inclusion and exclusion criteria, a total of 49 clinical trials were included for abstract review. The abstract review resulted in seven eligible studies for a full-length study assessment. Seven studies were found eligible for full-article review.^{56–62} Only two studies (Mayer⁵⁶ and Machtei⁵⁷) were found eligible for a quantitative analysis among seven studies (Table 1 and Flowchart 1). Whereas remaining five studies were considered for systematic review; among them, three had (Mozzati,⁵⁸ El-Chaar,⁶¹ and Canullo⁶²) no control group and two studies (Oliveira⁵⁹ and Cavdar⁶⁰) used the radiographic analysis. So, only two studies were considered for meta-analysis^{56,57} (Tables 2 and 3).

The study by Mayer et al. used the biphasic calcium sulfate (BCS) with β tri-calcium phosphate (β -TCP) and hydroxyapatite (HA) compared with the control group.⁵⁶ The study by Machtei et al. included 11 patients each in the test and the control group⁵⁷ and compared BCS/HA with control and xenograft. Whereas Mozzati et al.⁵⁸ used RegenOss [equine collagen I and magnesium (Mg)–hydroxyapatite (ratio: 40–60%)] and Canullo et al.⁶² used Mg-enriched nanohydroxyapatite powder in their study. All of them used synthetic GSM and performed the histomorphometric analysis. El-Chaar et al. study assessed less than 10 patients because of dropouts and had no control group.⁶¹ In Cavdar and Oliveira studies, only radiological assessment was performed.^{59,60} In both the studies, different synthetic GSMs were used.

Table 1: Sample size and intervention of included studies in the meta-analysis

S no	Author name	Study set up and region of study origin	Sample size—control	Sample size—test group/s	Intervention	Type of study	Inclusion/exclusion
1	Mayer ⁵⁶	Department of Dental School, Israel	15	14	BCS with TCP and HA	RCT	Included
2	Machtej ⁵⁷	Department of Dental School, Israel	11	11	BCS/HA	RCT	Included

Table 2: Characteristics of included studies in the systematic review with reasons for not considering in meta-analysis

S no	Author name	Study set up and region of study origin	Sample size—control and test	Intervention	Type of study	Reasons for not considering in meta-analysis	
1	Oliveira ⁵⁹	University Department, Brazil	26 patients divided into four groups, not mentioned the number of patients allotment to each	Deproteinized bovinebone mineral with 10% collagen (DBBM-C), poly(d,l-lactide-coglycolide) with hydroxyapatite/b-TCP scaffold (PLGA/HA), PLGA/HA/b-TCP with 2.0% simvastatin scaffold (PLGA/HA/S)	RCT	Only radiographic study	
2	Cavdar ⁶⁰	University Department, Turkey	11	41	Demineralized bone matrix + collagen membrane (CM)(N=14), hydroxyapatite bone substitute (HBS) + CM (N=14), CM (N=13), or left empty (N=11)	RCT	Only radiographic study
3	Mozzati ⁵⁸	University Department, Italy	00	32	Equine collagen I and Mg-hydroxyapatite	Single arm study no control group	No control group
4	El-Chaar ⁶¹	Private office, New York	00	8	15% hydroxyapatite, 85% b-TCP complex	Case series, single arm study, no control	No control group
5	Luigi Canullo ⁶²	Private office, Italy	00	20	Mg-enriched hydroxyapatite (MgHA)	Case series, no control	No control group

DISCUSSION

The use of SHA graft substitutes for bone formation has changed its mechanism of action from the scaffold^{63–65} to osteo induction.^{66–70} The synthetic graft material, in the beginning, was

used as a scaffold. The clinicians still think that it acts as a scaffold and bone defect-filling material. With evolution, synthetic GSM acted as an osteoconductive material.^{30,54,71} The mechanism of osteoconduction depends on the nature of origin, particle size, porosity, resorption rate, etc.^{68,69,72} The published literature showed the structure of HA is an important factor for an osteoinductive property. A few studies have shown osteoinductivity of HA in heterotrophic sites.^{66,73} The nanotechnology-assisted production made the clinicians dream for an artificial bone. The dream has come to a part reality for bone reconstruction. A few case reports emphasized the bone formation using block grafts for larger bone defects reconstruction in both animal and humans.^{66,73–75} The changing scenario has been well documented in the published literature.^{66,73–75}

There are many clinical reports, case series, and few original research, and RCTs that presented the benefits of synthetic GSM.^{5,29,30,54,56,57} But none of the systematic reviews nor the RCTs advised single material as an ideal graft substitute. This inconsistency in the conclusion might be due to a variety of commercially available GSMs and clinical scenarios which cannot be standardized like animal study defect models for conclusive remarks. The size and the nature of the lesion, along with patient factors, might be the reason for this inconclusiveness. So, this review addressed the focused question of SHAGSM use for socket grafting

Table 3: Quantity of bone formation in test groups among all the studies eligible for systematic review

S no	Author name	Total bone area in %	Connective tissue/marrow space in %	Residual graft in %
1	Mayer ⁵⁶	47.7 ± 10.6	36.3 ± 19.4	15.99 ± 11.4
2	Machtej ⁵⁷	44.15 ± 18.8	NF* and NR**	16.51 ± 16.2
3	El-Chaar ⁶¹	40.25	49.25	10.38
4	Luigi Canullo ⁶²	31.85 ± 6.99	27.33 ± 17.72	40.82 ± 6.71
	at 4th and 12th month	41.32 ± 9.37	32.40 ± 9.87	26.28 ± 11.49
5	Oliveira ⁵⁹	NA [#]	NA [#]	NA [#]
6	Cavdar ⁶⁰	NA [#]	NA [#]	NA [#]
7	Mozzati ⁵⁸	§NQ	§NQ	§NQ

*NF—not found in the article

**NR—no response from corresponding author

[#]NA—not applicable, radiological assessment only

[§]NQ—no quantitative analysis performed

Table 4: Internal quality assessment of included studies for meta-analysis

S no	Characteristics examined according to Maurits van Tulder et al. ^{55*}	Mayer ⁵⁶	Machtei ⁵⁷
A	Was the method of randomization adequate?	Yes	Yes
B	Was the treatment allocation concealed?	No	Yes
C	Were the groups similar at baseline regarding the most important prognostic indicators?	Yes	Yes
D	Was the patient blinded to the intervention?	No	Yes
E	Was the care provider blinded to the intervention?	No	Yes
F	Was the outcome assessor blinded to the intervention?	No	Yes
G	Were co-interventions avoided or similar?	No	Yes
H	Was the compliance acceptable in all groups?	Yes	Yes
I	Was the drop-out rate described and acceptable?	Yes	No
J	Was the timing of the outcome assessment in all groups similar?	Yes	No
K	Did the analysis include an intention-to-treat analysis?	Don't know	Yes

*It includes only the internal validity criteria (n = 11) that refer to characteristics of the study that might be related to selection bias (criteria a and b), performance bias (criteria d, e, g, and h), attrition bias (criteria i and k), and detection bias (criteria f and j)
A to K—scored as—yes/no/don't know

Table 5: Risk of bias assessment in the included studies for meta-analysis

Type bias	Points to be considered	Mayer ⁵⁶	Machtei ⁵⁷
Selection bias	Criteria a and b	Partly	No
Performance bias	Criteria d, e, g, and h	Significantly	No
Attrition bias	Criteria i and k	Partly	Partly
Detection bias	Criteria f and j	Partly	No

and emphasized on the histomorphometry of bone regeneration. The systematic review ascertained the objectives of the study. However, seven studies found eligible for systematic review; out of seven studies, four have discussed histomorphometry. One study exclusively described the effect of the synthetic graft substitute for bone regeneration pattern using various assays.⁷⁶

Mayer et al. study included BCS with b-TCP and HA for grafting.⁵⁶ The study assessed a combination of two alloplastic materials in comparison to natural socket healing in 40 extraction sites of 36 patients. The final assessment included 15 extraction sites in the control group and 14 in the test group.⁵⁶ The study did not mention the tooth number, instead mentioned anterior and posterior teeth in the mandible and the maxilla. The study involved the premolar and the molar region in both controls (12/15 sockets) and graft (14/14 sockets).⁵⁶ The graft group showed minimal bone loss compared to the control group. The histological evaluation revealed the mature lamellar bone in both groups.⁵⁶ But, the study had not mentioned how many bony specimens were taken for assessment. The study showed more connective

tissue in natural healing compared to the test group.⁵⁶ The study used a combination of materials but not the individual material, so it is difficult to comment on the effect of each component as both materials have different resorption kinetics. The author claims that the combination improved the quality of the material.⁵⁶

The study of Machtei et al. included 11 patients each in the test and the control group.⁵⁷ The study compared BCS/HA with control and xenograft. The study group involved premolars, canine, and incisors evenly presented both in the mandible and the maxilla.⁵⁷ The study showed a similar percentage of bone (44.15 ± 18.8%) as that of Mayer et al.'s study (47.7 ± 10.6%) but it was less compared with the control group. The study did not reveal the connective tissue component as mentioned in Mayer et al.'s study for comparison. Both studies consist of a similar sample size of 10 in Mayer et al.⁵⁶ and 11 in Machtei et al.⁵⁷

Histomorphometric analysis showed more of bone in the control group (81.72 ± 4.3%) than that in the BCS/HA group (44.15 ± 18.8%) which, in turn, was greater than in the xenograft group (22.50 ± 24.72%) in Machtei et al.'s⁵⁷ study. Residual scaffold material was significantly greater in the xenograft group (40.18%) than the BCS/HA group (16.51%). The BCS/HA group (44.15%) showed bone twice that of the xenograft group (22.50%).⁵⁷

The meta-analysis favors the use of SHAGSM over the control/natural healing group in terms of clinical and radiological outcomes. The histomorphometric analysis favored the grafting procedure compared to control (Fig. 1). Even though meta-analysis involved only two studies, the quantitative analysis favors grafting of the socket for the preservation of bone (Fig. 2). Advances in tissue-engineering techniques might soon provide novel biomaterials which are currently evaluated worldwide and will soon be introduced into the clinical

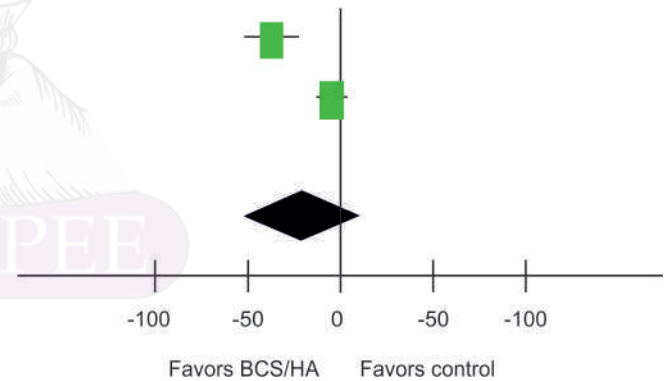


Fig. 1: Showing comparison of total bone formed among BCS/HA group and control. Comparison 1: BCS/HA vs control. Outcome changes 1.1 total bone area

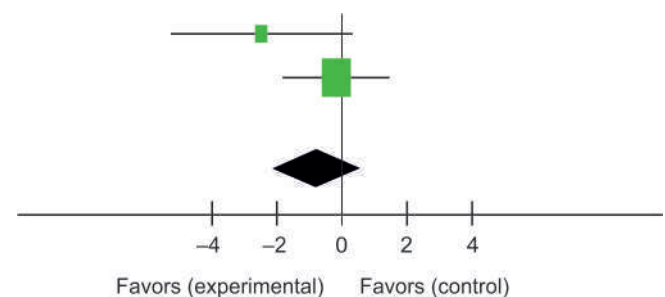


Fig. 2: Showing comparison of vertical ridge changes among BCS/HA group and control. Comparison 1: BCS/HA vs control. Outcome changes 1.2: vertical ridge



practice.⁷⁷ The newer GSM falls into the category of biomimetic scaffolds, as they stimulate bone formation, not only chemically but also structurally through micropores which connect each other. The osteoinductivity of these materials has been shown by published literature.^{66,76,78} The changing scenario and improved production technology may make the dream of artificial bone formation for grafting in the near future. The recent systematic reviews showed that the SHAGSM improved the bone regeneration along with the preservation of resorption.^{33,37,38,71} Long-term follow-up data are mandatory to elucidate the presence of grafted particles which would eventually interfere with the longevity of implant function.

CONCLUSION

Socket preservation using synthetic HA showed beneficial results compared to control group within the limitation of available studies for meta analysis. The use of GSM prevented alveolar bone resorption. The histomorphometric evaluation showed less residual graft material associated with SHA. Ridge preservation should become the standard of care for every extraction, so that healthy bone can be retained for successful restoration. To derive more robust evidence, we may need more number of RCTs with similar methodology and the same material for grafting in an economical way.

AUTHOR CONTRIBUTION

VSK and PSR drafted the protocol; VSK, PSR and KR developed a search strategy; VSK, PSR, KR and AAK searched for trials; VSK, AJ, MAK and KR obtained copies of trials; VSK, PSR, AJ, MAK, KR and AAK selected trials to include; VSK, AJ, AAK and KR extracted data from trials; VSK, AJ and AAK entered data into RevMan; RK, Specialist-Cochrane South Asia carried out the analyses; VSK, KR and AAK interpreted the analysis; VSK, PSR, AJ, MAK, KR and AAK drafted the final review.

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Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton

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Abstract (J Korean Assoc Oral Maxillofac Surg 2019;45:34-42)

Objectives: Various bone graft substitute materials are used to enhance bone regeneration in the maxillofacial skeleton. In the recent past, synthetic graft materials have been produced using various synthetic and natural calcium precursors. Very recently, eggshell-derived hydroxyapatite (EHA) has been evaluated as a synthetic bone graft substitute. To assess bone regeneration using EHA in cystic and/or apicoectomy defects of the jaws through clinical and radiographic evaluations.

Materials and Methods: A total of 20 patients were enrolled in the study protocol (CTRI/2014/12/005340) and were followed up at 4, 8, 12, and 24 weeks to assess the amount of osseous fill through digital radiographs/cone-beam computed tomography along with clinical parameters and complications. Wilcoxon matched pairs test, means, percentages and standard deviations were used for the statistical analysis.

Results: The sizes of the lesions in the study ranged from 1 to 4 cm and involved one to four teeth. The study showed significant changes in the formation of bone, the merging of material and the surgical site margins from the first week to the first month in all patients (age range, 15-50 years) irrespective of the size of the lesions and the number of teeth involved. Bone formation was statistically significant from the fourth to the eighth week, and the trabecular pattern was observed by the end of 12 weeks with uneventful wound healing.

Conclusion: EHA showed enhancement of bone regeneration, and healing was complete by the end of 12 weeks with a trabecular pattern in all patients irrespective of the size of the lesion involved. The study showed enhancement of bone regeneration in the early bone formative stage within 12 weeks after grafting. EHA is cost effective and production is environment friendly with no disease transfer risks. Thus, natural bioceramics will play an important role in the reduction of costs involved in grafting and reconstruction.

Key words: Apicoectomy, Wound healing, Grafts, Osteoconduction

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I. Introduction

Biomaterials are used as biocompatible scaffold systems, which allow the migration, proliferation, and differentiation of either resident or externally delivered cells to promote new bone formation¹⁻⁴. A wide variety of biomaterials have been used for craniofacial bone augmentation^{1,3,4}. These can be divided into organic and inorganic materials, where calcium phosphate bioceramics represent most inorganic scaffolds^{1,3}.

The basic rationale behind such materials use is an attempt to mimic an inorganic composition of native bone (hydroxyapatite [HA], a natural bioceramic) to form a strong and durable natural biomaterial for early mineralization^{1,3}.

Bioceramics is an advancing front in the reconstruction of maxillofacial skeleton defects. Advancing technology for the production of synthetic graft materials has led surgeons to quest for artificial bone regeneration¹⁻³. Various bone graft substitute materials are used to enhance bone regeneration^{1,2}. In the recent past, eggshell-derived hydroxyapatite (EHA) has been evaluated as a synthetic bone graft substitute and has changed the face of regenerative science^{5,6}. Thus, this study was planned to assess bone regeneration using HA synthesized from a natural calcium precursor derived from chicken eggshells for the grafting of cystic and/or apicoectomy defects in the mandible and maxilla using digital radiographs/cone-beam computed tomography (CBCT).

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II. Materials and Methods

1. Study design and materials

Our study was performed in the Department of Oral and Maxillofacial Surgery and the Department of Conservative and Endodontics of Sibar Institute of Dental Sciences (Guntur, India) from the year 2015 through 2017. A total of twenty-one patients (14 males and seven females; age range, 15-50 years) requiring grafting after cystectomy and/or apicectomy were enrolled. However, only 20 patients were assessed in the study, because one patient was excluded from the assessment. The study protocol was approved by the Institutional Ethics Committee of Sibar Institute of Dental Sciences (IEC 16/09/2014) and prospectively registered with the Clinical Trial Registry of India - CTRI/2014/12/005340.

1) Inclusion and exclusion criteria for patient selection

- Patients with moderately sized (more than 1 cm for the largest diameter) periapical cystic lesions of the maxilla and mandible involving one or more teeth, as confirmed by clinical and radiological evaluations, were included in the study.
- Patients who were readily available and willing to return for periodic recalls and reviews were assessed.
- All cases were screened for any systemic diseases such as diabetes, and hypertension, and medically compromised patients were excluded in the evaluation process.
- Patients with the gross mobility of involved teeth due to moderate bone loss or frankly infected cysts were excluded from the study.
- Vulnerable groups and special category people were excluded from the evaluation according to an Indian council for medical research guidelines.

2) Graft material used in the study

The EHA used in this study was synthesized from a cal-

cium precursor from chicken eggshells⁷. We prepared the HA using the microwave process published in a previous study^{7,8}. In this study, we used nanocrystalline eggshell-derived calcium-deficient HA measuring an average of 78 nm with a typical flower-like structure, which contained the Ca, P, and Mg trace elemental composition needed for bone formation⁷.

2. Surgical protocol

All patients were assessed preoperatively for the extent of their lesions using radiographs/CBCT, and if necessary, surgical stents were prepared for through and through (tunnel) lesions for palatal soft tissue support post-surgically. The patients and attenders received explanations about the study protocol and written consent was obtained. All patients underwent root canal treatments. Each apicectomy or cystectomy was performed under local anesthesia with all aseptic precautions within three days of obturation. A full-thickness mucoperiosteal trapezoidal or quadrangular flap was elevated. The cystic lining was enucleated, and the lesion was curettaged. Apicectomy was performed by cutting 2 to 3 mm of root end, and the retrograde filling was done using a glass ionomer cement/mineral trioxide aggregate.(Fig. 1. A) The defects were grafted with EHA particles, and care was taken so that no graft particles were placed outside the cavity under the mucoperiosteal flap.(Fig. 1. B) Wound closure was done using 3-0 Mersilk suture.(Fig. 1. C) In all cases, antibiotics and analgesics were prescribed for five days. Patients were recalled for suture removal seven days postsurgery.

3. Clinical and radiographic assessments

The patients were followed up over a period of 24 weeks post-surgically (Fig. 1. D) at intervals of 4, 8, and 12 weeks to assess the amount of osseous fill using digital radiographs (Fig. 2)/CBCT.(Fig. 3) Clinically, the patients were assessed

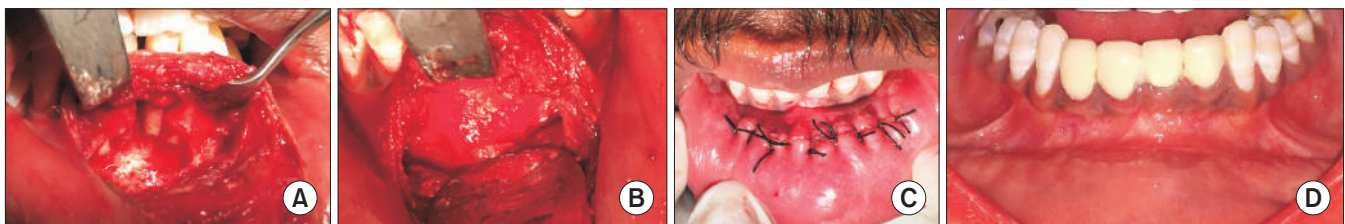


Fig. 1. A. Bony defect and loss of labial cortical plate. B. Bony defect filled with eggshell-derived hydroxyapatite. C. Wound closure. D. Well-healed wound with permanent crowns in place.

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. *J Korean Assoc Oral Maxillofac Surg* 2019

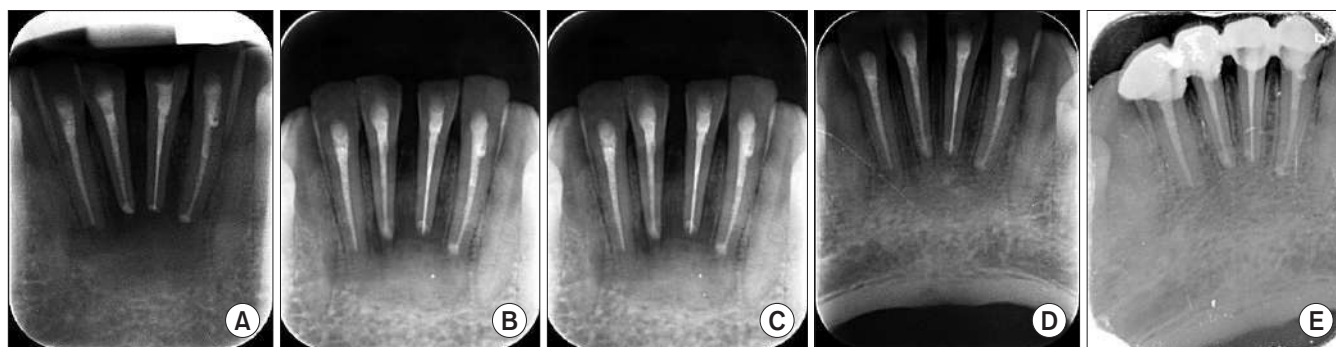


Fig. 2. Intraoral periapical radiographs showing radiological evaluations of lesion. A. Immediately after grafting. B. Four weeks postoperative. C. Eight weeks postoperative. D. Twelve weeks postoperative. E. Twenty-four weeks postoperative.

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

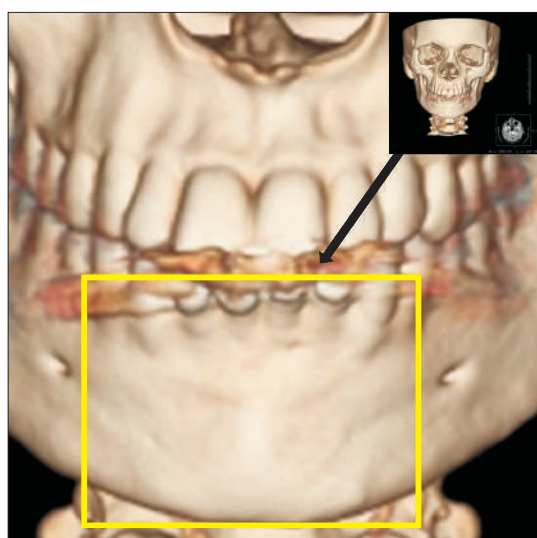


Fig. 3. Three-dimensional computed tomography of facial bone (frontal view) showing the complete bone formation after 24 weeks postoperative.

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

for wound healing/dehiscence, infection, persistent pain, sinus, or fistulas. Radiographically, the surgical site margin (Fig. 4. A), bone formation characteristics (Fig. 4. B), radicular healing patterns, and density of bone formation were evaluated^{9,10}. The radiographs were assessed by two endodontists who were blinded for the time duration of the follow-up and requested to fill out the assessment sheets. If a discrepancy existed, a third examiner (oral radiologist) was requested to assess the radiograph.

4. Statistical analysis

The results were tabulated using Microsoft Excel 2010

(Microsoft, Redmond, WA, USA). The means, percentages, and Wilcoxon matched pairs test were used for statistical analysis using IBM SPSS Statistics 20.0 (IBM, Armonk, NY, USA). Kappa correlation was considered to assess the degree of observer agreement for radiological assessment.

III. Results

1. Clinical observations

All patients (Table 1) showed well-healed wound, except in one female patient wound dehiscence was seen, who was excluded from the study protocol. The sutures were removed after seven days without any infections, persistent pain, sinus, or fistula irrespective of the sizes of the lesions (Table 1), which were measured intraoperatively.

2. Radiological observations

Significant changes were observed in the merging of the material and surgical site outline in all of the patients over a period of eight weeks; 95% of cases showed the absence of demarcation between material and the surgical outline and this process was almost complete by the end of 12 weeks. (Table 2) The comparison of the merging of material and surgical site outline was significant between the first week and the fourth, eighth, twelfth, and twenty-fourth weeks with significant ($P < 0.05$) P -values irrespective of the size of the lesion, the site, or the number of teeth involved. (Table 3) Bone formation was 90% specular and 10% trabecular in the grafted sites by the end of eight weeks (Table 4) with a significant P -value (Table 5), and 90% trabecular pattern was seen by the end of 12 weeks. The radiological evaluation of

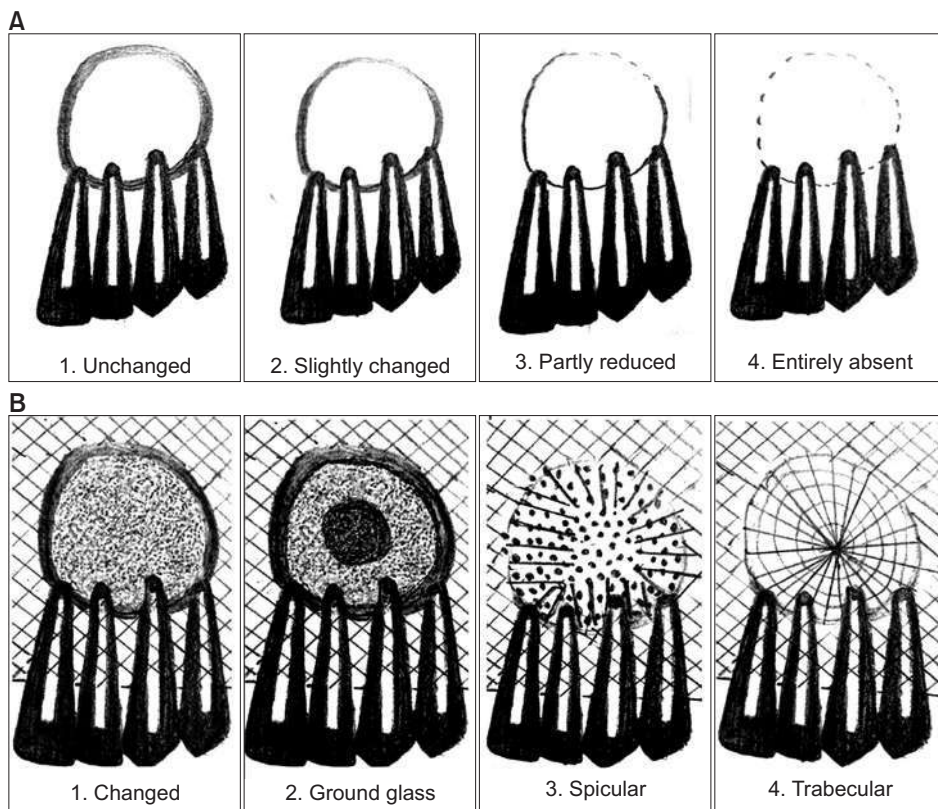


Fig. 4. Illustration showing the radiological evaluation of surgical site outline (A) and bone formation characteristics (B). Reproduced from the article of Kattimani et al. (Indian J Dent Res 2014;25:594-601)¹⁰ with original copyright holder's permission.
Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

Table 1. Gender and lesion size distributions of study subjects for assessment

Group	Value
Sex	
Male	14 (70.0)
Female	6 (30.0)
Size (cm)	
>1-2	5 (25.0)
>2-3	9 (45.0)
>3-4	6 (30.0)

Values are presented as number (%).

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

Table 2. Radiological evaluation surgical site outlines versus elapsed time postsurgery (n=20)

Elapsed time (wk)	Unchanged	Slightly changed	Partially reduced	Absent
1	20 (100)	0	0	0
4	0	12 (60.0)	8 (40.0)	0
8	0	0	15 (75.0)	5 (25.0)
12	0	0	1 (5.0)	19 (95.0)
24	0	0	0	20 (100)

Values are presented as number (%).

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

Table 3. Comparisons of radiological evaluation surgical site outlines over time intervals by Wilcoxon matched pairs test

Elapsed time (wk)	Mean	SD	Median	Mean diff.	% of change	Z-value	P-value
1	0.00	0.00	0.00				
4	1.40	0.50	1.00	-1.40	100.00	3.9199	0.0001*
4	1.40	0.50	1.00				
8	3.25	0.44	3.00	-1.85	-132.14	3.9199	0.0001*
8	3.25	0.44	3.00				
12	3.95	0.22	4.00	-0.70	-21.54	3.2958	0.0010*
12	3.95	0.22	4.00				
24	4.00	0.00	4.00	-0.05	-1.27	3.9199	0.0001*

(SD: standard deviation, diff.: difference)

*Statistically significant difference (P<0.05).

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

Table 4. Radiological evaluation of bone formation with time postsurgery (n=20)

Elapsed time (wk)	Unchanged	Ground glass appearance	Spicular	Trabecular
1	20 (100)	0	0	0
4	0	2 (10.0)	18 (90.0)	0
8	0	0	18 (90.0)	2 (10.0)
12	0	0	2 (10.0)	18 (90.0)
24	0	0	1 (5.0)	19 (95.0)

Values are presented as number (%).

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

Table 5. Comparisons of radiological evaluation of bone formation over time intervals by Wilcoxon matched pairs test

Elapsed time (wk)	Mean	SD	Median	Mean diff.	% of change	Z-value	P-value
1	0.00	0.00	0.00				
4	1.90	0.31	2.00	-1.90	100.00	3.9199	0.0001*
4	1.90	0.31	2.00				
8	3.10	0.31	3.00	-1.20	-63.16	3.9199	0.0001*
8	3.10	0.31	3.00				
12	3.90	0.31	4.00	-0.80	-25.81	3.5162	0.0004*
12	3.90	0.31	4.00				
24	3.95	0.22	4.00	-0.05	-1.28	3.9199	0.0001*

(SD: standard deviation, diff.: difference)

*Statistically significant difference (P<0.05).

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

complete radicular healing was observed in 80% of cases by the end of the eighth week and almost all subjects (90%) exhibited complete healing by the end of the twelfth week.(Table 6)

IV. Discussion

Bioceramics is an advancing front in the reconstruction of defects of the maxillofacial skeleton. Various biomaterials have been evaluated as bone substitutes¹⁻³. The volume needed for the reconstruction of maxillofacial defects without causing secondary morbidity in a patient with an autogenous graft is difficult to acquire, so alternative bone substitute materials have been investigated for a long time¹. The cost involved for synthetic materials and autogenous grafting is not affordable to the common man, so the search for an economical ideal substitute material is ongoing⁴. In the recent past, eggshell derivatives have been assessed as scaffold materials¹¹⁻¹⁴. More recently, an eggshell-derived bone graft substitute material was introduced and studied as a synthetic bone graft substitute in a few animal¹⁵⁻¹⁷ and human clinical studies^{5,6,12}.

Eggshell has been used for various medical formulations^{7,8}. The eggshell contains calcium precursors, typically calcium carbonate (94%), calcium phosphate (1%), organic matter (4%), and magnesium carbonate (1%)^{7,8}. The weight percentages of the constituents are Ca (35.11%), P (3.66%), C (15.28%), O (44.59%), and Mg (1.37%)⁷. Every day, a million tons of eggshells are produced as biological waste around the world⁷. Various production technologies for the synthesis of HA from different raw materials have been tried with varying success. Important issues in the industrial process are the minimization of waste and ability to recycle waste materials into useful products⁷. The synthesis of HA from chicken egg-

Table 6. Radiological evaluation of bone healing with elapsed time postsurgery (n=20)

Elapsed time (wk)	Incomplete	Complete
1	20 (100)	0
4	18 (90.0)	2 (10.0)
8	4 (20.0)	16 (80.0)
12	2 (10.0)	18 (90.0)
24	0	20 (100)

Values are presented as number (%).

Vivekanand Sabanna Kattimani et al: Natural bioceramics: our experience with changing perspectives in the reconstruction of maxillofacial skeleton. J Korean Assoc Oral Maxillofac Surg 2019

shell has been tried in the laboratory with different methods like hydrothermal, wet precipitation, sol-gel, and microwave irradiation processes⁷. The nanotechnology-assisted production of HA as a bone substitute is a dream come true for the surgeons working on artificial bone regeneration¹⁵⁻¹⁷. The advantage of HA is its great compositional similarities to the main constituent of human bone and its ability to process osteoprogenitor cells^{1,3}. The complex yet highly effective intracellular signaling of osteogenesis will be triggered by the presence of soluble calcium and inorganic phosphates^{1,3}.

The ideal bone substitute should be biocompatible, osteo-inductive or at least osteo-conductive, and have satisfactory mechanical properties¹¹. This bone substitute should be available in unlimited quantities with low cost¹¹. To accomplish these goals, the use of synthetic bone substitutes has been increasingly considered¹¹. Eggshell powder has shown promising results in preliminary animal studies¹¹⁻¹⁴. The experiments with animals in four groups have shown satisfactory healing, but osteo-induction has not been observed^{11,12}. Eggshell powder has demonstrated no toxicity in animal experiments¹¹⁻¹⁴. This natural product has already been used as a mineral additive in a chewing gum without adverse reaction; it is inexpensive and can be found in an unlimited quantity¹¹. Recently, the eggshell powder has been processed and converted into HA, which has been evaluated in different animal models with promising results¹⁵⁻¹⁷. EHA has been produced by our associates using a domestic microwave process and this environmentally friendly conversion technique of conversion has been patented⁷. The preliminary studies have shown promising results in a small group of patients^{5,6}.

In the present study, the surgical site outline is the outline of the surgical defect after cystic enucleation, which will change with time because of creeping substitution from the periphery and will merge with the material margin in the due course of time^{9,10}. The surgical site outlines were categorized as unchanged (altered less than 25%), slightly changed (more than 25% and less than 75%), partially reduced (more than

75%), or absent (inability to differentiate between graft material outline and surgical site outline)^{9,10}. In the present study, the surgical site outline was slightly changed in 12 patients and partially reduced in eight patients by the fourth week and the eighth week, respectively. The surgical site margin visibility was further reduced to partially by the eighth week in 15 patients and was completely absent in five patients, indicating the material diffusion into the adjacent bone or the creeping of bony substitute from the periphery; this is similar to previous studies published in the literature^{5,6}. The transition from the slightly reduced category to the partially reduced category was seen in seven patients over an interval of four weeks (i.e., from the fourth week to the eighth week). (Tables 2, 3) HA derived from eggshells showed more new bone formation in the early stages than did the scaffolds made from commercially available powders in animal studies^{16,17}, and this is also evident in our radiographic observations of bone formation. Another animal study compared EHA and synthetic HA (SHA) using a rat model, which showed significantly higher bone regeneration in the EHA group than the unfilled control at eight weeks¹⁷. These results support our study findings. The merging of the material margin and the bony margin is indicative of new bone formation, as a creeping bone substitute and margins were invisible by the twelfth week. (Tables 2, 3)

The formation of bone and the blending of the bone margin with the material margin were well correlated with the density of bone formation in the previous study⁶, which supports our study observations. The margin blend with commercially available materials like bovine and SHA in the previous studies are similar to those of the present study, indicating that EHA is equally efficient in the enhancement of bone regeneration¹⁰. The rapid bone regeneration associated with grafting could be explained by the ability of the HA to enhance the selective adsorption of attachment proteins and growth factors that stimulate osteoblast adhesion and bone deposition¹⁸. Another mechanism by which the graft could enhance bone formation is through ion release, as supported by the work of Matsuoka and associates¹⁹.

The bone formation characteristics are the radiological appearance of the surgical site as changed, ground glass, spiculed, or trabecular^{9,10}. These radiological appearances depend on the visible characteristics of radiographs in terms of radio-opacity in comparison with the adjacent bone and trabecular pattern. The study showed 90% specular appearance at the eighth week postoperatively, indicating the new bone formation. The initial radio-opacity of the graft material

was increased within four weeks, which is indicative of the osteoconductive nature of the material. (Table 4) The study showed a trabecular pattern in 18 patients by the twelfth week, which indicated complete bone formation in 90% of the patients. (Table 4) The bone formation was significant during the time intervals from the fourth to the eighth week and from the eighth to the twelfth week. (Table 5) Kim et al.¹⁶ showed that the bone formation around the HA scaffolds fabricated from eggshells was more active than that of the control groups in rabbits. Similarly, Lee et al.'s animal study¹⁷ showed very similar results by the end of eight weeks, with 41.99%±8.44% of new bone formation in SHA, a significantly higher amount, when compared with the control. Small-sized particles have a larger surface area than large-sized particles with the same weight. Therefore, small-sized particles may be more easily removed from the body¹⁷, as our HA is in the form of nanoparticles, which might be the reason for early bone regeneration. A microwave process with low temperature was used to prepare the HA in our study⁷. The crystallinity of the EHA was higher than that of the SHA used in animal studies in the published literature¹⁷. Highly crystalline HA substrate supports higher osteogenic cell proliferation than low-crystalline HA substrates¹⁷. Most defect areas were filled with regenerated bone in the EHA group, and the remaining EHA particles were incorporated into the regenerated bone at eight weeks after the operation¹⁷. This might be the reason for the complete trabecular patterns of radiographs in our study. The trabecular pattern was similar with the adjacent normal bone. Similar findings have been reported in an animal study by Kim et al.¹⁶. The levels of new bone formation, lamellation, and maturation were greater in an EHA group compared to a synthetic group¹⁶. In addition to these obvious advantages of the enhancement of bone regeneration^{5,6}, the results of these studies suggest that EHA have excellent bone formation ability^{16,17}. EHA was associated with a significantly higher bone formation in this study also.

The bone densities of the grafted sites were examined using Digora (Soredex, Helsinki, Finland). Digital intraoral periapical radiographs acquired at 4, 8, 12, and 24 weeks post-surgery were tabulated for changes in density with reference to the initial densities immediately after grafting, which were considered the baseline reference densities. The changes in density were correlated with bone formation characteristics at relevant time intervals. These changes in density were due to new bone formation and graft material resorption^{9,10}. The marked density changes that were observed, indicating bone formation, are similar to results in the published literature^{5,6}.



The trabecular pattern of bone formation had the highest density attained at 12 weeks of follow-up; these findings are supported by our previous studies^{5,6,10} and other published literature using HA-modified bioglass¹⁸.

Calcium-deficient HA with surface microstructures is more favorable as a bone substitute compared to stoichiometric HA, because of its faster biodegradability and rapid formation of a surface apatite layer, enabling rapid bone-bonding microstructures¹⁵. Our EHA is calcium deficient, which might be the reason for the enhancement of bone regeneration. Park et al.¹⁵ showed that surface characteristics may contribute to the increased osteo-conduction of hydrothermally treated eggshells in the healing of rat calvarial defects, thus, the HA derived from chicken eggshell is an osteoconductive material for the enhancement of early bone regeneration¹⁵⁻¹⁷. Recent studies provided proof of concept for the clinical application of “smart” biomaterials, because the HA’s osteo-inductive nature displayed superior biological performance through the modulation of cell behavior²⁰⁻²². EHA-enhanced bone formation, and healing was complete by the end of 12 weeks.(Table 6) This enhancement might be because of the calcium-deficient nature of EHA. The calcium-deficient characteristics of the surface HA layer in the new bone graft substitute (N-HA) derived from hen eggshell in Park et al.’s study²³ showed promotion of new bone formation by increasing protein adsorption and supplying sufficient Ca, which enhanced the reprecipitation and formation of calcium phosphate layers on its surface and subsequently influenced osteoblast responses. Even the histomorphometric observations revealed new bone formation and new bone islands with N-HA derived from hen eggshell prepared through hydrothermal processing, which is osteoconductive in nature²³. Our radiographic bone formation characteristics might be enhanced due to these above-mentioned facts at early stages within 8 to 12 weeks. The study samples were divided into three groups depending on the size of the lesions: >10-20 mm, >20-30 mm, and >30-40 mm.(Table 1) However, the healing of bone was complete in all of the patients irrespective of the sizes of their lesions. The literature showed that tunnel lesions will heal with scarring or fibrous connective tissue if grafting is not attempted^{24,25}. The published literature showed the necessity of grafting for early bone formation and enhancement, which supports our findings²³⁻²⁵. The majority of our cases were tunnel lesions; 45% of the patients had lesions >20-30 mm in size, and 30% had lesions >30-40 mm in size. In all cases, the healing was uneventful; 80% of patients showed complete bone healing by the eighth week, and 90% by the twelfth week. Within the

group, the study didn’t show any significant differences in the healing among all of the lesions. Regenerative therapy is essential for the enhancement of bone healing so that grafting prevents scar tissue formation^{24,25}. The published literature supports the notion of grafting/regenerative techniques for early bone healing²⁶⁻²⁹. However, none of the published studies used EHA. They used different grafting materials, including two studies that had control groups with Chitra granules²⁷ and anorganic bovine bone²⁸, and one study that used a freeze-dried bone allograft without a control group²⁹. All of these studies suggested that grafting or regenerative techniques enhance bone formation compared to control groups^{28,29}.

In our study, wound dehiscence was seen in one case, which was attributed to improper surgical planning and an existing sinus within the flap. This case has been excluded from the evaluation due to seepage of graft material through the defect. None of the animal studies in the published literature showed any undue reactions to the EHA bone graft substitute^{15-17,23}. Eggshells are by products of food waste, so recycling them as a raw material for bone graft material production; is not only economical but also environment friendly because of ready availability of eggshell waste^{17,23}. EHA is cost-effective and production is environment friendly with no disease transfer risks^{5,6}. This economical graft material can be used as raw material for the production of various shapes and sizes of grafts in various biomedical applications including three-dimensional construction³⁰.

V. Conclusion

Our study showed early bone regeneration by the twelfth week and EHA-enhanced bone formation. The grafting prevented dead space and undue complications associated with a large hematoma in the surgical defect. The EHA acts as a space filler and osteoconductive economic graft material. It is a versatile biocompatible graft substitute that does not cause any chronic inflammatory, allergic, or toxic reactions. Thus, bioceramics derived from natural calcium precursors like chicken eggshell (natural bio-waste) will play an important role in the reduction of the cost involved with grafting because of the abundant availability of this raw material.

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Authors' Contributions

V.S.K. performed study, participated in data collection and wrote the manuscript. V.S.K. and K.P.L. designed and analyzed the study. All authors read and approved the final manuscript.

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Ethics Approval and Consent to Participate

The study protocol was approved by the Institutional Ethics Committee of Sibar Institute of Dental Sciences (IEC 16/09/2014) and prospectively registered with the Clinical Trial Registry of India - CTRI/2014/12/005340.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Botulinum Toxin Application in Facial Esthetics and Recent Treatment Indications (2013-2018)

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INTRODUCTION

Ever-changing perception of beauty from childhood to old age is changing with the revolution in cosmeceuticals science. Esthetics is an individual's perception since time immemorial. Standards of beauty have changed through centuries with increased awareness about esthetics. The youthfulness despite advancing age includes smooth, charming skin without skin folds, volume loss, and skin laxity.^[1] According to Ayurveda aging is defined as “to become

ABSTRACT

Background: Ever-changing perception of beauty from childhood to old age is changing with the revolution in cosmeceuticals science. Esthetics is an individual's perception since time immemorial. Standards of beauty have changed through centuries with increased awareness about esthetics. The face remains main source of information for identification and discrimination. It constitutes a structural ground for many nonverbal messages including the emotional state of a person, so the proverb “Face is an index of mind” holds good. The wrinkles and laxity are considered to be one of the factors for aging. Hence, escalating demand for cosmetic treatment to reduce facial wrinkles and laxity has stimulated us to search for published literature for nonsurgical techniques for enhancement of facial beauty. The review analyzed the published data to provide narrative basic review in a concise way to the beginners, clinicians, and students.

Materials and Methods: We have adopted search criteria using keywords: Botox, Botulinum toxin, incobotulinumtoxinA, esthetics, face, uses of Botox, with various Boolean operators and or in title, and abstract using PubMed search engine. The database search limited to PubMed only from January 2013 to June 2018.

Results: Various search results have been appended as annexures at the end of the article for further reference for the readers. Finally, 17 references were selected to write narrative review to meet our objectives.

Conclusion: The advancing front in the use of toxins is an emerging science for the beautification of a face. Botox exploded in to market because of efficacy, tolerability, and minimally invasive nature. The present review gives brief about the history of Botulinum toxin, types, mechanism of action, clinical indications, preparations, storage, and technique for various uses with a brief note on patient selection, contraindications, and complications.

KEYWORDS: *Aging, botulinum toxin, cosmetics, esthetics, face, noninvasive procedure, wrinkles*

old by the act of wearing out” which is a synonym for “Vardhakya.”^[2] Aging is an inevitable biological process, in which both intrinsic and extrinsic determinants led progressively to a loss of structural integrity and physiological function.^[2] The desire to look younger and in turn, vital has been a lure to

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humanity for ancient times, though aging remains as a rule and a fact of life.^[3]

The face remains main source of information for identification and discrimination. It constitutes a structural ground for many nonverbal messages including the emotional state of a person, so the proverb “Face is an index of mind” holds good. The wrinkles and laxity are considered to be one of the factors for aging. The wrinkles in hyperfunctional muscles sometimes may be misinterpreted, when these types of habitual repeated contraction accompanied by a lack of shortening of skin which will produce a wrinkle.^[4] The pursuit of youth and beauty has undergone a resurgence of interest as evidenced by increased visits of people to cosmetology clinics. The field of esthetics is expanding with advances in the area of facial rejuvenation. In the present era, multiplicity of lasers to cosmeceuticals and other innovations are complemented to the armamentarium of cosmetologist. The limitations and strength of these various tools must be understood by the physician and the patient; so that one can tailor the most effective treatment plan appropriately.^[5]

Escalating demand for cosmetic treatment to reduce facial wrinkles and laxity has stimulated the search of published literature for nonsurgical techniques for enhancement of facial beauty. The advancing front in the use of toxins is an emerging science for the beautification of a face. This miracle poison is enormously used nowadays, after the Food and Drug Administration approval in 2002 by the Government of the United States.^[6] Botox exploded on to market because of efficacy, tolerability, and minimally invasive nature.^[7] The present review gives brief about the history of Botulinum toxin (BT), types, mechanism of action, clinical indications, preparations, storage and technique for various uses with a brief note on patient selection, contraindications, and complications. The review also briefs few published literature on the use of Botox in various facial esthetics procedures.

MATERIALS AND METHODS

The search using Botox (All Fields) OR OnabotulinumtoxinA (All Fields) AND (“face” [MeSH Terms] OR “face” [All Fields]) AND (“2015/01/01” (PDAT): “2018/12/31” (PDAT)) AND “humans” [MeSH Terms]) in PubMed search engine from January 2013 to December 2018. We have even activated additional filters such as number of years to recent 5 years, clinical trials, published in English language only with human studies and clinical trials, and reviews have been considered for writing the narrative review. All remaining data excluded from the writing purpose. The finalized articles were downloaded from various sources

for reading completely and analyzing the necessary data for extraction and synthesis of narrative review.

RESULTS

Various search results have been appended in Annexure 1 showing references of total 1832. The results have been refined using filter of recent 5 years (2013–2018) period yielded 556 published articles [Annexure 2]. The search further activated additional filters of only English and humans resulted in 325 published articles [Annexure 3]. Later, the search has been restricted to only reviews yielded 120 results [Annexure 4]. We have activated one more additional filter of clinical trials which yielded 51 results [Annexure 5]. All the resulted references and abstracts have been screened for usefulness to write a narrative review was identified. Finally, the search criteria of Botox (All Fields) OR OnabotulinumtoxinA (All Fields) AND (“face” [MeSH Terms] OR “face” [All Fields]) with time duration was activated to find the results [Annexure 6]. Of these final 63 references, the abstract has been read by the team members for suitability of inclusion in the study; and assorted the articles for writing a narrative review. Table 1 shows references used for writing the review

Table 1: Selected recent and few past studies involved for revision of esthetic and nonesthetic uses of Botulinum toxin

Reference number	Author	Year	Use
15	Paulo Keiki R <i>et.al.</i>	1996	Fronto-glabella Wrinkles
16	Andrewblitzer <i>et.al.</i>	2001	Facial lines and wrinkles
18	Achih H <i>et.al.</i>	2003	Altering brow contour
19	Benjamin A <i>et.al.</i>	2003	Glabellar rhytids
20	David W <i>et.al.</i>	2003	Lateral periorbital rhytids
22	Makram Ziadeuse <i>et.al.</i>	2013	Management facial wounds
31	Chen H <i>et.al.</i>	2018	Treating sunk scar
32	Jones DH <i>et.al.</i>	2017	Glabellar frown lines
33	Solish N <i>et.al.</i>	2016	Forehead lines
34	Hsu Ak <i>et.al.</i>	2017	Chin projection modification
39	Erickson BP <i>et.al.</i>	2015	Periorbital and midfacial areas
41	Steinsapir KD <i>et.al.</i>	2015	Forehead lift
43	Charles PD	2004	Noncosmetic uses
44	Awan KH	2017	Noncosmetic uses
46	Halpern L <i>et.al.</i>	2016	Noncosmetic uses
47	Vikelis M	2018	Noncosmetic uses
48	Bolshinsky V	2018	Noncosmetic uses

briefly to meet out the criteria set. Out of all search mentioned above references, our team members selected results only related to esthetics and humans; which have been considered to write a narrative review to provide the necessary outlook for the intended users.

DISCUSSION

The advancing front of the use of toxins is an emerging science for the beautification of a face. This miracle poison is enormously used nowadays after the Food and Drug Administration approval in 2002 by the Government of the United States. Botox exploded on to market because of efficacy, tolerability, and minimally invasive nature. The discussion has been divided into history, types of Botox, mechanism of action, clinical scenarios for use, preparation and storage, selection of patients, indications, and contraindications.

HISTORY AND TYPES OF BOTOX

BT is a powerful neurotoxin produced by bacterium *Clostridium botulinum*. First time in 1897, it was identified in Belgium by Professor Emile van Ermengem, following the investigation of fatal food poisoning caused by macerated ham consumption.^[8] It was named after the disease botulism originally associated sausage meat (Latin-botulus for sausage). There are seven types of Botulinum toxin (A, B, C, D, E, F, and G). Flaccid paralysis of motor and autonomic nerves occur due to classic foodborne disease caused by BT serotypes of A, B, and G. Type B-BT discovered in 1910, and isolation of type A-BT begun in 1920.^[8] During the second world war, the research continued at Chemical Warfare Laboratories of Fort Detrick, Maryland in the United Kingdom for biological warfare as a potent neurotoxin (agent X). In 1989, Ipsen pharmaceuticals bought Porton chemicals and are the only commercially available forms of Botulinum toxin (Dysport)[®].^[8]

Dr. Allen Scott performed a first clinical test in 1978 using type-A BT.^[8] His results were published in 1980 led to the extensive use of BT by ophthalmologists for the treatment of blepharospasm. With the advent of BT-A in 1990, effectively launched as nonsurgical esthetic medicine in this modern era.^[6] In 1973, a small dosage (35–50 units) of BT-A has been shown to be safe and effective for hyperfunctional lines and facial rhytids. The median lethal dose is considered to be 2500–3000 units which are approximately 100th of the lethal dose. Therefore, BT-A has an extremely high therapeutic index.^[9] It is commonly used as a part of overall facial rejuvenation. A myriad of application for BT-A has been explored not only for aging but also for a long list of neuromuscular, glandular disorders, muscular countering, and various pain syndromes.^[10]

Out of seven serotypes of BT, five are useful in human neuromuscular junction (BT-A, B, E, F, and G). Three types of BT are commercially available. Botox[®] and Dysport[®] (both BT type A) and Neuroblock[®] (BT type B). Allergan produces Dysport[®] by Ipsen pharmaceuticals and Neuroblock[®] by Elan, Ireland.^[8]

MECHANISM OF ACTION

Working knowledge of BT-A pharmacology is necessary to understand complications of treatment and contraindications.^[11] BT is a polypeptide comprises a protein molecule with a heavy and light chain held together by a heat-labile disulfide bond. Disruption of disulfide bond inactivates the neurotoxin; so BT storage at the correct temperature is necessary.^[8,11] Reconstitution should be carried out carefully to preserve the integrity of both the chains.^[8,11] BT blocks the release of Acetylcholine at the skeletal neuromuscular junction and induces paralysis by inhibiting transmission of a nerve impulse across the synaptic junction to the motor end plate. The chemodeneration results in weakness or classic paralysis.^[9,12,13] The BT heavy chain attaches to the nerve membrane which allows the light chain transportation to its site of action, that is, the protein complex. The light chain enzyme then cleaves the protein specific to the particular neurotoxin. Hence, neuromuscular transmission ceases, and reversibly target muscle atrophies.^[8] If the handling of BT is not proper, the fragile bond splits and makes the molecule ineffective. The binding of a molecule is permanent to the motor end plate, and it requires 24–48 h for therapeutic action. This delay is due to the time required to deplete acetylcholine storage in the presynaptic motor end plate. Although binding is permanent paralytic effect persists only for 2–6 months. The reason for this reversal is the reestablishment of neurotransmitter pathway due to new axonal sprouts formation. This neurogenesis process allows complete recovery of transmission pathway which results into the muscle function.^[14]

BT-B acts on the different cytoplasmic complex. The light chain of BT-B molecule cleaves vesicle-associated membrane protein. BT-B is effective with cervical dystonia and those resistant to BT-A.^[8]

CLINICAL USE OF BOTULINUM TOXIN

BT used successfully in cosmetic and noncosmetic indications. The cosmetic indications such as crow's feet, vertical and horizontal frown, wrinkles on the nose, upper lip rhytids, nasolabial fold, horizontal and vertical neck bands, scar management, and pebbly chin.^[7,15-42] The noncosmetic indications are migraine, strabismus, hemifacial spasm, bruxism, blepharospasm, spasmodic torticollis, postfacial nerve palsy synkinesis, hyperhidrosis, and esophageal achalasia.^[43-48]

PREPARATION, STORAGE, AND TECHNIQUE

BT is delivered in an insulated glass vial as 100 units of freeze-dried powder. The manufacturer's guidelines should be followed very strictly to prevent denaturation and maintain a maximum efficacy. The storage before reconstitution either frozen at -5° or in a refrigerator at 2° – 8° ; once reconstituted must be stored at 2° – 8° . If the clinic is not having a refrigerator thermos flask or vaccine transporter can be used. Companies such as Allergan do not recommend this type of storage. Dysport[®] comes in a plastic hinged box which contains two glass vials. Ipsen recommends the use of Disport[®] within 8 h and should not be frozen.

BT reconstitution should follow the standard aseptic precautions. The product is vacuum sealed, so air can be injected to avoid the rapid constitution. Saline must not be pushed into the vial to prevent agitation of solution mechanically. Rotating of the vial assists the gentle reconstruction. After reconstitution, during transport, cold pack insulation box should be used or else, it should be reconstituted after the journey as agitation denatures toxin and reduces the duration of action.

BT injections are used intramuscularly and not subcutaneous unless specifically stated. Injections around dangerous areas require careful analysis of the dose and depth of the injection. Intramuscular placement is essential for maximum effect and control, but in areas like corner of the mouth, subcutaneous treatment can be considered.

The dose required for cosmetic use has been discussed and established independently by many researchers based on their experiences^[22-41] [Table 1]. Review of literature provides more information on dosage preferences by practitioners.^[42] BT is potent and very expensive, so each drop must be used cautiously even 0.0125 mL is effective in certain areas.^[8] The comparison of the average doses of different BT products for different esthetic purposes showed a significant deviation from accepted 1:1 equivalence coefficients for OnaBTXA: IncoBTXA and 1:2.5 for OnaBTXA: AboBTXA, depending on a particular area of the face.^[42] Hence, the clinician can follow national guidelines with their experience during various esthetic procedures.

The cosmetologists should have a thorough knowledge of the muscles of the area to be injected.^[23] The beginner should mark the injection sites with a washable skin marker. It is important to follow aseptic precautions. During injections, it is vital to avoid nerve, vein, and artery complex in the region of glabella. It is better to know the surface anatomical landmarks such as supraorbital nerves for injection on the glabellar

area.^[23] The injection in the forehead region usually starts from frontalis zone. During injection, assess the bulk of muscle by asking the patient to frown, then relax and inject at the noted point.^[24-26] Do not touch the periosteum, take care to point the needle away from the danger area, avoid pointing toward orbital septum while injecting at the lateral canthus. Sometimes, it is useful to hold the muscle between two fingers in the glabellar area. In lateral orbital skin, spread the skin to observe the orbital veins clearly during injection. Look carefully to avoid superficial veins.^[23] Slow insertion during injection significantly reduces the pain perception by minimizing mechanical stimulation.^[27,28] Few authors reconstitute with lidocaine, but the efficacy of BT-A has not been tested.^[8] Postinjection, the patient should be asked to press on the site of injection with a tissue which minimizes bruising. If any bruise occurs, an ice pack should be used. Makeup can be used as a camouflage before the patient leaves the hospital. Few believe that ptosis may happen because of untoward diffusion due to flying or lying down after the treatment.^[8] Usually, the duration of appointment lasts for a maximum of 10 min excluding counseling visits. It is advisable that nurse may prepare the patient that reduces time spent with the practitioner.

SELECTION OF PATIENT

During esthetic procedures, it is very much advisable to understand the requirements of patients. Few patients should be assessed thoroughly when they do not have a clear idea of their need and good esthetic sense. They might have motivated because of their friends or parents advice. If this is the situation, the clinician should not commit. The golden rule for the use of Botox is a perceivable pleasure after their expenditure.^[8] The patient trust in the clinician will reduce where the patient think their demands has not been met. In such a scenario, it is better for the clinician to refuse the treatment. If the patient is unhappy; the procedure cannot be reversed in a week. Hence, it is essential to listen to the complaint and decide quickly whether the patient can be treated with BT or not. The careful psychological assessment is essential. Spend time with a patient by providing verbal and written information if the patient seems suitable. Always examine the patient closely and discuss likely outcome of the injection. Make sure the patient understands fully, before the patient is taken for injection. The preoperative photography is the must.

The clinician should decide before the injection whether alone BT will eliminate or reduce, or prevent further rhytids or any other adjunctive treatments is required. If so, a patient should be conveyed the fact and the outcome.^[26] Observe the patient face, neck, and hands while talking and in action.

The clinical examination before consideration for injection is as follows: look for a general skin conditions such as sun damage, pigment spots, and tonicity of the skin. The investigation of the cardiovascular system, skin perfusion, and the overall tone is necessary.^[27] Brow position, change with expression should be observed. Crow's feet at rest and in motion should be examined along with its extension toward perioral lines when smiling. Hooding should be examined and its disappearance when the patient lifts the brow. All these should be reviewed at rest and in action for successful execution.

INDICATIONS FOR TREATMENT

Wrinkles of the face

If the dynamic wrinkles are present with frowning and smile it can be treated.^[33] If the patient has wrinkles at rest and sun damage, and elderly patient better to take precautions because it will reduce lines, but will not eliminate them.^[41]

Glabella rhytid

If it is dynamic, the treatment will be successful but if it is at rest caution, the patient as it may not be eliminated completely.^[32] If the patients have a heavy bruise after treatment which may increase medial sagging. Eyebrow and lash distance is an important factor, as it may cause tired appearance if the distance is less. In asymmetrical brows motion, the results of treatment will be asymmetrical. If the frontalis is active, the procedure leads to lateral brow to elevate and creates unattractive peaking of eyebrows.

Horizontal rhytids

If the patients lash – brow distance is good at rest will do well at treatment. In case of high eyebrows, relaxing the horizontal rhytids will prevent high resting tone of brows.^[38,39]

Crow's feet

Wrinkles in action are ideal for treatment, but wrinkles at rest may require modification. Wrinkles extending over zygomatic arch will need more care which usually seen in older patients and creates Mickey Mouse appearance. Hooding of lateral eyebrows will remain same even after the treatment which should be explained to the patient that it may require adjunctive therapies.^[37]

Masseteric hypertrophy

Hypertrophy of the masseter can create a square face of the lower third of the face and convey an impression of heaviness to the face. The square face appearance gives a masculine look. So, many of the female patients wish to reduce the same without surgical intervention. BT provides better noninvasive treatment.^[37]

Many more uses of BT have been noted in dentistry such as hyperactive mentalis, nasolabial furrow, hyperactive

upper lip, and so. The activity can be reduced, and facial harmony can be brought during the action by use of BT injection.^[24,25,39]

The review provided mainly outline and brief about esthetic uses of BT in the maxillofacial area. The knowledge of BT for the clinician is essential to educate the patient and provide insights into esthetic treatment. Increased awareness of esthetics among public, made the necessity for learning and practice of esthetic procedure by a dentist which is increasing because of noninvasive nature of pharmacopeia. BT is becoming boon for the practicing dentist with thorough knowledge of anatomy and indications, contraindications of use of BT successfully without any undue complications. In this review, we have not discussed noncosmetic uses of BT-A which can be read elsewhere in the published literature^[43-48] [Table 1].

CONTRAINDICATIONS

Contraindications of BT are pregnancy, breastfeeding, neuromuscular junction disorders (myasthenia gravis), amyotrophic lateralizing sclerosis, myopathies, and drug interactions.^[36]

CONCLUSION

The success of BT treatment depends on patient satisfaction despite good results. It is better to ask ourselves will this make a patient happy; if not, then better to reassess the patient whether the patient is suitable for BT or not. The patient factors such as identification and avoidance of low intelligence, unreasonable expectations, depression, and dysmorphophobic patients are crucial factors for successful management of esthetic patients. Knowledge of anatomy, proper technique, and exposure for different scenarios of treatment using BT is much essential for successful treatment.

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Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.


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ANNEXURE

Annexure 1: Showing search result of 1832 published articles on Botox in all fields in PubMed search engine without any filters activated. Download link: http://www.jispcd.org/articles/2019/9/2/images/JIntSocPreventCommunitDent_2019_9_2_99_256006_sm2.pdf

Annexure 2: Activation of filter published in the past 5 years' duration yielded into 556 published articles on Botox in all fields. Download link: http://www.jispcd.org/articles/2019/9/2/images/JIntSocPreventCommunitDent_2019_9_2_99_256006_sm3.pdf

Annexure 3: Activation of filters English and humans resulted 325 published articles. Download link: http://www.jispcd.org/articles/2019/9/2/images/JIntSocPreventCommunitDent_2019_9_2_99_256006_sm4.pdf

Annexure 4: Activation of filters reviews resulted 120 published articles. Download link: http://www.jispcd.org/articles/2019/9/2/images/JIntSocPreventCommunitDent_2019_9_2_99_256006_sm5.pdf


Annexure 5: Activation of clinical trial filter resulted in 51 results. Download link: http://www.jispcd.org/articles/2019/9/2/images/JIntSocPreventCommunitDent_2019_9_2_99_256006_sm6.pdf

Annexure 6: Result of search criteria Botox (All Fields) OR OnabotulinumtoxinA (All Fields) AND (“face” [MeSH Terms] OR “face” [All Fields]) with 5 years yielded 63 published articles. Download link: http://www.jispcd.org/articles/2019/9/2/images/JIntSocPreventCommunitDent_2019_9_2_99_256006_sm7.pdf



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Use of eggshell-derived nano-hydroxyapatite as novel bone graft substitute—A randomized controlled clinical study

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Abstract

Background: Use of various bone graft substitutes are advised to prevent bone loss, periodontal problems, and enhance bone formation after surgical removal of the mandibular third molar. Choice of graft substitute depends on biological properties and the cost of the material. So, a preliminary study was planned to evaluate and compare the effectiveness of eggshell-derived nano-hydroxyapatite as an emerging graft substitute for the enhancement of bone regeneration.

Materials and methods: Twelve patients (out of 20 enrolled) were finally assessed for bone healing after surgical removal of bilateral mandibular third molars in a split-mouth randomized controlled clinical study. All of them were assessed and compared at baseline, first, third, and sixth month. Clinically wound healing and probing depth were assessed. Radiographically the changes in density, bone formation characteristics, and bone level were evaluated. The observers were blinded for the surgical procedure and follow-up duration. The study protocol was approved by Institutional Ethics Committee and registered with National clinical trial registry.

Results: Wound healing was uneventful in all 24 extraction sites of 12 patients. Trabecular bone pattern with increased bone density was observed indicating complete bone healing in the graft group at third month. Probing depth was gradually decreased over a follow-up period and remained within normal limits during the third-month follow-up. These changes in the graft group were significant compared to the control group. The grafting with eggshell-derived nano-hydroxyapatite showed better bone regeneration properties without any infection or undue reactions.

Conclusion: Eggshell-derived nano-hydroxyapatite showed enhancement of bone regeneration compared to the control group. Grafting maintained bone height and prevented periodontal problem emergence. Eggshell-derived nano-hydroxyapatite is a safe synthetic graft substitute because it is derived from eggshell without any diseases transfer risks, unlike allografts. The process of preparation is environment-friendly. The beneficial effects of eggshell-derived nano-hydroxyapatite may be extrapolated to develop an ideal economic graft substitute.

Keywords

Guided bone regeneration, periodontal pocket, second molar, socket preservation, synthetic graft

Introduction

Use of different origin graft materials to assist bone healing has been well-documented.^{1–3} These materials have shown varying degrees of success as graft substitutes for bone regeneration.^{2–5} The synthetic graft substitutes available in the market for cranio-maxillofacial applications are limited because of the costly production process. One more reason is a relatively low number of clinical studies comparing bone graft

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substitutes.⁵ Consequently, only few graft substitutes are dominated in the material market. Nonetheless, the demand for bone graft substitute is increasing. This leads to the production of new generation synthetic graft substitutes with better bioactivity and mechanical strength.⁵ The advantage and disadvantages associated with various biomaterials explained well in the published literature and stressed on the need for a new generation of materials to overcome these deficiencies.⁵

Therefore, researchers and scientists are in search of an economic ideal bone graft substitute which uses cost-effective and environment-friendly raw materials. Emerging technology has made it possible to use eggshell waste as raw material to produce nano-hydroxyapatite (EnHA) and use it as an emerging bone graft substitute for various biomedical applications.⁶⁻⁸ The EnHA meets the required criteria and properties of an ideal graft substitute as the size of EnHA is in nanoscale and porous in nature with calcium deficiency similar to native bone HA.^{7,8}

The historical study associated with healing of third molar socket showed intra-bony defects and periodontal pockets.⁹ Healing may be delayed or result in scar formation, which might require grafting for enhancement of bone regeneration and prevent periodontal complications and dry socket.¹⁰⁻¹² American association of oral and maxillofacial surgery task force and published literature advise grafting to prevent periodontal complications and enhance bone regeneration.^{10,12} The graft substitute selection and clinical use depend on the cost of graft material, availability, and ease of use. Therefore, various synthetic graft materials are emerging as bone graft substitutes.^{1,13,14} A recent addition to synthetic bone graft substitute is EnHA.^{6,15,16} Nano-hydroxyapatite synthesized from eggshell biowaste showed promising results in vitro.^{6,15-17} Clinical studies have used nano-HA derived from eggshell waste as a graft substitute in apicectomy and extraction defects successfully.¹⁸⁻²¹

So, the study was aimed to evaluate and compare the efficacy of EnHA for enhancement of bone regeneration after surgical removal of the bilateral mandibular impacted third molar teeth. The study followed the null hypothesis. The study hypothesized that EnHA grafted sites will show a similar type of bone at third month compared to the control site. The time for evaluation was considered at third month to provide early regenerative insights as early bone modulation happens within the first three months. The study was evaluated and compared clinically for wound healing, probing depth and radiographically for the changes in density, bones formation characteristics, and bone level.

Materials and methods

The total of 20 patients enrolled in this pilot study (Figure 1) were referred to Oral and Maxillofacial surgery outpatient department for surgical removal of impacted bilateral mandibular third molars between January 2016 and December 2017 with following inclusion and exclusion criteria.

Systemically healthy patients (ASA-I) between the age group of 18 to 45 years of either gender willing to take part in the study protocol and readily available for follow-up visits were included. Pregnant patients, vulnerable group of patients, patients with poor oral hygiene, and smokers were excluded from the study protocol.

A prospective, split-mouth randomized controlled clinical study with the blinded protocol was followed to evaluate and compare the efficacy of EnHA. Each patient acted as self-control because the test and control sites were in the same patient. The split-mouth design reduced the confounding patient factors such as age and gender, which were automatically controlled and eliminated the bias. The coin toss method was used for randomization of defect site before the surgical procedure. After surgical removal of impacted third molars, the bone defect on test side (graft group) was filled with EnHA and the control side (control group) was left unfilled.

The study was approved by the Institutional Ethics Committee IEC-16/09/2014 and registered with our national clinical trial registry CTRI/2014/12/005340. The protocol was explained to the patient and written informed consent was obtained. The study followed the Helsinki Declaration and the guidelines.

Materials

The EnHA used in this study was indigenously prepared nanocrystalline HA derived from hen's eggshell under waste management technology development project. Eggshells were collected and immersed in boiling water to remove the inner membrane and surface contaminants. After drying, they were ground into a powder and immersed in sodium hypochlorite (NaOCl) to remove organic components. Then, they were extensively washed with Millipore water and dried in a vacuum oven for 5 hrs. at 110°C. So formed eggshell powder was converted into calcium-EDTA complex by reacting it with EDTA. This Ca-EDTA reacted with disodium hydrogen orthophosphate while maintaining the pH above 10 and subjected to microwave irradiation to produce HA^{7,8} (Figure 2). So produced HA size ranged from 0.5 to 1 µm in length and 100 to 200 nm in width. The physicochemical



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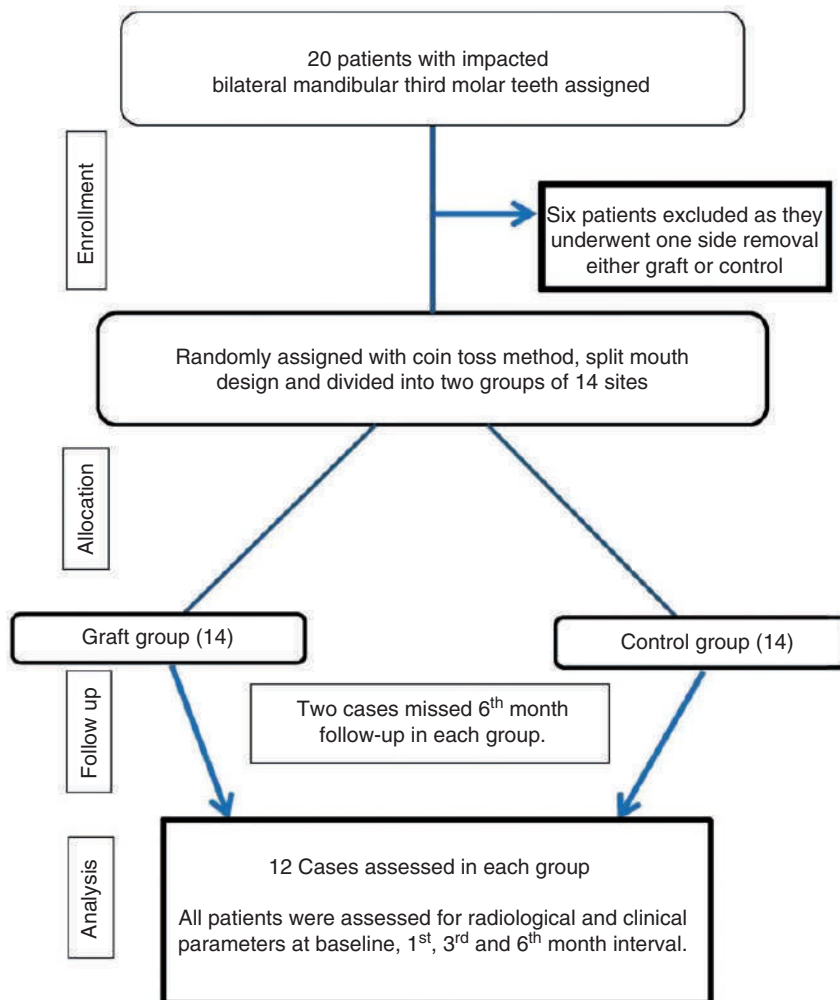


Figure 1. CONSORT diagram showing protocol scheme, patient assignment, and evaluation.

characterization showed superior material properties compared to commercially available synthetic HA.^{7,18}

Surgical procedure. Under local anesthesia, surgical removal of the tooth was performed using standard aseptic operating protocol. A mucoperiosteal flap was elevated using an envelope incision (in few cases, position C and horizontal bony impactions, Ward's and modified Ward's incision were used). Buccal bone guttering was performed under copious saline irrigation, and, if necessary, the tooth was sectioned prior to removal (Figure 3). All extractions were performed by a single operating surgeon. Wound toileting was done using saline, and hemostasis was achieved. Grafting was done on test side incrementally; graft particles were condensed using wet gauze piece which also removed excess fluid. Wound closure was done using Mersilk (3-0) suture in a simple interrupted manner (Figure 4). Pressure pack was placed for

30 min. Standard post extraction instructions were given. Antibiotics and analgesics were prescribed for five days.

Clinical and radiological assessment

Clinically, probing depth and wound dehiscence were evaluated by an oral and maxillofacial surgeon (VK; Figures 5, 6, and 7). Standardized digital intraoral periapical radiographs (IOPAR), with fixed exposure parameters for all images, were obtained with a calibrated 1-mm² x-ray mesh in place (with 120 millisecond exposure using BLUE-X Intra OS 70, 70 kVp and 7 mA for production of X-rays Make-Fona S.R.L, Italy) for pre- and postoperative assessment. Preoperatively patients were assessed for radiological similarity and difficulty based on Winter's and Pell and Gregory's classification (Figure 8(a)). Postoperatively, all patients were observed for clinical

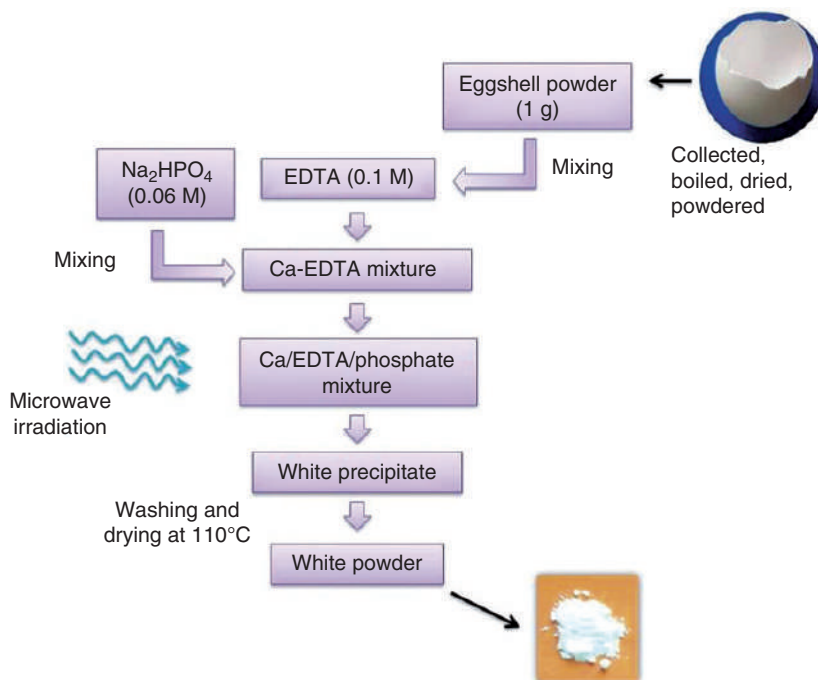


Figure 2. Flowchart for the microwave conversion of eggshell powder into nanoHA.



Figure 3. Showing intraoral view immediately after surgical removal and grafting on the test side of the defect with EnHA and non-grafted empty defect on the control side.

and radiological parameters over six months period with time intervals of the first and third month (Figure 8(b) to (e)). Evaluation of radiographs was carried out blindly. The observers were blinded for the procedure and time interval of follow-up. The radiographic observers (two oral and maxillofacial radiologists) assessed changes in bone density, radiological bone formation characteristics, lamina dura/surgical/socket outline, and bone level at different time intervals.

Bone density was measured using the Digora[®] Optime digital radiograph software (Digora for windows 2.8.107.458 Network Client, Soredex Orion Corporation, Finland). Two examiners observed the images, and the density was noted at a predetermined point in both control and test sites. Two midcoronal and two mid radicular points were considered for measuring density, at a fixed distance from the second molar taken as a reference point for reproducibility (Figure 9). Digora[®] system

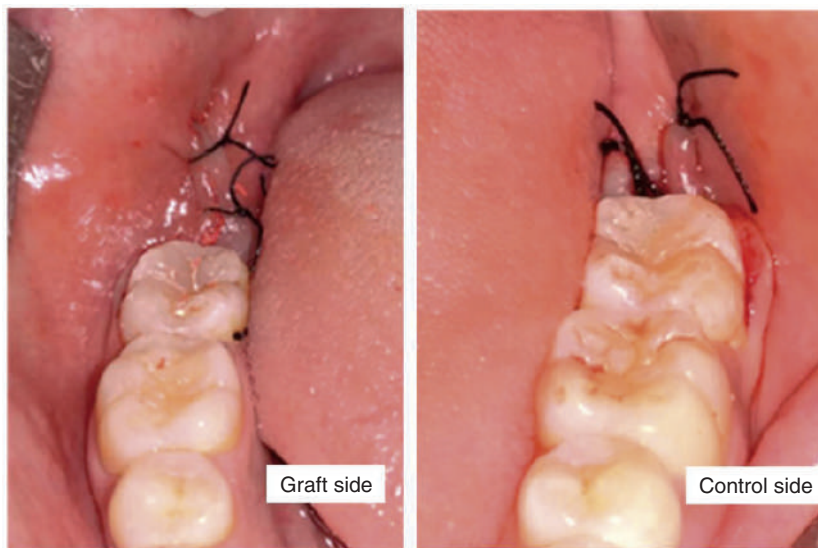


Figure 4. Showing intraoral view immediately after suturing using 3-0 mersilk.



Figure 5. Showing intraoral view at first-week follow-up immediately after suture removal with Grade 2 wound.



Figure 6. Showing intraoral view at first-month follow-up both test and control side with Grade 1 wound.

provides bone density values in pixels ranging from 0 to 255 for the region of interest.²²⁻²⁴ These observations are reproducible with a subsequent follow-up period because of the X-ray mesh (Figure 10).

The radiological bone formation characteristic is a qualitative assessment based on the appearance of the radiograph. These parameters have been modified for third molar socket graft healing as ground glass, spicular, and trabecular.²⁵ These changes can be



Figure 7. Showing intraoral view of well-healed wound at third-month follow-up both test and control side.

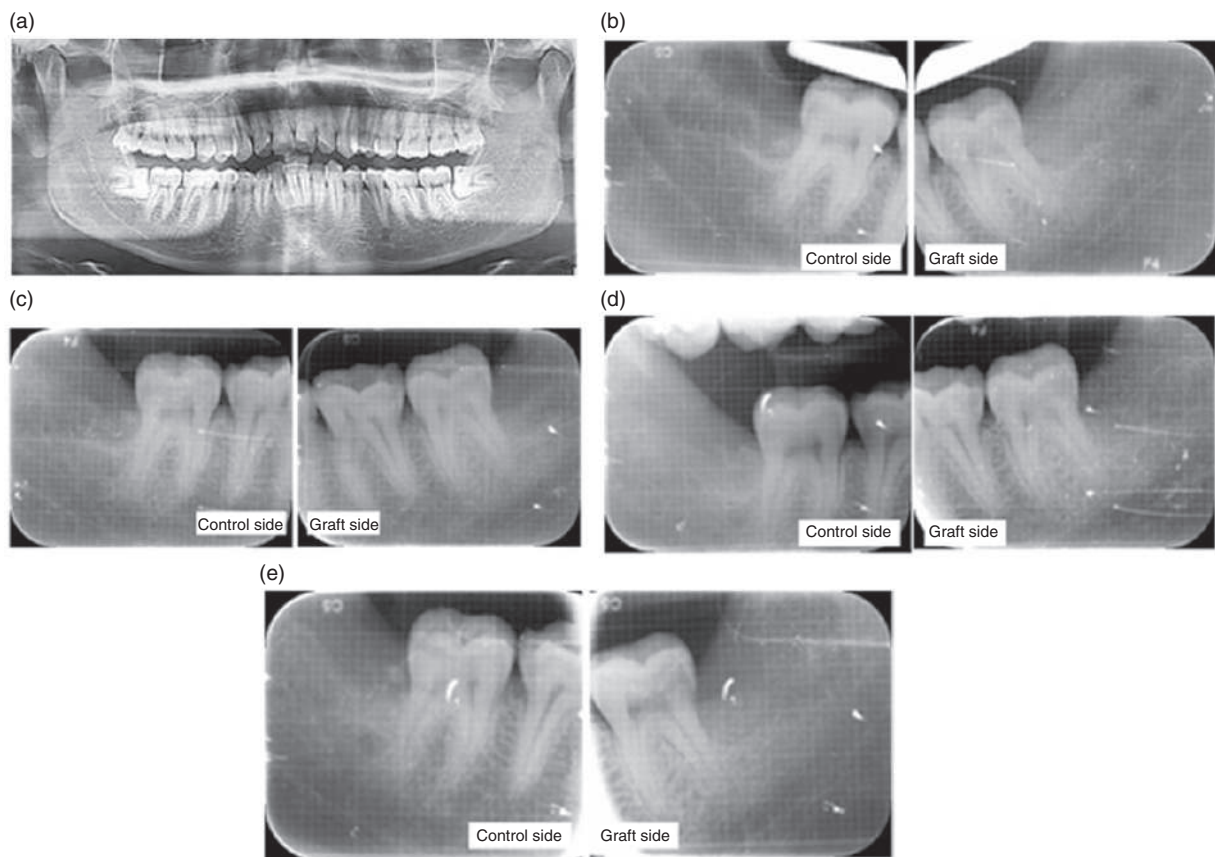


Figure 8. (a) Preoperative orthopantomograph showing similar impacted bilateral mandibular teeth. (b) IOPAR of control and grafted side immediately after grafting at baseline. (c) IOPAR of control and grafted side at first month after grafting. (d) IOPAR of control and grafted side at third month after grafting. (e) IOPAR of control and grafted side at six months after grafting.

appreciated during follow-up which indicates the bone modulation changes.

The changes in lamina dura (surgical site outline) observations have been modified and examined for the presence or absence of lamina dura of socket after extraction with a duration of follow-up (Figure 11). The visual observations of bone formation characteristics have been modified and adapted for correlation.^{25,26} Immediately after removal of the tooth, the complete appearance of the socket with lamina dura has been considered as present (category 1). The other categories like slightly changed—the disappearance of lamina dura more than one-fourth and less

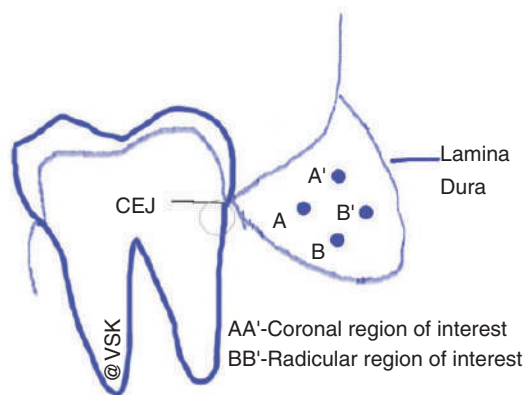


Figure 9. Schematic diagram showing points for assessment of density and bone level parameters.

than or equal to half of the lamina dura observed at baseline (category 2), partly reduced—the disappearance of more than half of the lamina dura observed at baseline (category 3). Complete disappearance of the lamina dura is considered as absent (category 4) indicating complete bone regeneration.

The crestal bone level was observed with reference to the cemento-enamel junction (CEJ) of the second molar, which was considered as a reference point to assess the bone level on the distal aspect in millimeters.²⁶ The bone level above the CEJ was noted as positive (+), below the CEJ level as negative (–), and at the level of CEJ was considered as zero.

Probing depth was measured using William's probe (Coloruven probe-Hu-Friedy 1,2,3,5,7,8,10). The measurements were performed at three points: distobuccal, middle, and distolingual of the second molar. Average of three measurements were considered at first, third, and sixth month follow-up. First month follow-up observations were considered as the baseline for the changes in probing depth with subsequent follow-ups.

The criteria of wound healing were adopted from Rakprasitkul and Pairuchvej.²⁷ Grade 1: no wound breakdown. Grade 2: slight wound breakdown explorable with a blunt instrument. Grade 3: moderate wound breakdown, with the exposed socket. Grade 4: severe wound breakdown, with exposed socket and visible nonvital bone.²⁷

The diagnosis of localized osteitis or dry socket was performed using the criteria of Blum IR.²⁸ Signs and

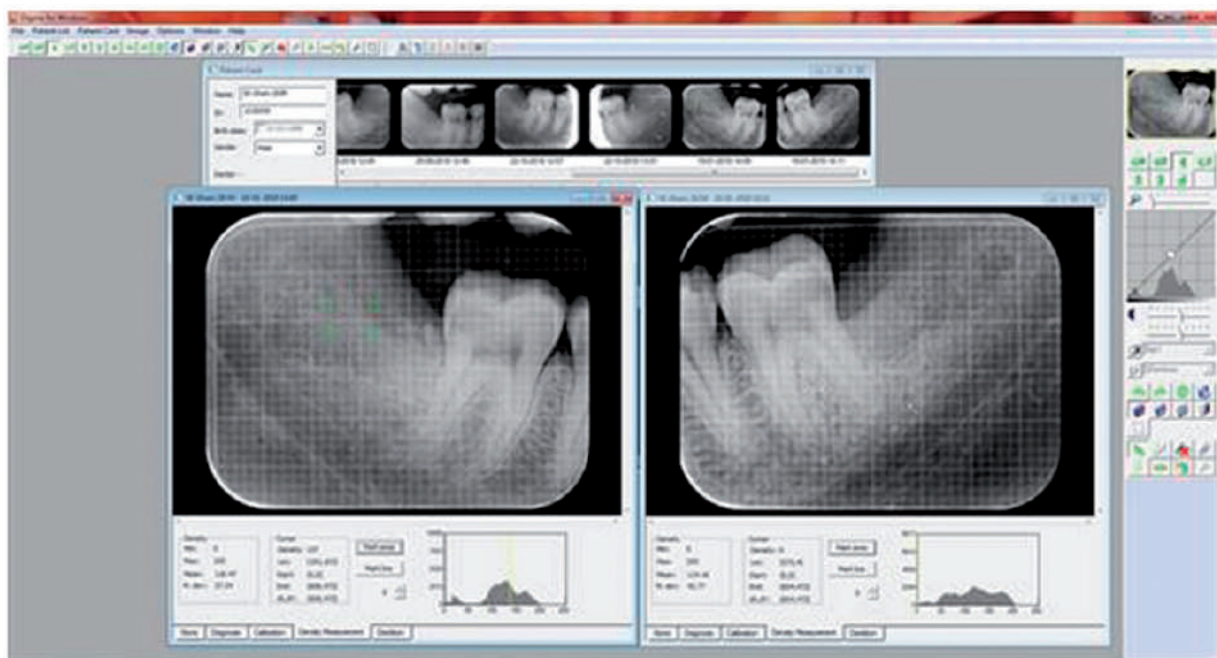


Figure 10. Representational image of Digora Window for the measurement of density at predetermined points for evaluation and comparison of bone formation.



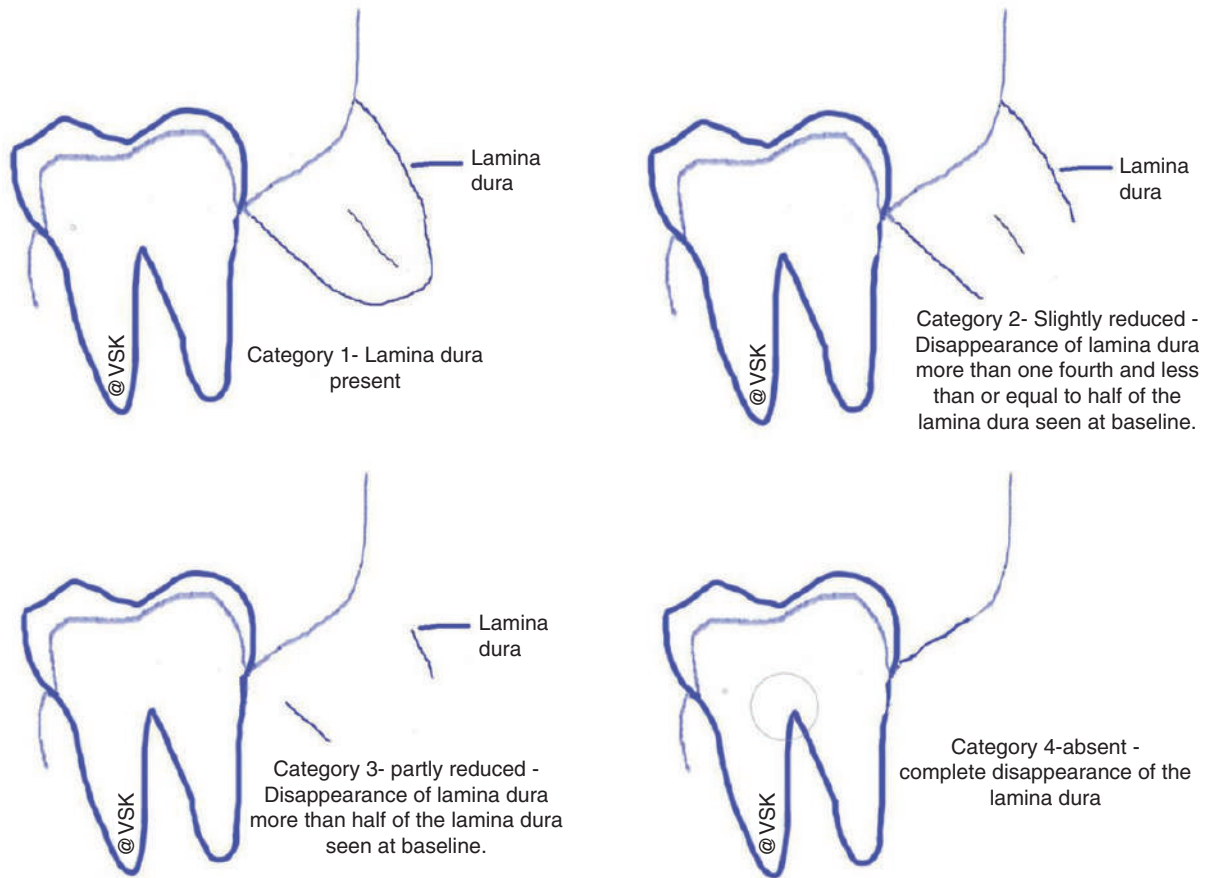


Figure 11. Schematic diagram showing criteria for the assessment of lamina dura/surgical site/socket outline.

symptoms to diagnose dry socket were the start of throbbing, continuous type pain after 48–72 hrs. of extraction, not relieved even after medication. The pain radiating to the ear, temple, and neck, with a foul taste, bad breath, socket devoid of a blood clot, localized swelling, and lymphadenopathy were considered for the diagnosis.

Purulent discharge from the wound was regarded as an infection.²⁹

Statistical methods

The observations were tabulated using Microsoft Excel for both groups. Independent *t* test and dependent *t* test were used to assess the probing depth and bone density within and among groups between coronal and radicular portion. Demographic, clinical variables, bone formation characteristics, and wound healing were evaluated using descriptive analysis, chi-square test, and Cochran's Q test. Bone level was assessed using Friedman ANOVA test using Statistical Package for the Social Sciences software. A *p* value less than 0.05% was considered significant. The

kappa (κ) correlation was considered to assess the degree of observer agreement for radiological assessment.

Results

Out of 20 assigned patients, a total of 14 patients (seven male and female each) were recruited, of which 12 patients were assessed (two patients lost for follow-up at the sixth-month interval; Figure 1). The mean age of the patients was 25 ± 8.71 years. Female patients were younger than males (Table 1). The study showed 71.43% of mesioangular, 14.29% horizontal, and 14.29% vertical impactions. The procedure involved envelope (21 cases), L-shaped (4 cases), and modified Ward's (3 cases) incision for flap reflection, almost equal in proportion in both the groups (Table 1).

The density values at baseline for the radicular portion were relatively higher than the coronal portion in both the groups. The mean density at a coronal and radicular portion in the control group was 96.83 ± 31.16 and 117.21 ± 26.69 , whereas in graft group it was 107.67 ± 23.54 and 116.88 ± 17.72 , respectively

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Table 1. Patients study characteristics grouped by demographic and operative variables.

Study variables	Control group	%	Graft group	%	Total
Demographic					
Age in years—male	31.29 ± 9.41				
Age in years—female	20.00 ± 1.15				
Total	25.64 ± 8.71				
Operative					
Winter's classification type					
Mesioangular	10	71.43	10	71.43	20
Horizontal	2	14.29	3	21.43	5
Vertical	2	14.29	1	7.14	3
Chi-square = 0.5331; $p = 0.7662$					
Relation of tooth to ramus of mandible—Pell and Gregory classification					
Class I	4	28.57	2	14.29	6
Class II	8	57.14	7	50.00	15
Class III	2	14.29	5	35.71	7
Chi-square = 2.0192; $p = 0.3641$					
Relative depth of the third molar—Pell and Gregory classification					
1	10	71.43	11	78.57	21
2	4	28.57	3	21.43	7
Chi-square with Yates's correction = 0.000; $p = 1.000$					
Third molar decay					
No	14	100.00	11	78.57	25
Yes	0	0.00	3	21.43	3
Chi-square with Yates's correction = 1.493; $p = 0.222$					
Second molar decay					
Filled	1	7.14	1	7.14	2
No	11	78.57	10	71.43	21
Yes	2	14.29	3	21.43	5
Chi-square = 0.2418; $p = 0.8841$					
Type					
Bony	5	35.71	7	50.00	12
Soft tissue	9	64.29	7	50.00	16
Chi-square = 0.5832; $p = 0.4451$					
Type of incision					
Envelope	10	71.43	11	78.57	21
L shaped	2	14.29	2	14.29	4
Modified Ward's	2	14.29	1	7.14	3
Chi-square = 0.3811; $p = 0.8272$					
Total	14	100.00	14	100.00	28

(Table 2 and Figure 12). The density was increased gradually over a period of three months and remained the same at the six-month recall, whereas the control group showed lesser bone density. In comparison, the total density was significantly increased in graft group during the first-month follow-up (p value 0.0492) compared to control (Tables 2 and 3). The density increased earlier in the radicular portion than in the coronal in both the groups with time intervals, but the difference was not statistically significant. The graft group showed overall increased mean bone density compared to control. Even at the end of the follow-up period, the control group did not attain the same density as that of graft group. The null hypothesis was rejected because of p value <0.05, indicating graft group showed better

bone formation characteristics compared to the control group.

The graft group showed 83.33% of trabecular bone at the end of the follow-up period, whereas the control group showed 50%. Earlier bone remodeling and complete bone regeneration were seen with the graft group when compared to control (Table 4). The newly formed bone showed trabecular appearance within three months of duration in grafted sites, whereas in control sites it was not attained completely even at sixth-month follow-up.

The socket outline or lamina dura was changed during the follow-up period. In comparison, these changes were significant at the end of the first- and sixth-month follow-up. The lamina dura was



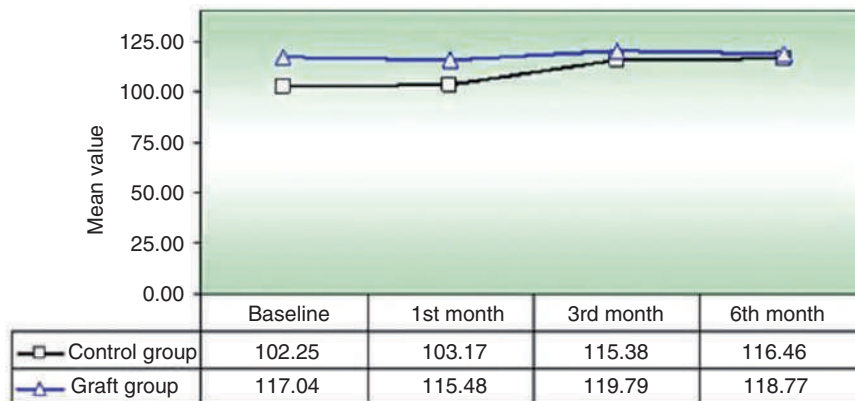
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Table 2. Comparison of coronal (AA¹) and radicular (BB¹) portion density and total density at different time points between two groups by independent t test.

Samples	Time points	Control group		Graft group		t value	p value
		Mean	SD	Mean	SD		
AAI	Baseline	96.83	31.16	107.67	23.54	-0.9610	0.3470
	1st month	97.96	21.93	108.38	9.87	-1.5005	0.1477
	3rd month	108.04	18.77	122.71	25.15	-1.6188	0.1197
	6th month	114.17	16.27	118.75	16.30	-0.6894	0.4978
BBI	Baseline	117.21	26.69	116.88	17.72	0.0360	0.9716
	1st month	112.04	26.66	118.92	12.72	-0.8063	0.4287
	3rd month	117.42	25.14	122.17	21.79	-0.4945	0.6258
	6th month	117.13	18.89	120.42	21.50	-0.3983	0.6942
Total	Baseline	102.25	23.80	117.04	19.82	-1.6543	0.1123
	1st month	103.17	12.45	115.48	16.27	-2.0815	0.0492*
	3rd month	115.38	16.66	119.79	22.84	-0.5411	0.5939
	6th month	116.46	14.81	118.77	19.48	-0.3273	0.7465

Note: *denotes statistically significant value at first month follow-up and $p < 0.05$ is significant.

**Figure 12.** Graph showing the density of the bone in both the groups at different time intervals.

appreciated in 41.67% of control cases, whereas in graft group it was seen only in 16.67% of the cases at first-month follow-up. At the end of the follow-up period, 91.67% of graft cases showed the absence of lamina dura, whereas 41.67% cases showed the absence of lamina dura in the control group. This change indicates complete bone modulation in the graft group (Table 5). Even at the end of the follow-up period, the lamina dura was appreciable in the control cases (Table 5).

The crestal bone level at different time intervals in both groups showed consistency in the modulation. The resorption followed after extraction in the control group, whereas it remained the same and increased in few cases of graft group (Table 6). The changes in the observations, in both the groups, were not significant.

The probing depth of 2.19 ± 0.63 mm seen with control group, whereas 2.00 ± 0.68 mm with graft group.

At the end of the follow-up period, the probing depth remained within normal limits (1.14 ± 0.33 mm in control group and 1.0 ± 0.19 mm in graft group; Table 7), but the difference of probing depth in the control and graft groups was statistically significant at different time intervals. The probing depth was decreased with the increase in follow-up duration (Figure 13).

None of the cases showed socket exposure (i.e. Grade 3 and 4 wound healing criteria) in both groups. All cases showed well-healed gingiva with significant scores indicating normal architecture of gingiva by first-month follow-up (Table 8). The observation of depression was evident with control group indicating the lack of support for the tissue edges after suturing. All the patients had complete healing by three-month follow-up. Both the groups showed 83.33% of complete healing by the end of the first month. None of the patients presented with any complications.

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Table 3. Comparison of density changes at different time points in two groups in relation to coronal (AA¹) and radicular (BB¹) portion and total density by dependent *t* test.

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	% of change	Paired <i>t</i>	<i>p</i> value
AAI								
Control group	Baseline	96.83	31.16					
	1st month	97.96	21.93	-1.13	17.60	-1.16	-0.2214	0.8288
Graft group	Baseline	96.83	31.16					
	3rd month	108.04	18.77	-11.21	22.35	-11.57	-1.7371	0.1103
	Baseline	96.83	31.16					
	6th month	114.17	16.27	-17.33	27.90	-17.90	-2.1524	0.0544
	Baseline	107.67	23.54					
	1st month	108.38	9.87	-0.71	17.93	-0.66	-0.1368	0.8936
Graft group	Baseline	107.67	23.54					
	3rd month	122.71	25.15	-15.04	37.12	-13.97	-1.4036	0.1880
	Baseline	107.67	23.54					
	6th month	118.75	16.30	-11.08	25.55	-10.29	-1.5026	0.1611
BBI								
Control group	Baseline	117.21	26.69					
	1st month	112.04	26.66	5.17	12.34	4.41	1.4503	0.1749
	Baseline	117.21	26.69					
	3rd month	117.42	25.14	-0.21	20.91	-0.18	-0.0345	0.9731
	Baseline	117.21	26.69					
Graft group	6th month	117.13	18.89	0.08	21.25	0.07	0.0136	0.9894
	Baseline	116.88	17.72					
	1st month	118.92	12.72	-2.04	20.55	-1.75	-0.3441	0.7372
	Baseline	116.88	17.72					
	3rd month	122.17	21.79	-5.29	23.10	-4.53	-0.7934	0.4443
Graft group	Baseline	116.88	17.72					
	6th month	120.42	21.50	-3.54	24.50	-3.03	-0.5009	0.6263
Total								
Control group	Baseline	102.25	23.80					
	1st month	103.17	12.45	-0.92	15.89	-0.90	-0.1999	0.8452
	Baseline	102.25	23.80					
	3rd month	115.38	16.66	-13.13	25.66	-12.84	-1.7717	0.1041
	Baseline	102.25	23.80					
Graft group	6th month	116.46	14.81	-14.21	23.48	-13.90	-2.0963	0.0600
	Baseline	117.04	19.82					
	1st month	115.48	16.27	1.56	13.38	1.33	0.4046	0.6935
	Baseline	117.04	19.82					
	3rd month	119.79	22.84	-2.75	18.76	-2.35	-0.5079	0.6216
Graft group	Baseline	117.04	19.82					
	6th month	118.77	19.48	-1.73	17.73	-1.48	-0.3378	0.7419

Discussion

Various bone regenerative materials are available in the market for grafting of defects. The published literature showed intra bony defect of at least 4 mm in 40% and 7 mm in 50% of mandibular second molars even four years after third molar extraction.^{12,30} To prevent these complications and residual bone defect, investigators proposed and performed various periodontal regenerative procedures. Regenerative techniques included in the published literature were osseous grafting, guided bone regeneration, and biologicals, which were evaluated for preventing

periodontal defects with varying results.¹² The search for a better economical graft material is an ongoing process. Various natural raw materials have been used to develop hydroxyapatite for clinical applications viz. coral, algae, and eggshell.^{7,31,32} Recently, eggshell-derived nano-hydroxyapatite is emerging as economic graft material substitute because of abundant availability of raw material.¹⁹⁻²¹ Hence, the study assessed and compared bone regeneration and healing potential of EnHA prospectively to introduce it as a safe, economically viable alternative graft substitute for bone reconstruction and for earlier bone healing.

Table 4. Comparison of bone formation characteristics at different time points between two groups.

Bone formation	Control group	%	Graft group	%	Total
1 month					
Changed	5	41.67	4	33.33	9
Ground glass	5	41.67	6	50.00	11
Spicular	2	16.67	3	25.00	5
Trabecular	1	8.33	1	8.33	2
Chi-square = 0.3653; $p = 0.9471$					
3 month					
Changed	0	0.00	0	0.00	0
Ground glass	6	50.00	5	41.67	11
Spicular	6	50.00	5	41.67	11
Trabecular	4	33.33	7	58.33	11
Chi-square = 0.9711; $p = 0.6161$					
6 month					
Changed	1	8.33	0	0.00	1
Ground glass	5	41.67	2	16.67	7
Spicular	6	50.00	4	33.33	10
Trabecular	6	50.00	10	83.33	16
Chi-square = 5.2572; $p = 0.1541$					

Bone density

The density values immediately after grafting at the test site were high when compared to the control site which might be attributed to material properties. The decrease in density was observed in the first month, possibly due to graft resorption, with a subsequent increase at the third month, indicating early bone formation. The density values remained the same at the end of sixth-month follow-up. The control site demonstrated a gradual increase in density, which was lesser when compared to the grafted site, even at the end of the follow-up period (Figure 1). This difference in density at the sixth month was not statistically significant (Tables 1–4). Similar observations were noted in published studies with hydroxyapatite from eggshells (EHA) in cystic grafting.^{18–20}

Munhoz et al. showed an earlier increase in bone density by the end of the second month using xenogenic graft material compared to the control group.^{22,23} de Melo et al. showed increased bone density at 30 and 60 days interval using an inorganic bovine bone graft.²⁴ Kaur and Maria³³ and Panday et al.'s³⁴ studies showed a significant increase of density compared to the control group at first and third month, similar to our timelines of study using G-bone as graft material. But none of these studies used nano-HA nor EnHA; so our study is unique to provide the insights of bone regeneration using EnHA in third molar extraction sockets.

As a part of ongoing research, Kattimani et al. used EnHA in extraction sockets of maxilla and mandible.

Table 5. Comparison of lamina dura changes (surgical site/socket outline) at different time points in both the groups.

Lamina dura	Control group	%	Graft group	%	Total
Baseline					
Absent	0	0.00	2	16.67	2
Present	11	91.67	6	50.00	17
Slightly changed	0	0.00	1	8.33	1
Partly reduced	1	8.33	3	25.00	4
Chi-square = 5.4710; $p = 0.1400$					
1 month					
Absent	0	0.00	3	25.00	3
Present	5	41.67	2	16.67	7
Slightly changed	1	8.33	5	41.67	6
Partly reduced	6	50.00	2	16.67	8
Chi-square = 8.952; $p = 0.0300^*$					
3 month					
Absent	3	25.00	8	66.67	11
Present	0	0.00	0	0.00	0
Slightly changed	5	41.67	2	16.67	7
Partly reduced	4	33.33	2	16.67	6
Chi-square = 4.2251; $p = 0.1212$					
6 month					
Absent	5	41.67	11	91.67	16
Present	0	0.00	0	0.00	0
Slightly changed	5	41.67	1	8.33	6
Partly reduced	2	16.67	0	0.00	2
Chi-square = 6.917; $p = 0.0310^*$					
Partly reduced	12	100.00	12	100.00	24
Between times,	7.7058		11.7907		
Friedman ANOVA					
p value	0.0525		0.0081		

* $p < 0.05$.

The results showed an increase in bone density. But the study not included third molars.²¹ The study evaluated the efficacy of EnHA for bone regeneration using histomorphometry and microCT and showed similar bone formation characteristics.²¹ But in this present study, only clinical and radiological observations were used for the evaluation. Kattimani et al. used platelet-rich fibrin as a barrier membrane for wound closure²¹ but in our study, we performed primary closure.

Bone regeneration using synthetic nano-HA by Gholami and Götz et al.^{35,36} showed the integration of the bone graft material into the physiological remodeling processes of the host. Synthetic nano-HA material showed nearly complete ossification with a small residue of material after four months,³⁶ but in the present study, eggshell-derived nano-HA was used. The density of bone was increased from coronal to radicular portion during socket healing using xenograft in Farina and Trombelli's study,¹¹ but our study showed a reverse tendency. The radicular density was consistently increased and was more notable in grafted sites,

Table 6. Showing bone level at different time points in both groups.

S No/ Time points	Control group				Graft group			
	Baseline	1st month	3rd month	6th month	Baseline	1st month	3rd month	6th month
1)	0	0	0	0	-1	-1	-1	1
2)	-1	-1	-1	0	-1	-1	-1	-1
3)	1	0	0	0	1	0	0	0
4)	0	-2	-1	-1	0	0	0	0
5)	-1	0	0	0	0	0	0	0
6)	-1	0	0	0	-1	0	0	1
7)	0	0	1	1	2	2	1	1
8)	-1	-1	-1	-1	-1	-1	-1	-1
9)	1	-1	0	0	-1	-1	-1	-1
10)	-4	0	-1	-1	1	1	2	2
11)	1	1	1	1	0	0	0	0
12)	0	2	2	1	1	2	2	2

Below CEJ: negative in mm; above CEJ: positive in mm; at CEJ: zero.

Table 7. Comparison of probing depth (in mm) at different time points in two groups by dependent t test.

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	% of change	Paired t test	p value
Control group	1st month	2.19	0.63					
	3rd month	1.78	0.57	0.42	0.32	18.99	4.4857	0.0009*
	1st month	2.19	0.63					
	6th month	1.14	0.33	1.06	0.57	48.10	6.4653	0.0001*
	3rd month	1.78	0.57					
Graft group	6th month	1.14	0.33	0.64	0.48	35.94	4.6000	0.0008*
	1st month	2.00	0.68					
	3rd month	1.64	0.48	0.36	0.44	18.06	2.8617	0.0155*
	1st month	2.00	0.68					
	6th month	1.06	0.19	0.94	0.62	47.22	5.3040	0.0003*
	3rd month	1.64	0.48					
	6th month	1.06	0.19	0.58	0.47	35.59	4.2625	0.0013*

*p < 0.05.

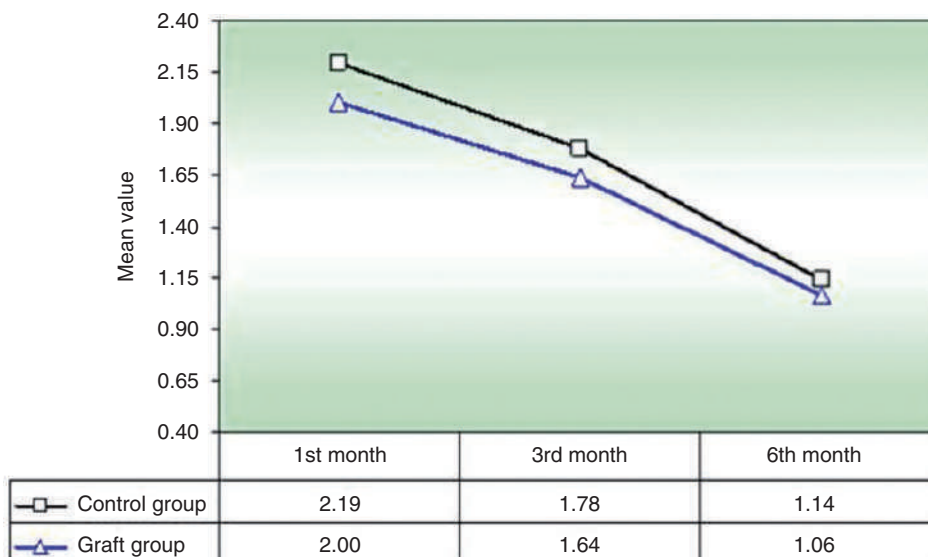


Figure 13. Graph showing probing depth in both the groups at different time intervals.

Table 8. Comparison of wound healing characteristics in two groups.

Wound healing	Control group	%	Graft group	%	Total
1st week					
Grade 1	4	33.33	5	41.67	9
Grade 2	8	66.67	7	58.33	15
Chi-square with Yates's correction = 0.0001; $p = 1.0000$					
1st month					
Grade 1	10	83.33	10	83.33	20
Grade 2	2	16.67	2	16.67	4
Chi-square with Yates's correction = 0.0000; $p = 1.0000$					
3rd month					
Grade 1	12	100.00	12	100.00	24
Grade 2	0	0.00	0	0.00	0
Chi-square with Yates's correction = 0.0000; $p = 1.0000$					
Total	12	100.00	12	100.00	24
Between times Cochran Q	Q = 13.0000		11.1428		
p value	0.0015*		0.0038*		

* $p < 0.05$.

indicating graft consolidation and earlier remodeling. The concept of differential bone density (coronal and radicular) studies are not available for comparison. Even though the comparative density results are not significant, coronal and radicular portion demonstrated a differential pattern of bone formation characteristics. The published literature showed more woven bone in the coronal portion, whereas lamellar bone was seen at the apical portion.^{11,37} This might be the reason for the differential characteristics in our study. The changes in the density revealed a similar cascade of events of the socket grafting and pattern of regeneration.

Radiological bone formation characteristics

Visual assessment of radiographs is critical and depends on the experience of the observer. To prevent the observer bias, both examiners were blinded for the procedure and time duration of follow-up. The bone formation characteristics were noted as granular, ground glass, and trabecular by the observers.²⁵ The bone formation characteristics at the grafted site and control site differ at baseline because of graft material, which is radioopaque. The material margin and lamina dura of the socket wall were well appreciated immediately after grafting. The internal portion of the socket soon after grafting was considered as changed. The grafted socket showed a higher degree of radiopacity when compared to the control. At third month follow-up, the control group showed 50% spicular and ground glass and only 33.33% trabecular appearance, whereas graft group showed spicular and ground glass in 41.67% and trabecular in 58.33% (Table 5). These

differential patterns of bone formation characteristics were supported by the density of the bone formation.

The comparative study in a rabbit calvarial defect model by Lee et al.⁶ showed that eggshell-derived HA exhibited better physicochemical properties and significantly higher bone regeneration than the control group at eight weeks postoperatively when compared to synthetic HA. The EHA is nanosize, having higher crystallinity which showed osteogenic cell proliferation than synthetic HA.⁶ The degradation velocity is an essential factor for bone regeneration. In EHA, the calcium is released rapidly from the surface because of rapid degradation which increased local calcium essential for bone regeneration.^{6,38} The study by Kattimani et al. showed trabecular bone formation as early as 12 weeks of grafting using EnHA in cystic defects.^{18–20} In another study, EHA showed significant bone formation compared to commercially available bone substitutes and control group using a rat calvarial defect model.³⁹ The notion of bone formation in these studies supports our observations of bone formation as early as 12 weeks after grafting.^{6,39,40} The assumption of microporous EnHA enhance bone formation by increasing protein adsorption, which increases the osteoblast adhesion and differentiation.^{6,39} The various bone morphogenic proteins, adsorption on the sub-micron level HA might have increased faster bone healing.³⁹ The study showed a gradual increase in density correlated with changes in the architecture of trabeculations.

The healing of the extraction socket mechanism applies for the healing of the third molar also, but the primary healing, along with grafting, accelerates the mechanism of healing and osteodeposition.⁴¹ The trabecular pattern observed in the test group was



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DEAN

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83.33%, whereas the control group was 50% at the end of the follow-up period. This difference in bone formation characteristics is attributed to the enhancement of bone regeneration by the graft material.^{11,42} The HA is an osteoconductive material which enhances osteodeposition and early bone maturation. Recent trends are changing because of material properties, which showed nHA as osteoinductive material in non-osseous sites.⁴³ Published literature of both animal^{6,16,40} and human trials¹⁸⁻²¹ support our study results with EnHA.

The concept of osteoconduction is changed to induction because of changes in the shape and size of the particle.^{43,44} The process of evolution made the HA as inductive material.^{43,44} In-vitro study showed increased expression of BMPs and osteoinductive biomarkers using nano-HA which may stimulate differentiation and proliferation of osteoblasts thus enhance bone regeneration.⁴⁵ HA grafted in well-vascularized bone incorporates into the clot and then releases phosphate into the surrounding environment, which stimulates neoangiogenesis. A critical factor for optimal integration is the size of HA crystals.⁴⁵ Natural crystals in the vertebrate bone range from 1 to 10 nm, but our study used nano-HA which ranged from 100 to 200 nm in width and 0.5 to 1 μm in length.⁷ In-vitro studies showed the stimulatory effect on mesenchymal stem cells. It also showed increased osteoblast adhesion and stimulation compared to micro-HA resulting in the bone formation and stimulation of periodontal ligament (PDL) attachment.^{45,46} These might be the probable mechanism involved in our study to enhance bone regeneration and PDL formation in distal bone defect of the second molar in deep horizontal and mesioangular impactions, but these studies used commercially available synthetic nanocrystalline hydroxyapatite (nano-HA) paste (Ostim®; Heraeus Kulzer, Hanau, Germany).^{47,48}

Lamina dura (surgical site/socket outline)

The surgical site or socket outline has been modified and used for the analysis of third molar socket healing.²⁵ Very similar findings were observed in our study at slightly earlier timelines which have been observed previously by Haghighat et al.⁴⁹ Our study showed significant changes ($p=0.0300$) in the lamina dura by the end of the first month ($p<0.05$; Table 6). The earlier disappearance of lamina dura was observed in the graft group compared to control at follow-up time intervals. These findings were similar to the observations made by other researchers using different origin HA.^{14,22,24,33,34,50-53} Almost complete disappearance of lamina dura (91.67%) was observed in grafted sites at the end of sixth-month follow-up, whereas control sites showed only 41.67% (Table 6). These changes are

attributed to the osteoconductive property of the material and remodeling of bone.

Bone level

The bone level is one of the critical indicators of bone formation. Use of the mesh is a reliable and economical method to measure bone level, which helps in the calibration of the viewers for assessment. In our study, the CEJ of the second molar is taken as a reference point for assessing the bone level at different follow-up time intervals. The grafted sites showed lesser bone resorption, whereas control sites showed more resorption and were statistically significant (Table 7). Similar observations were found in the published literature using other alloplastic material.^{13,22,53,54} The bone level at the distal part of the second molar is critical for good periodontal support. Attachment loss and probing depth are directly related to the crestal bone level. Our study results showed proper bone contour because of grafting, whereas the control sites showed depression at the distal part of the second molar.

Probing depth

Probing depth is a critical indicator for periodontal health of the involved tooth. The pocket formation, periodontal degeneration, and attachment loss are directly related to the bone loss and prognosis of tooth involved. The socket grafting has improved probing depth compared to control sites, which were well correlated with wound healing and bone level. The probing depth of 2.19 ± 0.63 mm and 2.00 ± 0.68 mm in control and graft group, respectively, were measured at baseline (Table 8). These baseline values appear near to normal and might be attributed to the patient variables. Our patients had no signs of periodontal disease nor the initial bone loss might be attributed to the younger age group of patients with good oral hygiene. The probing depth values gradually reduced with a duration of follow-up period which showed 1.14 ± 0.33 mm and 1.06 ± 0.19 mm, respectively, in control and graft groups at sixth-month follow-up and remained within normal limits. The decrease in probing depth indicates the improvement in periodontal health.⁵⁵

Wound healing

The published literature reported one to three weeks for complete epithelialization for extracted socket healing.¹¹ In primary intention healing, the epithelization will start by the second day.¹¹ In both the groups, 16.67% of wound breakdown (Grade 2) was observed during the fourth week of follow-up. These patients were instructed to rinse the mouth with lukewarm



salt water for 2–3 times a day after meals to prevent food lodgment. These patients were instructed to return to the clinic after eight days for further evaluation. By the end of the sixth week, all patients showed complete wound healing. The patient showed no leaching of material nor infection in grafted sites. The study showed no complications, such as a dry socket, infection, bleeding, or paraesthesia. These might be attributed to meticulous planning and preoperative evaluation with adherence to aseptic standard operating procedures.

The results showed a beneficial effect of EnHA for early bone regeneration, which reduced probing depth, in turn, prevented attachment loss and periodontal pocket formation. Nano-hydroxyapatite has paved the way for a newer dimension of reconstruction in bone tissue engineering;^{45,56} nano-HA can be successfully used for the preparation of composite material as a substrate.^{56,57} Both density and visual characteristics can be opted as a standard method for evaluation of bone healing at the control and grafted site which provides insights into graft healing in an economical way.⁴⁹

Literature has shown the distinctive advantage of the use of HA because of abundant availability, the absence of antigenic response and biocompatibility, low risk of infection, and high rate of good results⁵⁸ with ease of use. These properties were also observed in our study with EnHA.^{18–21} Within the limitations of this study like smaller sample size, shorter duration of follow-up, and younger age group of patients, the EnHA showed better results compared to the control group.

Future directions

Our results warrant the multicenter study involving a large number of elderly, high-risk patients for periodontal disease to evaluate the efficacy of EnHA in different clinical scenarios. The histomorphometric analysis may provide a deeper understanding of bone regeneration pattern to delineate material interaction with host bone. The results can be extrapolated for various grafting procedures in maxillofacial, orthopedic, and neurosurgery defects for functional and esthetic needs like ridge preservation, implant rehabilitation, and filling bone defects in an economically viable way.

Conclusion

EnHA showed promising results for the enhancement of bone regeneration in comparison with the control group at early bone modulation phases. The complete bone healing with increased density and reduced probing depth with meager bone loss indicates its efficacy

for early bone regeneration compared to control sites. No undue complications were observed during study protocol indicating EnHA as a safe synthetic bone graft substitute. The process of preparation of EnHA is environment-friendly. The eggshell waste is a valuable raw material for EnHA production in a cost-effective way with no disease transfer risks, unlike allografts. Therefore, EnHA may emerge as an alternative and economically viable graft substitute for grafting in the maxillofacial skeleton.

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Review Article

Basal Implants: A Review

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ABSTRACT:

Basal implants were developed primarily for immediate use in the atrophied jawbone. Basal implants are used to support single and multiple unit restorations in the upper and lower jaws. As the use implants can help avoid risky and expensive bone augmentation procedures, these implants are the therapy of first choice in moderately or severely atrophied jaws as well as in those cases, where immediate loading or cheaper treatments are desired. From this review article, we explain about the various aspects of basal implants.

Key words: Basal implants, atrophied jawbone, bone augmentation.

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Introduction: Originally, dental implants were considered as “last resort” for treatment of the edentulous patients. As implant dentistry progressed, the original Brånemark protocol required long healing periods of several months for osseointegration to take place before beginning fabrication of the definitive prosthesis¹. The term ‘basal implant’ refers to the principles of utilizing basal bone areas free of infection and resorption, and the employing of the cortical bone areas. Treatment with Basal implants is simpler and quicker than conventional implant therapy since no augmentation procedures are involved².

Basal implantology also known as bicortical implantology or just cortical implantology is a modern implantology system which utilizes the basal cortical portion of the jaw bones for retention of the dental implants which are uniquely designed to be accommodated in the basal cortical bone areas. The basal bone provides excellent quality cortical bone for retention of these unique and highly advanced implants. Because basal implantology includes the application of the rules of orthopedic surgery, the basal implants are also called as “orthopedic implants” to mark a clear distinction between them and the well-known term “dental implants.” These implants when placed in this bone can also be loaded with teeth immediately. This principle behind has already been proved in orthopaedic implants (Hip/Knee replacements).

Once the patient is fitted with the artificial joint, he/she is asked to start using immediately.³

Historical Background: Over the years basal implants have been developed and improved in several stages by majorly the German and French dentists. The first single piece implant was developed and used by Dr. Jean-Marc Julliet in 1972 with no basal plate resilience.⁴ In 1983 French dentist Dr. Gerard Scoretcci improved the basal implant system with matching surgical tools and external and internal connections for the prosthetic superstructure; he called them “Diskimplants”.⁵ In 1997 Dr. Stefan Ihde started manufacturing lateral basal implants like the Diskimplants. These implants had limited shapes and sizes and their surface was initially roughened. Soon Dr. Stefan Ihde improved the basal implant; the round base plates got edges, preventing early rotation of the implants in the bone before integration, in 2002 fracture-proof base plate was invented and later patented in Europe and United States, bending zones in the vertical implant shaft were introduced, in 2005 screwable designs (BCS, GBC) were introduced.⁶ In 1999 vertical shaft surfaces were polished, from 2003 the whole basal implant was produced with polished surface, as polished surfaces show no tendency to inflammation, and in case of sterile loosening, reintegration of the implant was possible if the load was adjusted in time. The design was developed to

leave enough elasticity for the development and functional stimulation of bone.⁶

Types:

Basal implants are of two types: Basal osseointegrated and Basal cortical screw type implants. Despite acceptable success rates, these approaches involve unpredictable degree of morbidity at the donor and recipient sites and poor prognosis. The basal implants were specifically designed to utilize strong cortical bone of the jaw without risk of infection.²

Advantages of Basal implant

- Single/ monobloc unit
- Utilizes basal cortical bone for support.
- Efficiently used in atrophic and compromised bone conditions.
- Better distribution of masticatory forces.
- Lesser peri-implantitis evidence.
- Better results in medically compromised patients like Diabetics or patients having chronic periodontitis.³

Disadvantages of conventional implant

- Requires large amount of bone and hence, generally requires bone augmentation surgeries which increases the cost and time of surgery.
- Mostly placed into poor density spongy bone which cannot be loaded immediately therefore requires healing time of 3-8 months approximately.
- Has a screw connection which may lead to future screw loosening/ breakage under the prosthesis.
- Sensitive infection due to its rough surface area and vertical path of load distribution.
- Maximum load/ stress are over the crestal bone which results in crestal bone loss.
- Wider neck diameter makes it difficult for soft tissue re-epithelisation.^{3,8}

Indications of Basal Implant

- In situations when multiple teeth are missing or have to be extracted.
- When a bone augmentation procedure has failed.
- Cases of thin ridges – That is deficiency of bone in buccolingual thickness.
- Cases where bone height is insufficient.³

Contraindications of Basal Implants

- Medical conditions like recent myocardial infarction, cerebrovascular stroke, immunosuppression.
- Patients on chemotherapy and antiplatelets.⁸

Basal Implants for Atrophied Ridges

- Restoring atrophied ridges poses a challenge for the prosthodontist. Restoration of such cases with basal implantology exclude the need for extensive surgeries. Before restoring atrophied maxilla and mandible following points must be considered:

1. General Systemic Conditions

- The patient should not have recent myocardial infarction, cerebrovascular accident, immunosuppressant therapy, chemo and/or radiotherapy and bisphosphonate therapy. Diabetes and smoking is not a huge concern.^{3,6}

2. Biomechanical Considerations

- Bone is a visco-elastic structure and so is this implant, therefore, the phenomena of stress shielding is avoided.⁶

3. Loading 4, 15, 16

- According to basal implantology the cranial bone is permanently in a state of torsion, i.e.; there are constant lateral stresses being applied to the cranial bone at all times due to action of the attached facial muscles, therefore, there is no such thing as an “unloaded” implant as lateral forces will always exist no matter the implant receives a superstructure or not. Considering this phenomena, basal implants can either be left without a superstructure till completion of the healing phase or they can receive a superstructure immediately, after 3 days, 1 week, 6-8 weeks, or temporary restoration can be done for 3-6 months followed by definitive restoration.^{6,9,10}

4. Choice of restoration between both jaws

- The stomatognathic system consists of stationery (maxillary bone) which absorbs a considerable amount of the forces applied and a mobile (mandibular bone) component, the role of which is to apply forces and the stationery component. Due to this it become important that the mandible should be restored first, also a conventional mandibular denture on an atrophied foundation is unstable, therefore, chewing function becomes poor and gradually the associated muscles lose their tonicity, because of fixed rehabilitation these adversities are avoided, thus, mandible should be restored first.⁶

5. Treatment modalities of Atrophied Ridges

a. Atrophied Mandible-

Over the years two schools of thought have developed regarding implant restorations in atrophied mandible, they are-

i. Multi-Implant Concept of French School

- Propagated and founded by Scortecchi this school favors a large number of basal implants in the mandible mostly around 7-12 implants. According

to this school basal and crestal implants are combined to result in a restoration that is so rigid that it does not permit any torsion across the mandible also this does not allow the jaw system to reorient forces. Since, it is almost impossible to stop mandibular torsion, there is generation of excessive forces on the implant body which leads to overload osteolysis and causes implant failure.^{5,6}

ii. Strategic Implant Positioning Concept of German School

- This school was founded by Dr. Ihde. According to this school 4 implants are placed in the mandible preferably in the canine and second molar regions this allows for mandibular torsion and reorientation of forces which gets compensated by flexibility of the prosthesis, thus, overload osteolysis and implant failure is avoided.^{6,9}

b. Atrophied Maxilla^{5,6,11}

- The pneumatized sinus and the porous bone make implant placement a challenging task. The porous bone is taken care of by the compression screw implants, whereas, for the sinus two techniques have been described, which describe alternate techniques of placement-

i. Sinus Section Technique- In this two/three walls of the sinus are sectioned to facilitate placement of the basal disk in the sinus. Basal implantologists leave the option of lifting the sinus membrane and grafting on the operator. The sole purpose of this technique is to gain bi-cortical support; also only one implant can be placed this way in each sinus.

ii. Tuberopterygoid Screws- These implants are placed in the pterygoid bone and aid in providing additional support to the prosthesis. These are used in conjunct with Sinus Section technique and are placed at 20°-45° in the bone and the angulation between Basal osseointegrated implant and **Tuberopterygoid** screw should not exceed 90° otherwise prosthesis placement becomes difficult.

iii. Zygomatic Screw Implant- These are zygomatic implants that are placed in the zygomatic bone and they also have sharp edged cortical screws that gain bi-cortical support.

c. Cortically Fixed -This is introduced by Dr. Henri Diederich in 2013; this protocol is based on basal cortical implantology and is specifically aimed at rehabilitating atrophied jaws irrespective of the amount of bone available without any need for augmentations. This is basically a plate form implant, which looks like mini plates with an abutment platform, this unique design allows them to be bent and adapt to any surface and is anchored to bone using bone expanding mini screws. The number of holes required can be reduced; another

advantage is their isoelasticity enabling them to mimic bone.^{12,13}

Complications

- Functional overload osteolysis is one of the complications of basal implants.
- As long as the bone substance is not torn away from the implant and the area is not superinfected, the loss of mineralization remains diffuse but usually reversible. Basal implants in this status have a good chance of getting reintegrated at a high degree of mineralization, if loads are reduced to an adequate amount.⁴

Conclusion

Basal implants can be used for patients with atrophic ridges which can be restored without any extra surgical interventions like bone augmentations thus, reducing the time and cost of the treatment plan and provide immediate loading and also they can be placed with a flapless technique and can be combined with any implant.

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Review Article

Lasers in Prosthodontics: A Review

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ABSTRACT:

Lasers have a wide range of applications in the field of dentistry and also became a boon to dentists from all over the world. Lasers are being used for general surgical procedures since long time but now it was introduced in dentistry to overcome the difficulties that occur due to conventional methods. Interaction of lasers to soft tissue allow bloodless surgery, least post-operative pain and scarring; since the laser incision is more broad and irregular so it blends better with the surrounding tissue while healing. The introduction of the lasers to the specialties of dentistry like Prosthodontics, Surgery, Periodontics and Endodontics has brought a revolution in the treatment delivery with increased precision of procedures and comfort for the patients. Lasers for hard tissues encourage efficient diagnosis of caries and improve the resistance of dental enamel to caries, etching of enamel, cavity preparations, photopolymerization of composite resin and sterilization of the root canal system. This review article describe the uses of lasers in Prosthodontics for treatment of Fixed And Removable Prosthesis, Implantology.

Keywords: Lasers, Prosthodontics, Implantology.

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INTRODUCTION:

The word LASER “Light Amplification by Stimulated Emission of Radiation” was used for the first time by an American Physicist, Gordon Gould in 1957 while recording his ideas with a title “Some rough calculations on the feasibility of a LASER”.¹⁻³ After years of work on lasers Miaman foresaw the use of the device for bloodless surgical tool for treatment of cancers and as dental equipment.⁴ A laser is a device that changes electrical or chemical energy into a very fine, intense beam of light energy that alters light of several frequencies into an

intense, small, and nearly non-divergent beam of monochromatic radiation, within the visible range.⁵ Two major types of lasers were introduced in terms of clinical applications; hard lasers such as carbon dioxide (CO₂), neodymium–yttrium aluminum garnet), and erbium–yttrium aluminum garnet (Er:YAG) with both hard and soft tissue usages. Because of high cost and a potential for thermal tissue damage, hard lasers have some limitations.^{6,7} On the other hand, soft or cold lasers have been predominantly used for biostimulation or low level laser therapy (LLLT).⁸ Lasers are used in various disciplines in

dentistry such as restorative dentistry where they are used for diagnosis of caries, improving the resistance of dental enamel, and photopolymerization of composite resin;^{9,10} endodontics for bactericidal cleansing of root canal;¹¹ periodontics for gingivectomy, gingivoplasty, frenectomy, and vestibuloplasty;¹² pedodontics to prepare tooth surfaces for sealant application;¹³ and oral and maxillofacial surgery to treat vascular malformation.^{14,15} Dental lasers are classified with regard to the lasting medium used such as gas laser or solid laser, application in different tissues such as soft tissue or hard tissue lasers, the range of wavelength, and the risk of laser usage.¹⁶ This review article describes the uses of lasers in Prosthodontics for treatment of Fixed And Removable Prosthesis, Implantology.

LASERS IN PROSTHODONTICS:

Lasers in prosthodontics can be used in fixed or removable prosthesis, implantology and in case of maxillofacial rehabilitation.

LASERS IN IMPLANTOLOGY:

Implantology: Dental lasers can be used in implantology in procedures like implant recovery, implant site preparation and removal of diseased tissue around the implant.

1. **Implant recovery:** Use of lasers in implantology is that because the implant can be exposed, impressions can be taken immediately after second stage surgery because there is little blood contamination in the field. There also is minimal tissue shrinkage after laser surgery, which will ensure that the tissue margins will remain at the same level after healing as they are immediately after the surgery.^{17,18}
2. **Implant site preparation:** Lasers can be used for the placement of mini implants in cases of patients with potential bleeding problems, to make bloodless surgery in the bone.¹⁸
3. **Removal of diseased tissue around the implant:** Lasers can be used. The Diode lasers alone or with CO₂ & Er: YAG lasers can be used to restore implants by sterilizing their surfaces with laser energy by removing granulation tissue in case there is inflammation around an Osseointegrated implant.^{18,19}

REMOVABLE PROSTHETICS

Lasers may now be used to perform most pre-prosthetic surgeries. These methods involve hard and soft tissue tuberosity reduction, torus removal, and treatment of inappropriate residual ridges involving undercut and irregularly resorbed ridges, treatment of unsupported soft tissues, and hard and soft tissue malformation. Lasers may

be used to treat the problem of hyperplastic tissue and nicotinic stomatitis under the palate of a full or partial denture and ease the irritation of epulis, denture stomatitis, and other problems related with long term wear of ill-fitting dentures.

1. **Treatment of unsuitable alveolar ridges:** To remove sharp bony projections and to smooth the residual ridge soft tissue lasers surgery to uncover the bone may be produced with any number of soft tissue wavelengths (CO₂, diode, Nd:YAG,) Hard tissue surgery can be produced with the erbium family of wavelengths.²⁰
2. **Treatment of undercut alveolar ridges:** Osseous surgery may be performed with the erbium family of lasers.²⁰
3. **Treatment of enlarged tuberosity:** The expanded soft tissue can be reduced with any of the soft tissue lasers. Erbium laser is the laser of choice for the osseous reduction.²⁰
4. **Surgical treatment of tori and exostoses:** Soft tissue lasers may be used to expose the exostoses and erbium lasers may be used for the osseous reduction.²⁰

Laser can be used as an adjunct to removable prosthetic care for many different procedures, including the following:

1. **Epulis fissuratum reduction:** Many researchers have reported the usage of CO₂ lasers in treatment of epulis fissuratum with much more satisfactory results than conventional scalpel.²¹⁻²⁴
2. **Vestibuloplasty:** Neckel has found that there is less post-operative pain in patients treated with lasers for vestibuloplasty. Many other researchers have reported the same results of less post-operative pain and no relapse without any complications.²¹⁻²²

USE OF LASERS IN FIXED PARTIAL DENTURE PROSTHODONTICS:

1. **Crown lengthening:** It refers to the surgical exposure of longer gingivoincisor length. It can be done by excising either soft tissue or hard tissue or at times both. Erbium lasers provide to be a master tool for bone removal without raising a flap.²⁷⁻²⁸ Lasers have an advantage in crown lengthening regard as they cut only at the tip and can be held parallel to long axis of the tooth to remove bone immediately adjacent to cementum without damaging it. Also, using lasers is less complicated and achieves maximum patient comfort.²⁹
2. **Soft tissue management around abutments:** Argon laser energy provides excellent haemostasis and efficient coagulation and vaporization of oral tissues. These characteristics are helpful in retraction and haemostasis of the gingival tissue in



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- preparation for an impression during a crown and bridge procedure.³⁰
3. **Modification of soft tissue around laminates:** The removal and re-contouring of gingival tissues around laminates can be easily done with the argon laser.³⁰
 4. **Osseous crown lengthening:** The water content and hydroxyapatite are responsible for the high absorption of the Er: YAG laser light in the bone. Er: YAG laser has very promising potential for bone ablation.³
 5. **Laser troughing:** Lasers can be used to create a trough around a tooth before impression taking. This can entirely replace the need for retraction cord, electrocautery, and the use of haemostatic agents.³⁰
 6. **Bleaching:** Bleaching using diode lasers results in immediate shade change and less tooth sensitivity and is preferred among in office bleaching systems.³¹
 7. **Veneer removal :** Lasers like Er:YAG and Er Cr:YSGG can be used remove failed veneers.³²
 8. **Crown fractures at the gingival margins:** Er:YAG or Er, Cr:YSGG lasers can be used to allow correct exposure of the fracture margin.³³
 9. **Formation of ovate pontic sites:.** This is easily formed with the use of an laser.³⁴
 10. **Pigmented Gingiva:** Diode, Nd:YAG, CO₂ and erbium lasers prove to be usage of choice for depigmentation procedures.³⁵⁻³⁹

LASERS IN MAXILLOFACIAL REHABILITATION

The use of lasers in the maxillofacial prosthetics is mainly for the initial work up of three dimensional attainment of optical data of the extraoral defects. Laser technology has proved to be particularly helpful for planning the shape and position of the prostheses. With the help of lasers the need for conventional impression techniques and associated disadvantages like deformation of the soft tissue and discomfort to patients can be eliminated. Lasers have advantage of 3D CT and MRI reconstruction as the patient is not exposed to considerable radiation and any stress.³²

LASER APPLICATIONS IN THE DENTAL LABORATORY

Lasers are used for deposition of hydroxyapatite (HA) thin films on titanium implants. Pulsed laser deposition (PLD) has proven to be a sure method to produce pure, crystalline and adherent HA coatings which show no dissolution in a simulated body fluid.³²

Lasers can be used for surface treatment of titanium castings for ceramic bonding and have shown improved bond strength when compared to acid etching techniques. Lasers can also be used for welding.⁴⁰

CONCLUSION:

Lasers become the technology that replaced the conventional methods. Advances in the use of laser devices in prosthodontics will continue. To use lasers safely in a clinic, the practitioner should have precise knowledge of each laser system & their applications, their uses and their adverse effects. The risks can increase in magnitude due to lack of knowledge about lasers.

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Review Article

Provisional Restoration in Prosthodontics: A Review

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ABSTRACT:

Provisional restorations are an essential phase in the treatment procedure for fixed provisional restoration. Provisional restorations are designed in order to protect oral structures and promote function and esthetics for a limited period of time, after which they are to be replaced by a definite prosthesis. Provisional restorations are important during the rehabilitation process, knowledge of the mechanical properties of the available materials allows us to predict their clinical performance. These materials should not only satisfy the mechanical requirements such as strength and resistance to wear but also meet the biologic and esthetic demands. The present review article describes the different materials used for these provisional restorations and also the techniques used to fabricate them along with their advantages and disadvantages.

Key words: Restoration, Rehabilitation, Prosthesis.

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INTRODUCTION:

Provisional or interim restorations are commonly used in dentistry during the time between tooth preparation and placement of the definitive restoration.¹ The provisional restorations have become a valuable tool for esthetic and functional diagnosis in dentistry. Dentists can gain their patients' confidence by handling this intermediate stage of treatment successfully, achieving the necessary predictability for a successful final restoration.² According to Shillingburg, the restorations should:

1. Proper pulp protection
2. To prevent supraeruption or tipping of the teeth
3. Serve proper occlusal function for the patient
4. Can be easily maintainable in a hygienic condition
5. The material should withstand occlusal forces and should be retentive
6. Be aesthetically pleasing and can be polished so as to prevent plaque accumulation
7. Margins should not intrude the gingival tissues and induce gingival pathosis.³

Fixed prosthodontic treatment, whether involving complete or partial coverage, natural tooth or dental

implant abutments, commonly relies on indirect fabrication of a definitive prosthesis in the dental laboratory. Fabrication of this definitive prosthesis, on an average takes about 7-10 days during which the prepared tooth need to be protected from the oral environment and also its relationship with the adjacent and opposing tooth need to be maintained. Thus, in order to protect these prepared abutment teeth, provisional restorations are fabricated and the process is called as Temporization.⁴

Requirements and concepts of provisional crowns:

The requirements can be biological, mechanical and esthetic requirements. The provisional crown protects the pulp from thermal and chemical insults after crown preparation and enamel removal.⁵ It serves to maintain gingival health and contour while providing for an esthetic and/or functional interim restoration.⁶ Provisional crown should also be easy to clean and not impinge on the tissues. Most importantly maintains interocclusal and intra-arch tooth relationships.⁷⁻⁹ Finally they should exhibit a good shade match and have a highly polished surface so that they are esthetically pleasing to the patient. All these factors are extremely important to the success or failure of treatment outcomes. Besides the

immediate protective, functional, and stabilizing value, interim restorations are useful for diagnostic purposes where the functional, occlusal and esthetic parameters are developed to identify an optimum treatment outcome before the completion of definitive procedures.¹⁰

Materials

Materials used to fabricate provisional restorations can be classified as acrylics or resin composites. Several types of acrylic resin materials are available for interim restorative treatment like polymethyl methacrylate resins, polyethyl methacrylate resins or combinations of unfilled methacrylate resins.¹¹

Acrylics:

These materials have been used to fabricate provisional restorations since the 1930s and usually available as powder and liquid. They are the most commonly used materials today for both single-unit and multiple-unit restorations.

The advantages are due to their low cost, acceptable esthetics, and versatility. The disadvantages include they produce acceptable short-term provisionals but tend to discolour over time, other disadvantages include an objectionable odour, significant shrinkage and heat generation during setting.

The three types of acrylics are polymethyl methacrylates, poly-R' methacrylates and epimines. Polymethyl methacrylates are commercially available as Jet (Lang), Alike (GC America), Temporary Bridge Resin (Dentsply/Caulk), Neopar (SDS/Kerr), and Duralay (Reliance). Advantages of this material include low cost, good wear resistance, good esthetics, high polishability, good colour stability whereas it also has certain drawbacks like significant amount of heat given off by exothermic reaction, high degree of shrinkage, objectionable odour, short working time, hard to repair and radiolucent. Plant et al. found that the intra-pulpal temperature rise associated with the polymerization of methyl methacrylate materials could be up to five times that associated with the normal consumption of thermally hot liquid.¹²

Poly-R' Methacrylates (R' = ethyl, vinyl, isobutyl) are commercially available as Snap (a polyethyl methacrylate from Parkell), Splintline (a polyethyl methacrylate from Lang), Trim II (a polyvinyl methacrylate from Bosworth), Provisional C&B Resin (a polyvinyl methacrylate from Cadco), and Temp Plus (a polyisobutyl methacrylate from Ellman). Despite various advantages like low cost, less heat given off during reaction, less shrinkage than polymethyl methacrylates and extended working time, Poly-R' Methacrylates have certain disadvantages which include less esthetic than other currently marketed materials, eugenol deteriorates the resin, poor wear resistance, poor colour stability, objectionable odour, hard to repair and radiolucent.

Epimines were the first two-paste acrylics, commercially introduced in 1968 as Scutan (ESPE). Although Scutan had relatively low shrinkage, heat production and lowest

pulpal irritability, it was weak and could not be altered or repaired.

Composites:

Composite provisional materials encompass a fairly variable category by virtue of the fact that they are chemically comprised of a combination of 2 or more types of materials. Most of these materials use bis-acryl resin, a hydrophobic material that is similar to bis-GMA. Composites are available as auto-polymerized, dualpolymerized and visible light polymerized. Bis-acryl provisional materials are resin composites and represent an improvement over the acrylics because they shrink less, give off less heat during setting, excellent esthetics, minimal odor and can be polished at chair-side. These products are provided in cartridges for use in an automix dispenser gun. Commercially available bis-acryl auto polymerized composite include Bis jet, Integrity Luxatemp, Protemp II, Protemp Garant, Protemp IV, Provitec, Smar Temp, Tempase, Turbotemp and Ultra Trim. Commercially available Bis-acryl composite (Dual-polymerized) are Iso temp, Luxatemp solar, Luxa-flow and Provipont DC. Urethane dimethacrylate composite, Visible lightpolymerized is available as Triad Bis-acryl materials are compatible with other composite materials, but alterations for repairs and addition are difficult.¹³ Koumjian and Nimmo showed an 85% decrease in transverse strength after repair of a bis-acryl material. They suggested that it might be more advantageous to make a new provisional restoration than repair this material.¹³

Preformed Crowns :

Preformed provisional crowns or matrices usually consist of tooth-shaped shells of plastic, cellulose acetate or metal. They are commercially available in various tooth sizes and are usually selected for a particular tooth anatomy. Nonetheless, available sizes and contours are finite which makes the selection process important for clinical success. They are commonly relined with acrylic resin to provide a more custom fit before cementation, but the plastic and metal crown shells can also be cemented directly onto prepared teeth. Compared with custom fabricated restorations, this treatment can result in improper fit, contour, or occlusal contact for a provisional restoration.^{14,15} Polycarbonate resin is commonly used for preformed crowns. These crowns combine micro-glass fibers with a polycarbonate plastic material. These serve as matrix material around a prepared tooth that is relined with acrylic resin to customize the fit.¹⁶ Polycarbonate resin is the commonly used for preformed crowns. These crowns combine microglass fibres with a polycarbonate plastic material.¹⁴ This material possesses high impact strength, abrasion resistance, hardness, and a good bond with methyl-methacrylate resin.¹⁶ Metal provisional materials are generally esthetically limited to posterior restorations. Aluminium shells provide quick tooth adaptation due to the softness and ductility of the material, but this same positive quality can also promote rapid wear that results in perforation.¹⁴

Techniques of fabrication

1. Indirect Provisional Fixed Partial Denture

The technique involves fabrication of the interim restoration outside the mouth. Fabrication of provisional restorations using the indirect technique eliminates the problems associated with the direct technique and also has the advantage that it can be partially delegated to auxiliary personnel.¹⁷ Fisher et al. describes the use of an indirect technique for provisional fabrication that uses a fast-setting plaster. The technique has several advantages over the direct procedures. There is no contact of free monomer with the prepared teeth or gingival which might cause tissue damage and an allergic reaction or sensitization. The technique avoids subjecting prepared tooth to the heat evolved from the polymerizing resin. Indirect technique produces restoration with a superior marginal fit and as an auxiliary is involved in fabricating the restoration in the lab, it frees the patient and dentist for considerable amount of time.^{18,19} Principal disadvantage of the technique includes increased chair side time and increased number of intermediate steps. It is a tedious task to perform if there is inadequacy of assistants or the laboratory facilities. In addition, the technique involves use and possible damage of diagnostic casts.²⁰

Procedure:

1. On the diagnostic cast, place a selected acrylic tooth on the area of the missing tooth, and seal it with the carding wax.
2. Following this, a silicone putty index is made involving at least one tooth each beyond the abutment teeth.
3. Prepare the patient's teeth in the usual manner.
4. Make a sectional impression of the prepared teeth and the adjacent structures and pour a check cast.
5. Lubricate the check cast with a petroleum jelly or any suitable separating media, mix the provisional restorative material, and place it in the tissue surface of the index and seat it on the check cast.
6. Try in the preformed restoration for its fit on the cast and intraorally.
7. Reline the temporary restoration to perfect the internal fit.
8. Finish, polish, and cement the restoration

Indirect-Direct Provisional Fixed Partial Denture

The technique produces a custom made preformed external surface form of the restoration but the internal tissue surface form if formed by the underprepared diagnostic casts. This indirect-direct procedure has several advantages. With the combination indirect-direct technique, chair time can be reduced, since the provisional shell is fabricated before the patient's appointment. Enhanced control over restoration contours minimizes the time required for chair side adjustments. In addition, a smaller amount of acrylic resin will polymerize in contact with the prepared abutment,

resulting in decreased heat generation, chemical exposure, and polymerization shrinkage compared to the direct technique.¹⁹ Another advantage is that contact between resin monomer and soft tissues is reduced and less chances of allergic reactions. The disadvantage of this procedure is the potential need of a laboratory phase before tooth preparation and the adjustments that are frequently needed to seat the shell completely on the prepared tooth.

Procedure:

1. Pour an accurate pretreatment diagnostic cast from an impression of the unprepared teeth. For FPDs, wax a pontic into the edentulous area of the study cast, and modify with wax to obtain ideal contours, contacts, and occlusion.
2. Lightly lubricate the modified diagnostic cast, and make an impression using a high-viscosity elastomeric impression material. To provide an adequate bulk of material at the margins of the provisional, trim the sharp edge on the elastomeric over impression that represents the gingival crevice with a round bur to allow for extra bulk of resin material in this area. The silicone putty index is made involving at least on tooth each beyond the abutment teeth.
3. Remove the acrylic tooth and prepare the abutments on mounted diagnostic casts.
4. Lubricate the prepared diagnostic cast with a petroleum jelly or any suitable separating media, mix the provisional restorative material, and place it in the tissue surface of the index and reseat it on the prepared diagnostic casts.
5. After the acrylic resin has polymerized, finish the restoration. The provisional restoration should be paper thin and correctly contoured, and it should precisely follow the gingival margins on the cast.
6. Prepare the patient's teeth in the usual manner (to the gingival margins).
7. Try in the preformed restoration. (If the amount of tooth reduction is adequate, the provisional restoration will show optimal marginal fit with no need for adjustment.)
8. Reline the temporary restoration to perfect the internal fit.
9. Finish, polish, and cement the restoration.^{17,21}

3. Direct Provisional Fixed Partial Denture

In the direct technique, patient's prepared teeth and the gingival tissues directly provide the tissue surface form eliminating all the intermediate laboratory procedures. This is convenient when assistant training and the office laboratory facilities are inadequate for efficiently producing an indirect restoration. However the direct technique has significant disadvantages like potential tissue trauma from the polymerizing resin and inherently poorer marginal fit. Therefore, the routine use of directly formed interim restoration is not recommended when indirect techniques are feasible.

Procedure:

1. Before the tooth preparation, place an acrylic tooth in place of the missing tooth and make an alginate impression or a putty index.
2. Prepare the patient's teeth in the usual manner.
3. Lubricate the prepared teeth and the adjacent gingival margins with petroleum jelly, and reseat the index or the alginate impression with provisional restorative material in the dough stage on the tissue surface of the impression.
4. Remove and reseat the restoration until it sets.
5. Finish, polish, and cement the restoration.
4. Alternative Techniques for Direct Technique

1. Acrylic Resin Block Technique for Direct Provisional Restoration

A useful, though seldom employed, method for making provisional restorations is the acrylic resin block technique. It provides a means of fabricating the interim restoration without the use of diagnostic casts and laboratory processing costs. The technique requires knowledge of dental anatomy and the patience and artistic traits inherent in dentists.

Procedure:

- i. Tooth Preparation is carried out in a usual manner.
- ii. Autopolymerizing acrylic resin of the suitable shade is mixed and allowed to set to a doughy consistency (the sheen of surface-free monomer has completely disappeared). After the abutments and surrounding gingiva have been lightly lubricated with petrolatum, the acrylic resin record is placed over the prepared abutments, and the patient is guided to closure in the centric occlusion position.
- iii. The acrylic resin record is removed and replaced a few times during the curing process to minimize the effect of the exothermic heat on the abutments. After polymerization, the occlusal surface of the resin record is analyzed for anatomic design and may be marked with pencil as to cusp location and buccolingual width to facilitate carving the crown forms.
- iv. Carbide burs and diamond stones are used to roughly develop contour and form of the provisional restoration.
- v. Since no impression matrix is used to carry the acrylic resin mix over the prepared teeth, the initial splint must be relined to assure adequate marginal adaptation and integrity. The inside of the crowns is relieved with a round carbide bur to provide space for the relining acrylic resin. The inner surfaces are moistened with monomer and filled with a fresh mix of acrylic resin. The splint is then replaced over the prepared abutments while the acrylic resin cures. The patient is again guided to closure in the centric occlusion position.

vi. The provisional restoration is carved to correct occlusal anatomy, crown contour, and embrasure form with burs, stones, and discs. This must be done with sufficient care and attention to detail so that it approximates the environment to be established by the final restoration. The provisional splint must be smooth and highly polished.

vii. The completed provisional restoration is now ready for placement with temporary cement. Zinc oxide and eugenol cements should be avoided, as they tend to soften the acrylic resin on contact and may weaken the restoration.²²

2. Before starting to make a crown preparation, an irreversible hydrocolloid impression is made and immediately poured while waiting for the anesthetic to take effect. Following this an acrylic teeth is placed in the missing tooth region on the diagnostic cast and a shell matrix is custom made from mouthguard material. Lubricate the prepared tooth and adjacent teeth. Add just enough tooth-colored acrylic resin to fill only the prepared tooth space in the shell matrix and place the matrix over the teeth in the patient's mouth, pressing down on the adjacent teeth. Wait for the material to set, finish, polish, and cement the restoration.

Alternatively, the restoration can be fabricated in a similar fashion outside the patient's mouth on the master cast after tooth preparation using the custom made shell matrix making it an indirect procedure.²³

3. After contours of badly broken-down teeth are restored with wax, a preliminary alginate impression with a stock dentulous tray is made of the area to be prepared. Preferably, a complete-arch impression is obtained. The borders and septa are trimmed away from the set impression to facilitate reseating in the mouth. If a posterior fixed partial denture is to be made, a strip of irreversible hydrocolloid is removed from the edentulous ridge area to form a pontic in the completed temporary restoration. If an anterior fixed partial denture is to be made, then a denture tooth (or teeth) may be fixed in place with a small piece of soft rope wax prior to fabrication of the impression.

In an another technique, instead of replacing the missing tooth in temporary restoration in posterior quadrants, alginate impression can be scored in the form of a bar running across the edentulous region connecting the abutment teeth, thus producing a final restoration with crowns on the abutment teeth connected by a bar maintaining the integrity of the restoration. Instead of scoring a bar, a reverse pontic can also be scored in the alginate impression.

4. In this technique, after removing the impression tray from the mouth, one should shorten the proximal projections of the impression material, and trim away the excess impression material palatally/lingually and buccally/facially to ensure complete reseating of the tray intraorally. Then, in this preoperative impression, grooves has to be created starting 1 mm buccally and lingually to the margin of the prepared tooth and continue towards the buccal and lingual flange areas to provide a pathway for the excess interim restorative material to escape.²⁴
5. In another technique, a provisional removable partial denture which is often used to replace anterior teeth prior to fixed prosthodontic treatment is used as an aid in making a provisional fixed restoration.


An irreversible hydrocolloid impression of the anterior segment of the provisional removable partial denture is made. Cold-cure acrylic resin of an appropriate shade is poured into the impression or placed into it with the powder-liquid method. The cured resin is removed from the impression as a block section of the anterior teeth and stored in water until needed. When the provisional fixed splint is being made, this block section of pontics is directly attached to the provisional crowns made for the abutment teeth. The block section of pontics may also be helpful if the provisional removable restoration is lost. The abutment teeth can then be prepared, individual provisional abutment crowns made, and the pontic section added. Alternatively, the unprepared abutment teeth can be acid etched and the block section of acrylic pontics directly attached to them with composite resin. Alternatively, in an indirect way, an impression can be made with the existing removable partial denture in place, and this impression may be used to make the temporary restoration by placing it on the master cast that would be made after the tooth preparation.

6. In any of these techniques, instead of building up the entire tooth with autopolymerizing resin, the acrylic tooth can be trimmed in the form of a labial veneer and the rest of the tooth built up with autopolymerizing resin. This tooth that has been trimmed in the shape of a veneer can be either used directly in the patient's mouth and rest of the tooth built up or can be used indirectly on a cast.
7. Using the existing prosthesis as a provisional restoration: when a cemented fixed prosthesis is to be removed for the reason of remaking it, damage to the prosthesis is of little concern. The important principle in such a case is to remove the prosthesis with minimum risk to the natural abutment teeth. It is possible to remove a cemented fixed prosthesis with little or no risk of damage to the abutment teeth by sectioning the prosthesis and expanding the retainer. Once removed, the prosthesis can be rebuilt to be used as a provisional or temporary prosthesis. The

advantages of using the existing prosthesis are that the long-span fixed partial denture is stronger with metal reinforcement; the prosthesis incurs less occlusal wear with a metal or porcelain restoration versus an acrylic restoration; less time is required for fabricating a temporary restoration.²⁵

Alternatively, an impression of the existing fixed partial denture may be made before attempting its removal, and this impression may be used to make the temporary restoration by placing it on the master cast that would be made after the tooth preparation.

8. In cases the patient presents with tooth preparation already being done and without a temporary, the following measures may be undertaken.
 - a. To build up the prepared tooth with the carding wax and place an acrylic tooth in the area of missing tooth and take an impression and use it to fabricate the temporary restoration.
 - b. To make the impression of the prepared tooth as it is and then score the impression in the form of reverse pontic and also in the area of the prepared tooth in an attempt to duplicate the unprepared tooth.
 - c. To use acrylic resin block technique forming direct provisional restoration.
9. Provisional restoration for post and core restorations:
 - i. If custom made post and core is to be used, the post and core portion can be instantly built and temporary crown be fabricated on it.
 - ii. If cast post is to be placed in the final restoration, the following measures may be taken.
 - a. A ball pin may be placed into the post space and an alginate over impression made that would pick up the ball pin and then the restoration fabricated on the cast.
 - b. Instead of placing the ball pin directly into the post space, it may be placed into the impression and the restoration fabricated.
 - c. In an alternative technique, a ball pin may be placed into the post space and the restoration fabricated intraorally using acrylic resin block technique. A tooth trimmed in the form of a labial veneer can also be used to serve the purpose.


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Conclusion:

One of the most important aspects of dental profession is to provide a predictable outcome to any oral rehabilitation, and the use of the provisional restoration is a critical phase in the treatment. Making adequate provisional restorations requires significant time and effort. Provisional restorations should be delegated to qualified dental assistants to reduce the overhead cost of producing crowns and fixed prostheses. The future research activities will need to focus on technological advancements to provide improved materials that demonstrate improved biocompatibility, physical properties, ease of use and esthetically pleasing appearance to the patients.

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Review Article

Esthetics in Implant Dentistry: A Review

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ABSTRACT:

The evolution of Implant dentistry occurs over the years. Re-establishment of lost function, esthetics, and harmony of dentition is the prime concern of implantology. Esthetics is very important for the success of implant-supported prostheses. Morphology of the peri-implant soft tissue adjoining the implant components plays a important role in displaying the implant esthetics. The ultimate goal of a creating an implant restoration that cannot be distinguished from the rest of the natural dentition. This article aims to review the various considerations that help the dentists in increasing the esthetics in implants.

Key words: Implant dentistry, Esthetics, natural dentition.

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Introduction:

The success of osseointegration of a dental implant is not enough for treatment success because esthetics of restoration are important for patient satisfaction.^{1,2} Esthetics depend on some factors that should be assessed during diagnosis to reveal and overcome previous limitations of the treatment. After this phase, the implant can be safely positioned for prosthesis insertion according to the biological distance and satisfactory architecture of the peri-implant tissues.³ The ability to preserve the architecture, modify and even improvise the soft tissue contour lie in the hands of the periodontist and this can greatly improve the overall restorative result. Four potential time points can be differentiated for soft/hard tissue are: at the time of implant placement, during healing of the implant, during second stage surgery, and finally at the maintenance phase.⁴ This article aims to review the various considerations that help the dentists in increasing the esthetics in implants.

Treatment planning

When the case diagnosed to be implant compatible one, the next phase is treatment planning. It is basically the primary and most important phase concerned with esthetics. During treatment planning rechecking the following fundamentals are considered essential:

adequate bone volume i.e., horizontal, vertical and contour; optimal implant position i.e., mesiodistal, apicocoronal and buccolingual angulation; stable and healthy peri-implant soft tissues; aesthetic soft tissue contours and ideal emergence profile.⁵

I. Optimal implant positioning

Proper positioning of implant fixture and restoration are important requirements for functionally and esthetically successful implant rehabilitation. The implant insertion occurs in an optimal 3 dimensional position that relates to final restorative phase of treatment.⁶

Mesiodistal position

Spacing is influenced by periodontal width of adjacent teeth although it fails to consider aesthetically important factors like cervical and coronal width of replaced tooth, presence or absence of diastema and necessity of maintaining the interdental papillae.⁷ Now it has been recommended to keep a distance of 2 mm between cervical implant face and natural tooth and greater than 3 mm cervical distance between two implants to minimize the amount of crestal bone loss, better soft tissue fill and proper papilla bone support.⁸ If this distance is compromised there is a chances of resorption of interproximal alveolar crest to the level of implants. This

loss of interproximal bone causes reduction of papillary height, impede the emergence profile, and leads to compromised clinical outcomes.⁹

Buccolingual position

Spray et al had proposed critical bone thickness of 1.8 mm buccolingually to maintain optimum aesthetic outcome.¹⁰

Apico-coronal position

According to Saadoun et al the apicocoronal positioning of implant shoulder is dependent on cervical bone resorption morphology, the diameter of the implant, the size discrepancy between the root and diameter of the implant, the thickness of the marginal gingival and proximal tissues. The implant collar to be located 2 mm apical to the cemento-enamel junction of the adjacent teeth if no gingival recession is present and 3 mm from free gingival margin when there is gingival recession, for proper emergence profile maintenance and better aesthetics.⁸

II. Adequate Bone Volume

The essential conditions to be considered are the ridge height, width and trajectory to the proposed location of the final restoration. Comparable ridge height to adjacent teeth is important in establishment of natural mucogingival architecture. The adequate width of alveolar ridge is judged as 1.5 mm bone on both labial and lingual implant surfaces. Leaving a thin labial bone plate at the time of implant placement may lead to periimplantitis or an unaesthetic metal showing through the gingiva.¹¹

Hard tissue reconstruction

To achieve natural aesthetic results, it is necessary to determine whether adequate bone is available for the planned prosthesis.^{12,13} The choice of the graft material depends on the objective of the surgical procedure. If the objective is to fill an osseous defect then any graft material can be used, but if it is to restore with a living bone^{14,15}, an osseointegrative material like autograft or freeze dried bone must be the choice.¹⁶⁻¹⁸

III. Soft tissue grafting

The lack of crestal soft tissue and an intact papillae, advocates the need for soft tissue grafting. The two common situations requiring adjunctive soft tissue procedures are gingival recession around implants and concave ridge profile caused by thin, deficient gingiva.¹⁹ Soft tissue augmentation procedures using patient's masticatory mucosa (palate) have been routinely performed, to create a new zone of attached keratinized gingiva.^{20,21} Depending on the cause of recession, various surgical procedures such as, double split papillae⁵, lateral sliding pedicle flaps²² and coronally repositioned flaps^{23,24} are used. Soft tissue augmentation procedures are used when a concave rather than a convex profile of gingiva at the implant site is seen after the resolution of swelling, following implant placement.²⁵ Soft tissue augmentation

procedures using epithelial-connective tissue graft²⁶, interpositional connective tissue graft²⁶, roll technique²⁷, double papillae repositioned flap²⁸ etc. can be used for papillary reconstruction.

IV. Emergence profile

Development of proper emergence profile begins after second stage surgery, with placement of a properly contoured provisional restoration. This restoration should facilitate ideal gingival scalloping and papilla formation while creating a natural emergence profile to a great extent²⁹⁻³⁵.

Conclusion:

The biological, functional, esthetic needs of the individual patient are very important for the implant prostheses. The selection of better implant and implant placement techniques help is to achieve a naturally looking and esthetically appealing gingiva and associated structures.

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Original Article

Effect of 17% ethylenediaminetetraacetic acid and 0.2% chitosan on pushout bond strength of biodentine and ProRoot mineral trioxide aggregate: An *in vitro* study

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Abstract

Aim: The purpose of this study was to evaluate the effect of 17% ethylenediaminetetraacetic acid (EDTA) and 0.2% chitosan on pushout bond strength of biodentine and ProRoot mineral trioxide aggregate (MTA).

Materials and Methods: Midroot dentin of single-rooted human canine teeth were sectioned into 2-mm-thick slices horizontally ($n = 60$). The canal space of each dentin slice was enlarged with a 1.3-mm-diameter diamond bur. The samples were divided into two groups ($n = 30$) based on the type of perforation repair material placed, i.e., Biodentine and ProRoot MTA. The samples were wrapped in wet gauge for 10 min, and based on the type of chelating agent used for removal of smear layer, each group is further divided into three subgroups ($n = 10$), to be immersed into saline (control), 17% EDTA and 0.2% chitosan for 30 min, and a wet cotton pellet was placed over each test material. After 48 h of incubation, the dislodgement resistance of the samples was measured using a universal testing machine.

Statistical Analysis: Data were analyzed using one-way analysis of variance and *post hoc* Tukey tests. The level of statistical significance was set at 0.05.

Results: Biodentine showed significantly higher pushout bond strength than ProRoot MTA. Biodentine and ProRoot MTA lost strength when exposed to 0.2% chitosan.

Conclusion: Biodentine showed considerable performance as a perforation repair material than ProRoot MTA even after being exposed to various endodontic chelating agents.

Keywords: Biodentine; chelating agents; mineral trioxide aggregate; pushout bond strength

INTRODUCTION

Perforation is a procedural complication that can occur during endodontic therapy or post space preparation of teeth.^[1] Delayed perforation repair therapy can cause periodontal or endodontic lesions and lateral periodontal abscesses occurring secondary to delayed diagnosis,

which usually prognosticates a high failure risk. Hence, immediate repair of perforation is important to avoid contamination and thereby preventing endodontic failure.^[2]

An ideal endodontic root repair material should be biocompatible, radiopaque, antibacterial, dimensionally stable, easy to manipulate, and unaffected by blood contamination.^[3] It should also remain in place under dislodging forces, such as mechanical loads of occlusion

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Prasanthi, *et al.*: Effect of 17% EDTA and 0.2% chitosan

or the condensation of restorative materials over it. Therefore, the pushout bond strength is an important factor for perforation repair materials.^[4]

Although many perforation repair materials such as amalgam, cavity, composite resin, glass ionomer cement, calcium hydroxide, super EBA, intermediate restorative material, and mineral trioxide aggregate (MTA) have been used, most of these materials show significant shortcomings in one or more of the following areas: solubility, leakage, biocompatibility, handling properties, and moisture incompatibility.^[1] Despite the numerous favorable properties of MTA that support its clinical use when compared with other traditional materials, there are several critical drawbacks of MTA such as the prolonged setting time, difficult handling characteristics, high cost, and potential of discoloration. A variety of new calcium silicate-based materials have been developed recently aiming to improve MTA shortcomings.^[1]

Biodentine is introduced as an alternative to MTA. Biodentine (septodont) is a high-purity calcium silicate-based dental material composed of tricalcium silicate, calcium carbonate, zirconium oxide, and a water-based liquid containing calcium chloride as the setting accelerator and water-reducing agent.^[5]

After repairing the furcal perforation, endodontic treatment should be performed with various irrigants to disinfect the root canal system, including chelating agents for smear layer removal.^[6] During biomechanical preparation, the smear layer is formed over the cut dentinal surfaces.^[7] Various agents, such as sodium hypochlorite, ethylenediaminetetraacetic acid (17% EDTA), mixture of tetracycline acid detergent, and organic acids (e.g., citric acid), have been introduced for smear layer removal.^[7] The alternating use of 17% EDTA and sodium hypochlorite has been recommended for the efficient removal of the smear layer. However, the use of these solutions may cause periapical inflammatory reactions and reduce periapical healing.^[7] 17% EDTA, which is the most commonly used calcium chelator, has inhibited the setting of MTA.^[8] To minimize their harmful effects on periapical tissues, the use of biocompatible solutions is essential.^[7]

Chitosan is a natural polysaccharide that is biocompatible, nontoxic, and having chelating property.^[7] Chitosan is natural polysaccharide obtained by the deacetylation of chitin, but it has limited solubility. Chitosan and chitin do not cause any biological hazard, and both are inexpensive. Chitosan exhibits many biological actions such as antimicrobial, wound healing, mucoadhesive, sustained drug releasing property. Chitosan is used as a chelating agent and as an irrigating solution during endodontic treatment.^[7]

Hence, in this study, smear layer removing agents 17% EDTA and 0.2% chitosan were used. Root canal treatment procedure may cause unavoidable contact of chelating solutions with the repair materials. Thus, the purpose of this *in vitro* study was to evaluate the effect of chelating agents, i.e., 17% EDTA, 0.2% chitosan on the pushout bond strength of biodentine in comparison with ProRoot MTA.

MATERIALS AND METHODS

Freshly extracted single-rooted human canine teeth were selected. The crowns of all teeth were removed, and the midroot dentin was sectioned horizontally into slices with a thickness of 2 mm ($n = 60$) using hard tissue microtome. In each slice, the space of canal was enlarged with a 1.3-mm-diameter diamond bur. The specimens ($n = 60$) were randomly divided into two groups ($n = 30$) and the following test materials were used: Group 1: Biodentine and Group 2: ProRoot MTA. The test materials were incrementally placed into canal spaces of the dentin slices and condensed. Excess material was trimmed from the surface of the samples with a scalpel. Subsequently, the samples were wrapped in wet gauze placed in incubator and allowed to set for 10 min at 37°C with 100% humidity. Setting time of biodentine is 10 min, so it was allowed to set for 10 min. As the initial setting time of ProRoot MTA is 10 min, it was allowed to set for 10 min only. Immediately after incubation, the samples were divided into three subgroups ($n = 10$) to be immersed into saline solution (control), 17% EDTA, 0.2% chitosan. After 30 min of immersion, all samples were removed from the test solutions, rinsed with distilled water, and were placed in incubator at 37°C with 100% humidity for 48 h.

Pushout test

After 48 h, the pushout bond strength values were measured using a universal testing machine (Instron Universal Machine) [Figure 1]. The samples were placed on a base of a metal slab of universal testing machine to allow the free motion of the plunger. The compressive load was applied by exerting a downward pressure on the surface of test material in each sample, with the Instron probe moving at a constant speed of 1 mm/min. The plunger size of 1 mm had a clearance of approximately 0.2 mm from the margin of the dentinal wall to ensure contact only with the test materials. The maximum force applied to materials at the time of dislodgement was recorded in newtons. The pushout bond strength in megapascal (MPa) was calculated by dividing this force (N) by the surface area of the test material where $N/2\pi \times r \times h$, π is the constant 3.14, r is the root canal radius, and h is the thickness of the dentin slice in millimeters. Data were analyzed using one-way analysis of variance and *post hoc* Tukey tests. The level of statistical significance was set at $P < 0.05$.

RESULTS

Table 1 shows the mean values and standard deviations of the pushout bond strength (MPa) of all groups. The lowest pushout bond strength was observed in the ProRoot MTA group ($P < 0.05$). Biodentine displayed a significantly higher resistance to displacement than the ProRoot MTA group. In biodentine, saline (control) group showed the highest pushout bond strength whereas chitosan group showed the least pushout bond strength next to EDTA group. In ProRoot MTA, saline (control) group showed the highest pushout bond strength, whereas chitosan showed the least pushout bond strength next to EDTA group [Figure 2].

DISCUSSION

After repair of the perforation, the success of the endodontic therapy depends on a well-placed coronal restoration as well as the resistance of the repair material to displacement forces happening while undergoing condensation of permanent restorative materials. Thus, the bond strength of the perforation repair materials is an important factor in clinical practice.^[1] To assess the bond strength, the pushout bond test has been shown to be efficient, practical, and reliable.^[1,4]

The presence of smear layer may inhibit or significantly delay the penetration of irrigating solutions, sealers,

and medicaments into the dentinal tubules. There is a controversy regarding the presence and removal of smear layer. It is now generally advocated that the smear layer should be removed prior to the root canal obturation to facilitate better adaptation of the filling material to the root canal wall and to improve adhesion.^[5]

Alternate use of EDTA and NaOCl as retro smear layer removing agents may cause periapical inflammatory reactions at surgical site. Calt *et al.* observed that usage of EDTA for prolonged periods caused excessive tubular and intertubular dentin erosion.^[9] Hence, the use of biocompatible retro smear layer removing agents is essential. Chitosan is a natural polysaccharide that is biocompatible, nontoxic, and having chelating property.^[7]

This study evaluated the pushout bond strength between biodentine and ProRoot MTA after exposure to endodontic chelating agents, i.e., 17% EDTA and 0.2% chitosan. In the present study, among ProRoot MTA groups, saline-treated ProRoot MTA samples resisted dislodgment forces $>17\%$ EDTA-treated ProRoot MTA samples and 0.2% chitosan-treated ProRoot MTA samples. This was in accordance with the results of the study done by Loxely *et al.* who reported that the compressive strength of MTA increased when immersed in saline solution because of the remaining unreacted mineral oxides. These may be solidified after additional supplied hydration and may result in the increased strength of material.^[10]

ProRoot MTA samples treated with 17% EDTA showed lower resistance to dislodgement forces. Lee *et al.* showed the adverse effects of 17% EDTA on calcium silicate-based cement (CSC) hydration.^[11] Yan *et al.* showed that 17% EDTA decreased the bond strength between dentine and CSC.^[12] Nagesh *et al.* reported that sealing ability of MTA with chitosan was less.^[7]

Table 1: Mean pushout bond strength values and standard deviations of all test groups

Groups	Mean	SD	SE	CV
Biodentin-Control	43.46	6.90	2.18	15.87
Biodentin-Chitosan	12.41	4.19	1.32	33.77
Biodentin-EDTA	16.66	4.37	1.38	26.23
MTA-Control	20.37	3.31	1.05	16.27
MTA-Chitosan	11.20	3.35	1.06	29.89
MTA-EDTA	15.94	1.98	0.63	12.44

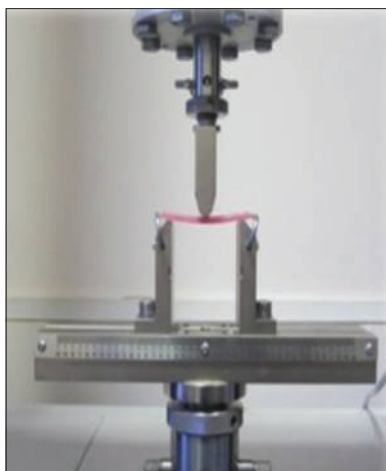


Figure 1: Pushout bond test

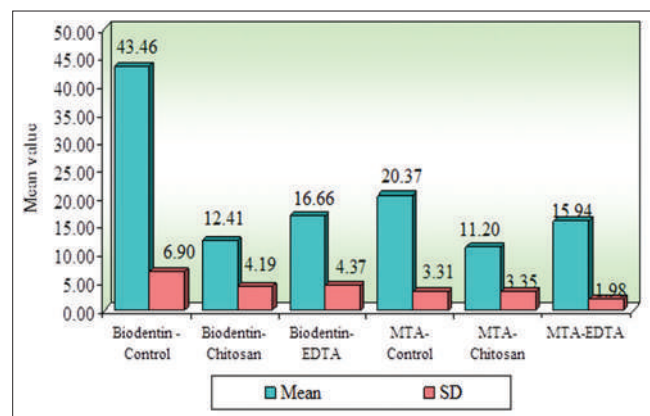


Figure 2: Comparison of six groups (biodentine – control, biodentine–chitosan, biodentine–ethylenediaminetetraacetic acid, mineral trioxide aggregate – control, mineral trioxide aggregate – chitosan and mineral trioxide aggregate – ethylenediaminetetraacetic acid)

Prasanthi, *et al.*: Effect of 17% EDTA and 0.2% chitosan

Biodentine has a similar composition to MTA, differing mostly by being aluminum-free and having tantalum oxide as a radiopacifier in place of the bismuth oxide. This is claimed to be associated with improved biological property.^[2] The presence of calcium chloride, and the concordant reduced setting time and contact time, probably provided the high-bond strength in biodentine group.^[1]

Biodentine was more resistant to dislodgement forces than MTA in the present study. The biomineralization ability of biodentine, most likely through the formation of tags, may be the reason of the dislodgement resistance.^[1] The higher bond strength values of biodentine may, in part, result from its smaller particle size, which has the potential to enhance penetration of the cement into the medicament-free dentinal tubules, leading to improved bond strength.^[13] This effect might be further reinforced through the formation of dentinal bridges as a result of crystal growth within the dentinal tubules, leading to increased micromechanical retention (Han and Okiji 2011, Atmeh *et al.* 2012).^[14] Biodentine may have a more prominent biomineralization ability than MTA, as biodentine specimens showed wider Ca and Si rich dentine areas and larger incorporation depths than MTA. This could be because of the amount of Ca and Si dissolution that could be larger in biodentine than in MTA. Chemical analysis of the interfacial dentine layer confirmed increased Ca levels and Ca/P ratios in the biodentine and MTA specimens.^[15] This finding is related to Ca incorporation. As such, the quality of the interfacial dentine layer appeared to be improved. The higher content of calcium-releasing products in biodentine than in MTA may promote to higher biomineralization and higher bond strength.^[16]

Biodentine also displayed a lower resistance to dislodgement after exposure to 17% EDTA and chitosan solutions. Lee *et al.* showed the adverse effects of 17% EDTA on CSC hydration, microhardness, and cell adhesion. In addition, Yan *et al.* showed that EDTA decreased the bond strength between dentine and CSC. Uyanik *et al.* examined the effect of different irrigation regimens on the sealing ability of CSC and showed that using EDTA significantly increases leakage of CSC.^[17] In another study, CSC showed significantly higher leakage when smear layer was removed before placing the material.^[3] This might be because of the reaction between CSC and residual EDTA inside the root canal.^[3] Pushout bond strength is reduced when exposed to chitosan due to more chelation property which interferes with the setting reaction of CSCs.

CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that:

1. The force needed for dislodgement of biodentine from root dentin was significantly higher than ProRoot MTA
2. Endodontic chelating agents influence the resistance to dislodgement of biodentine and ProRoot MTA.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Effect of NaOCl and EDTA Solutions on Topography of ESX, TruShape and ProTaper Gold NiTi Rotary Instruments – An Atomic Force Microscopic Study

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Abstract

Aim: The study was aimed to evaluate the effect of 5.25% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA) Solutions on Surface Topography of ESX, TruShape, and ProTaper Gold nickel-titanium (NiTi) Rotary Instruments using Atomic Force Microscope (AFM).

Materials and Methodology: A total of 27 each of three commercially available brands of endodontic NiTi instruments were analyzed, which were divided into three groups as follows: Group I: ESX (Brasseler, USA) Group II: Tru shape (Dentsply Tulsa, USA) Group III: Pro Taper Gold (Dentsply Tulsa, USA). These files were further divided into three subgroups containing nine files each, no immersion, immersion in 5.25% NaOCl for 5 min, and immersion in 17% EDTA for 5 min. Surface areas along 3 mm sections at the tip of the files (perfect squares of 10 μm \times 10 μm) were analyzed using AFM operating in contact mode under ambient conditions.

Statistical Analysis: Data were analyzed using two-way ANOVA and pairwise comparison of three main groups with respect to Ra and RMS by Tukey's multiple posthoc procedures.

Results: Three-dimensional AFM images of the surface of all the rotary NiTi instruments, including new and those immersed in 5.25% NaOCl and 17% EDTA solutions, revealed topographic irregularities at the nanometric scale. Ra and RMS values of instruments treated with 5.25% NaOCl and 17% EDTA solutions were statistically higher than that of the new ones ($P < 0.05$).

Conclusion: Using AFM for analysis indicated that short-term contact between 5.25% NaOCl and 17% EDTA solutions and NiTi instruments caused alterations in the topography of instruments.

Keywords: Atomic force microscope; nickel-titanium rotary instruments; sodium hypochlorite; surface roughness

INTRODUCTION

Over the past few years, mechanical instrumentation of root canals has been carried out conventionally with the help of carbon steel instruments, which were later replaced by stainless steel instruments due to their poor corrosion resistance. However, stainless steel

instruments have advantages such as better cutting action and superior corrosion resistance, but its modulus of elasticity was found to be high, resulting in the lack of flexibility, which led to the advent of rotary nickel-titanium instruments (NiTi).^[1] In recent years, the popularity of NiTi instruments has increased because of their superior elasticity and resistance to torsional fracture compared to stainless steel hand files. Despite these advantages of NiTi instruments, they were prone to fracture during their clinical performance.^[2,3] Manufacturers of rotary NiTi

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instruments generally claim a single use, but there are many studies that suggest and support the multiple uses of rotary NiTi instruments.^[2]

The NiTi alloys are generally made up of 56% by wt nickel and 44% by wt titanium. Some amount of nickel generally disappears from the surface due to its unstable oxide resulting in the highly irregular long island like structures indicating their selective dissolution. This tendency during mechanical usage and autoclaving cycles results in the unequal dissolution of nickel and titanium, thus leading to an easy predilection for surface irregularities.^[4]

Due to the pseudoelastic nature of these alloys, NiTi instruments are manufactured by the machining process rather than twisting like stainless steel hand files. Grinding of rotary NiTi instruments during the manufacturing process often results in a surface that is irregular, stressed, and showing cracks, milling grooves, pits, and fissures and regions of metal rollover (Alapati *et al.* 2005).^[5] These surface irregularities may act as a nidus for the crack propagation whenever there is an increased fatigue load on the instrument (Kuhn *et al.* 2001).^[6] Usually, endodontic instruments during endodontic treatment are used in the presence of lubricants or irrigants, which are mainly chlorine-containing compounds, which tend to cause pitting corrosion due to the localized attack on passive metals.^[4,7] Although direct evidence regarding the failure due to pitting corrosion and manufacturing defects were not reported, these can alter the fracture mechanisms during their usage leading to instrument separation.^[7,8] The field emission Scanning Electron Microscopic (SEM) images and Energy Dispersive X-Ray Spectroscopy clearly revealed the presence of non-corroded zones and of corroded areas where the localized attack had created pitting and cracks close to the fracture surface on the NiTi instruments. The phenomenon observed may be attributed to galvanic corrosion, also known as “dissimilar metal corrosion.”^[9]

Hence, various developments were made in the metallurgical aspects of NiTi instruments, to improve the properties, which made them more resistant to corrosion and fractures. Therefore, various surface treatment techniques have been tried to create smoother surfaces to enhance the wear resistance of rotary NiTi instruments.^[10]

To date, the surfaces of NiTi rotary instruments have been analyzed using a variety of different techniques. SEM was widely used to evaluate the surface topographic characteristics of different types of materials. Recently, the atomic force microscope (AFM) for imaging the three-dimensional surfaces of different materials has become increasingly popular and has been recommended as a valuable research tool for investigating the topography of various endodontic instruments.^[3,8] This

study involves the testing of three new commercially available NiTi instruments, i.e., ESX (Brasseler, USA), TruShape (TS) (Dentsply Tulsa, USA) and ProTaper Gold (PTG) (Dentsply Tulsa, USA), which were recently introduced with improved mechanical properties and to date, there appear to be no studies that evaluated the effect of 5.25% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA) solutions on the topography of these files. Hence, the aim of this study was to evaluate and compare the effects of 5.25% NaOCl and 17% EDTA solutions on the surface nanostructure of three rotary NiTi instruments with different manufacturing processes using AFM analysis.

MATERIALS AND METHODOLOGY

This study was conducted in the Department of Conservative Dentistry and Endodontics, Sibar Institute of Dental Sciences, Guntur. The purpose of this study was to evaluate the effect of 5.25% NaOCl and 17% EDTA on the surface of three different rotary NiTi instruments. A total of 81 NiTi rotary endodontic files, of 27 each of ESX (Brasseler, USA), TS (Dentsply Tulsa, USA) and PTG (Dentsply Tulsa, USA) rotary instruments were used for this study.

These files were divided into three groups as follows:

- Group I: ESX (Brasseler, USA) rotary NiTi instruments
- Group II: TS (Dentsply Tulsa, USA) rotary NiTi instruments
- Group III: Pro Taper Gold (Dentsply Tulsa, USA) rotary NiTi instruments.

These files were further divided into three subgroups containing nine files each: (a) new un-immersed instruments were kept as controls, (b) instruments immersed in 5.25% NaOCl for 5 min, and (c) instruments immersed in 17% EDTA for 5 min.

With the fine orthodontic pliers, the active part of these instruments, i.e., (apical 3 mm) was separated. Nine active parts from each group were kept as controls, the other nine active parts were immersed in 5.25% NaOCl for 5 min, and another nine active parts were immersed in 17% EDTA for 5 min, respectively. After the stipulated duration of immersion, these samples were dried, and then, un-immersed and immersed instruments were attached to a silicon wafer with the help of rapid setting cyanoacrylate glue.

Image analysis

These samples were subjected to atomic force microscopic (3D standalone, Asylum Research, IIT Mumbai) analysis. For each sample, the surfaces were analyzed on a 3-mm section starting at the tip of the file. The AFM images were recorded in contact mode operation under ambient conditions. Atomic force

microscopy probes (curvature radius <20 nm) mounted on cantilevers (250 μm), with a spring constant of 0.1 Nm², were used. Scanned areas were perfect squares (10 μm × 10 μm). This was possible by visualizing a monitor displaying an image of the specimen at 500x magnification, providing data on the topography of the analyzed area in nanometers (nm). Three-dimensional images (256 lines × 256 lines) were processed with C-AFM (3D standalone, Asylum Research, IIT Mumbai). For comparison, Roughness (Ra) and Root Mean Square (RMS) values were chosen to investigate the surface features of endodontic files. The Ra and RMS values of the scanned surface profiles were recorded [Figure 1]. An increase in Ra and RMS values indicates alterations of NiTi instruments surface caused by the 5.25% NaOCl and 17% EDTA.

Statistical analysis

Comparison of three main groups (ESX, TS, PTG) and three subgroups (Control, NaOCl, EDTA) with respect to Roughness values and RMS values was made by two-way ANOVA, and pairwise comparison of three main groups (ESX, TS, PTG) and three subgroups (control, NaOCl, EDTA) with respect to RMS values and roughness average (Ra) values by Tukey's multiple posthoc procedures. The level of statistical significance was set at $P < 0.05$.

RESULTS

There was a significant difference between the main groups and between the subgroups. Controls of ESX, TS, and PTG instruments had significantly lower Ra and RMS values when compared to instruments immersed in 5.25% NaOCl and 17% EDTA, where values of ESX were highest followed by PTG and least for TS. For instruments

immersed in 5.25% NaOCl for 5 min, RA and RMS values were highest for TS, followed by PTG and least for ESX. For instruments immersed in 17% EDTA for 5 min, RA and RMS values were highest for ESX, followed by PTG and least for TS [Figures 1-3].

DISCUSSION

Over the years, various attempts to enhance the surface characteristics of NiTi instruments were made by manufacturers to minimize or eliminate inherent defects, increase surface hardness, flexibility, and to improve resistance to cyclic fatigue, corrosion, and cutting efficiency of endodontic instruments. Some of these techniques, such as plasma immersion ion implantation, thermal nitridation, cryogenic treatment, and electropolishing, have improved the properties of NiTi instruments.^[10]

Till date, a variety of different techniques have been used to analyze the surface topographic properties, such as SEM. However, SEM produces two-dimensional images of NiTi rotary instruments but cannot directly provide any quantitative data. On the contrary, AFM provides both qualitative and quantitative information by scanning the surface topography.^[3] Therefore, in the present study, topographic surface changes in all NiTi rotary instruments were evaluated using AFM that were immersed in NaOCl and EDTA solution. AFM records the data of samples in digital form as sets of x, y, and z values. These sets can be analyzed with digital software to give all the data pertaining to the examined surface in quantitative form by using vertical topographic parameters. Ra and RMS values of instrument surfaces were analyzed. These values are also known as the quadratic mean, which is a statistical measurement of the magnitude of a variable quantity in nanometers (nm), of the surface topography and the area in square micrometers (μm²).^[11]

In the present study, both of the solutions, i.e., 5.25% NaOCl and 17% EDTA caused significant deterioration of all instrument surfaces resulting in an increase in RMS and Ra values compared to their controls. The surface irregularities of the controls may be due to the industrial process (i.e., manufacturing) with the highest Ra and RMS values recorded for ESX, followed by PTG and lowest for TS. These nanostructure alterations may have a considerable impact on resistance to fracture of endodontic files and probably make them more prone to corrosion.^[8]

The results for instruments immersed in 5.25% NaOCl for 5 min, Ra and RMS values were highest for TS followed by PTG and lowest for ESX. According to Topuz *et al.* (2008) it was shown that immersion in 5.25% NaOCl for 5 min caused localized surface pitting and cracks that modify the integrity and resistance to fracture of NiTi instruments.^[12]

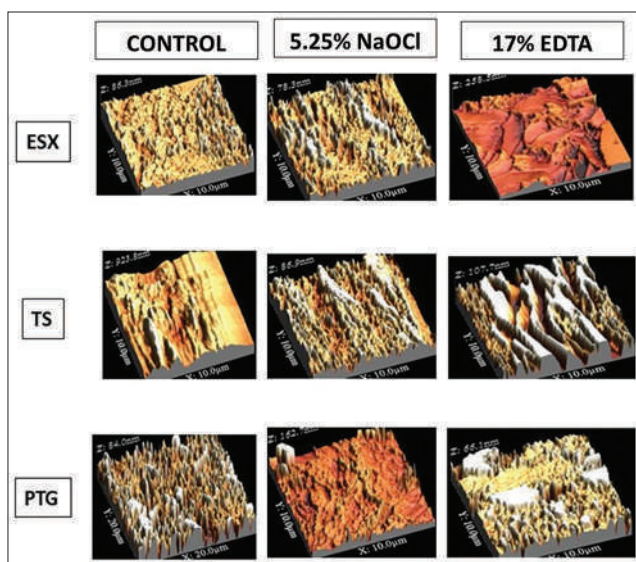


Figure 1: Three Dimensional Atomic Force Microscope Image of New and Immersed ESX, TruShape and ProTaper Gold Nickel-Titanium Rotary Instruments

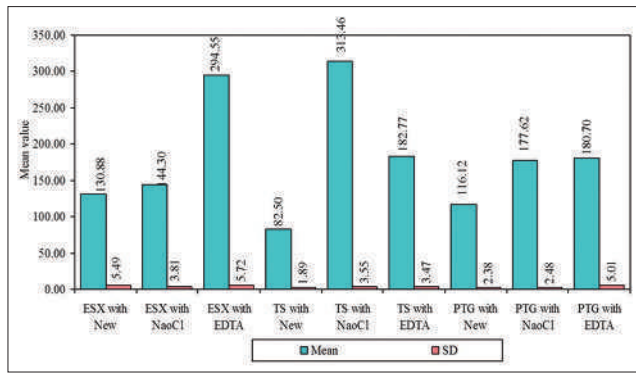


Figure 2: Comparison of three main groups (ESX, TruShape, and ProTaper Gold) and three subgroups (New, 5.25% NaOCl, 17% ethylenediaminetetraacetic acid) with respect to Roughness Values

In a study, conducted by Saglam *et al.* Protaper Rotary NiTi Instruments treated with 2.5% NaOCl demonstrated less surface alterations compared to the group treated with 5% NaOCl and concluded that the lower concentration demonstrated less surface alterations, and the use of 2.5% NaOCl may be recommended to avoid weakening of the ProTaper file.^[7] Sodium Hypochlorite is corrosive to metals involving the selective removal of nickel from the surface, creating micro pitting. It was evident that microstructural defects can lead to areas of stress concentration and crack formation, that weakens the structure of the instrument.

According to a study conducted by Pedulla *et al.* 2014, 5.25% NaOCl did not influence the cyclic fatigue of all instruments tested, instead 17% EDTA reduced the cyclic fatigue resistance of files after 3 min immersion.^[13] According to Haikel *et al.*, neither the mechanical properties of NiTi instruments were affected by NaOCl nor was the cutting efficiency.^[14]

PTG files (Dentsply Tulsa, USA) were developed using gold Wire technology, which is an advanced metallurgical process that makes files significantly more flexible and resistant to cyclic fatigue.^[15] In the present study, PTG showed an increase in Ra and RMS values when exposed to irrigating solutions than controls.

In this study, the ESX file, which was evaluated, had electropolished finish surface. Electropolishing (EP) is a process in which surface titanium of NiTi instruments is oxidized to titanium dioxide (TiO₂), which protects the underlying material from further corrosion.^[16] The electro-polished finish of ESX files did not inhibit the development of micro-fractures on its surface and showed increased RMS and Ra values when immersed in irrigating solutions when compared to the control group. According to an SEM study done by Herold *et al.* Electropolishing did not inhibit the development of microfractures in endosequence rotary instruments.^[17]

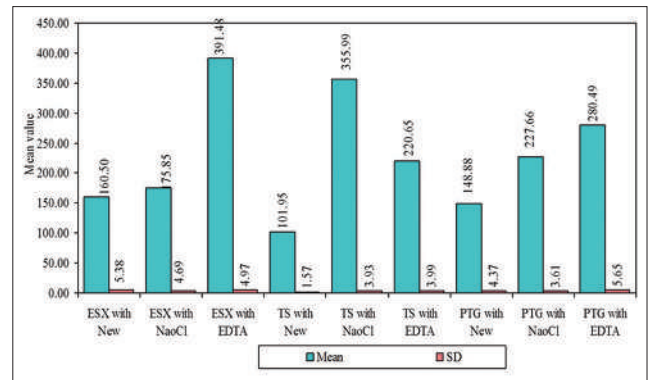


Figure 3: Comparison of three main groups (ESX, TruShape, and ProTaper Gold) and three subgroups (New, 5.25% NaOCl, 17% ethylenediaminetetraacetic acid) with respect to Root Mean Square Values

In the group of instruments immersed in 17% EDTA for 5 min, Ra and RMS values were highest for ESX, followed by PTG and lowest for TS. Interestingly, results for instruments immersed in 17% EDTA for 5 min, RMS and Ra values suggest that EDTA alters the surface of ESX and PTG more than NaOCl except for TS. These differences can be due to the lower pH of EDTA; compared to NaOCl (Bayramoglu *et al.* 2000).^[3,18]

There is not much literature available regarding surface finish and topography of TS files. The lower RMS and Ra values for TS immersed in EDTA could be due to smoother surface finish compared to ESX and PTG which could be explained by Reinhard *et al.* 1992 as that the larger molecules of EDTA, have greater difficulty in concentrating and orienting within small pits to increase the acidity to adequate values that could trigger corrosion.^[19]

The possible limitation of the present study could be the variable time of contact with irrigant, which is more compared to the clinical scenario during cleaning and shaping. On the other hand, we have evaluated topography in a stagnant irrigant solution, but while performing cleaning and shaping, the instrument will be continuously rotating with irrigant solution surrounding it. This situation may result in a different alteration of surface topography and it has to be evaluated in further studies.

CONCLUSION

Within the limitations of this study, short-term contact of 5.25% NaOCl and 17% EDTA solutions with ESX, TS, and PTG Rotary Files showed an increase in their surface roughness values indicating surface deterioration. AFM proved to be a practical method to directly characterize the endodontic file surface, allowing enhanced knowledge of the manufacturing quality of NiTi rotary instruments.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

Scanning electron microscopy evaluation of chitosan and carboxymethyl chitosan as retrograde smear layer removing agents

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Abstract

Background: The smear layer acts as a physical barrier against penetration of root canal medicaments and sealers, thus compromising the seal leading to microleakage.

Objectives: This study was conducted to evaluate the efficacy of 17% ethylenediaminetetraacetic acid (EDTA), 0.2% chitosan solution, and 0.2% carboxymethyl chitosan (CMC) used as smear layer removing agents in retrograde root canal preparation using scanning electron microscopy (SEM).

Materials and Methodology: Eighty single-rooted teeth extracted for periodontal reasons were collected for the study. Root canals were prepared and obturated with gutta-percha coated with AH plus resin sealer. Apical 3 mm of each root resected and Class I retrograde preparation carried out using ultrasonic handpiece and ultrasonic retro tips to a depth of 3 mm along the root long axis. In Group 1 (control), normal saline solution alone was used for smear layer removal. In Group II, 17% EDTA, Group III and IV were treated with 5 ml of 0.2% chitosan and 0.2% carboxyl methyl chitosan, respectively, for 3 min. Blinded evaluation of specimens using SEM was performed independently by two operators who registered the amount of the smear layer present on the surface of the canal walls based on the score described by Hülsmann *et al.*

Results: Group I (saline) was least efficient in the removal of the smear layer. Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC) efficiently removed the smear layer from the retrograde cavity with mean scores 1.35, 1.60, and 1.35, respectively. Statistically, no significant difference found in Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC).

Conclusions: About 0.2% CMC and 0.2% chitosan can be better alternatives to 17% EDTA for smear layer removal due to their biological advantages.

Keywords: Carboxymethyl chitosan; chitosan; ethylenediaminetetraacetic acid; smear layer

INTRODUCTION

When the root canals are instrumented during endodontic therapy, a layer of material composed of dentine, remnants

of pulp tissue and odontoblastic processes, and sometimes bacteria, is formed on the canal walls. This layer has been called the smear layer. It has an amorphous, irregular, and granular appearance under the scanning electron microscope.

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Surgical endodontic treatment should be considered when nonsurgical root canal treatment fails to treat

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periapical infections of endodontic origin.^[1] Apicoectomy and retrograde cavity preparation always associated with smear layer formation on dentinal surfaces. The smear layer consists of the necrotic pulp tissue, inorganic debris, microorganisms, and their by-products.^[2] The presence of such smear layer acts as a physical barrier against penetration of root canal medicaments and sealers, thus compromising the seal leading to microleakage. The smear layer also serves as a reservoir for bacterial proliferation.^[3]

Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), the mixture of tetracycline acid detergent, organic acids (citric acid), have been advocated for the removal of smear layer for both orthograde and retrograde root preparation.^[4] The alternating use of EDTA and NaOCl has been recommended for the efficient removal of the smear layer. However, this combined irrigation regimen causes inadvertent erosion of the intraradicular dentin.^[5] Silva *et al.* proposed that the use of 15% EDTA, 0.2% chitosan, and 10% citric acid effectively removed the smear layer from apical thirds of the root canal.^[6] The use of EDTA has been shown *in vitro* to inhibit the substrate-adherence capacity of macrophages when extruded into the periapical tissues, thus reducing both periapical inflammatory reactions and periapical healing. Hence aiming at minimizing its harmful effect on periapical tissues, the search for more biocompatible solutions than EDTA continues.

Chitosan is a natural copolymer of glucosamine and N-acetylglucosamine produced by the alkaline and the partial deacetylation of chitin from shrimps and crustaceans shells.^[7] Chitosan has a high chelating capacity for various metal ions. It has properties of biocompatibility, bioadhesion, biodegradability, and antimicrobial activity.^[8] Carboxymethyl chitosan (CMC) was introduced to overcome the lower solubility of chitosan.^[9] Hence, this study was evaluated smear layer removing capacity of chitosan and CMC solutions from retrograde cavity preparation.

MATERIALS AND METHODOLOGY

The sample size calculated by keeping the power of the study at 80%, alpha error 5%, effect size 0.92, and mean standard difference (d) is 0.54. The formula used was:

$$n = \frac{2(S^2)}{d^2} (Z_{\alpha/2} + Z_{1-\beta})^2$$

Where, $Z_{1-\alpha/2} = Z$ - value for α level = 1.96 and $Z_{1-\beta/2} = Z$ - value for β level = 1.96

Eighty single-rooted teeth, extracted for various orthodontic and periodontal reasons have been chosen for the study. Teeth were stored in physiologic saline until the sample was completed. Teeth were decoronated at the level of the cemento-enamel junction using a diamond saw. Root canal preparation was completed in a crown-down manner with ProTaper rotary Ni-Ti files (Dentsply Maillefer,

Switzerland) up to F3 size. After each instrumentation, 3 ml of 3% NaOCl (Prime Dental Products Pvt., Ltd., Thane, Maharashtra, India. Lot No. 111130-01) was used for irrigating root canals.

Canals were obturated with 6% gutta-percha coated with AH plus resin sealer. Apical 3 mm of the root was resected using a carbide bur (Zekrya; Maillefer Dentsply, Baillagues, Switzerland) at a plane perpendicular to the long axis of the root. Following resection of the root end, a class I retrograde preparation carried out using ultrasonic handpiece (Mini Piezo; EMS, Nyon, Switzerland) and ProUltra (Maillefer Dentsply) ultrasonic retrotips to a depth of about 3 mm along the root long axis. Debris was initially washed off using a normal saline solution.

Based on the solution being used to treat the retro smear layer, the specimens were categorized into four groups of twenty teeth each. In Group 1 (control), normal saline solution alone was used for smear layer removal. In Group II, 17% EDTA was used for about 30 s. Experimental Groups III and IV were treated with 5 ml of 0.2% chitosan and 0.2% carboxyl methyl chitosan for 3 min. After treating with these agents, the specimens were sectioned longitudinally with the help of hard tissue microtome (Leica SP 1600, Leica Biosystem, Germany) for the scanning electron microscopy (SEM) (Carl Zeiss, Japan: Neon 40) evaluation.

The blind evaluation was performed independently by two operators who registered the presence of the smear layer on the surface of the canal walls based on the following score described by Hülsmann *et al.*^[10] score 1: Dentinal tubules completely open, score 2: More than 50% of dentinal tubules open, score 3: <50% of dentinal tubules open, and score 4: Almost all dentinal tubules are covered with the smear layer. When the scores attributed by the observers were not coincident, the worst score was chosen.

The recorded data were transferred into IBM SPSS software version 20.0 (IBM, Armonk, NY, USA) for the statistical analysis. The data were then analyzed statistically using Kruskal–Wallis ANOVA and pairwise comparison was performed using Mann–Whitney U-test.

RESULTS

The present *in vitro* study evaluated the smear layer removal efficacy of 0.2% chitosan, 0.2% CMC with 17% EDTA from retrograde root canal preparation using scanning electron microscope. Scores were noted from photomicrographs of SEM images, and results were tabulated [Figure 1].

Group I (saline) was least efficient in the removal of the smear layer and most of the specimens were covered with

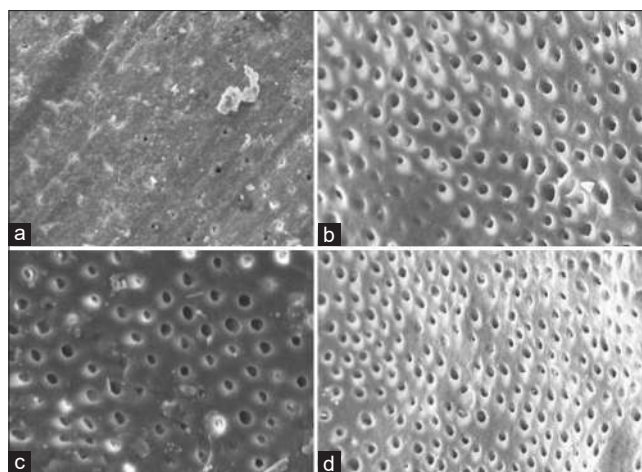


Figure 1: Scanning electron microscope images of remaining smear layer scores at apical level: (a) Saline (b) ethylenediaminetetraacetic acid (c) 0.2% Chitosan, and (d) 0.2% carboxymethyl chitosan visualized at $\times 1000$

of smear layer [Table 1]. Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC), resulted in the efficient removal of the smear layer from the retrograde cavity with mean scores 1.35, 1.60, and 1.35, respectively [Table 1]. Smear layer removal efficacy between groups was compared using Mann–Whitney U–test [Table 2]. Statistically, a significant difference was found in Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC) when compared to Group I (saline) [Table 2]. Group II (17% EDTA) and Group IV (0.2% CMC) equally efficient in the removal of the smear layer followed by Group III (0.2% Chitosan). Statistically, a significant difference was not found between Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC) [Table 2].

DISCUSSION

During apicoectomy and retrograde cavity preparation, a smear layer formed on the prepared root dentinal surfaces. This smear layer contains microorganisms and necrotic pulpal tissues; bacteria can survive and reproduce inside or below the smear layer.

The presence of the smear layer can also inhibit the penetration of intracanal irrigants and medications into the dentinal tubules. Smear layer may also act as a physical barrier between obturating material and the canal wall that results in poor seal leading to microleakage.^[3] Thus, removal of the smear layer from retrograde cavities enhances the adaptation of root-end filling material and potentially eliminates or minimizes microleakage.^[11] EDTA combined with NaOCl is the most commonly used for effective removal of the smear layer from root canals.^[12] EDTA reacts with calcium ions of smear and forms soluble calcium chelate, thereby removes the inorganic component of the smear layer. Crumpton *et al.* reported that the smear

Table 1: Summary of standard error of the mean scores in four groups (saline, ethylenediaminetetraacetic acid, chitosan, and carboxymethyl chitosan)

Materials	Mean	SD	Median	IQR	Mean rank
Saline	3.40	0.50	3.00	0.50	69.9
EDTA	1.35	0.49	1.00	0.50	28.65
Chitosan	1.60	0.68	1.50	0.50	34.8
CMC	1.35	0.49	1.00	0.50	28.65
<i>H</i>			49.4620		
<i>P</i>			<0.001		

EDTA: Ethylenediaminetetraacetic acid, CMC: Carboxymethyl chitosan, SD: Standard deviation, IQR: Interquartile range

Table 2: Pair-wise comparison of four groups (saline, ethylenediaminetetraacetic acid, chitosan and carboxymethyl chitosan) with respect to standard error of the mean scores

Groups	Mean	SD	Median	IQR	Mean rank	<i>U</i>	<i>Z</i>	<i>P</i>
Saline	3.40	0.50	3.00	0.50	30.50			
EDTA	1.35	0.49	1.00	0.50	10.50	0.00	-5.4100	<0.001
Saline	3.40	0.50	3.00	0.50	29.90			
Chitosan	1.60	0.68	1.50	0.50	11.10	12.00	-5.0854	<0.001
Saline	3.40	0.50	3.00	0.50	30.50			
CMC	1.35	0.49	1.00	0.50	10.50	0.00	-5.4100	<0.001
EDTA	1.35	0.49	1.00	0.50	18.65			
Chitosan	1.60	0.68	1.50	0.50	22.35	163.00	-1.0009	0.3169
EDTA	1.35	0.49	1.00	0.50	20.50			
CMC	1.35	0.49	1.00	0.50	20.50	200.00	0.0000	1.0000
Chitosan	1.60	0.68	1.50	0.50	22.35			
CMC	1.35	0.49	1.00	0.50	18.65	163.00	-1.0009	0.3169

$P \leq 0.05$ is statistically significant. EDTA: Ethylenediaminetetraacetic acid, CMC: Carboxymethyl chitosan, SD: Standard deviation, IQR: Interquartile range

layer was removed most effectively by with 1 mL of 17% EDTA for one min.^[13]

However, irrigation with a higher concentration of EDTA for prolonged time results in erosion of dentin and damages periapical tissue when extruded beyond the apex.^[14,15] Drawbacks associated with EDTA led to the search for more biocompatible solutions.

Chitosan is a natural polysaccharide, which has attracted attention in dental research because of its biocompatibility, bioadhesion, biodegradability, and the lack of toxicity.^[16] Chitosan is obtained by acetylation of chitin from crustacean shells.^[17] It has a high chelating ability for various metal ions in acidic conditions and has been applied widely for the removal or recovery of metal ions in different industrial areas.^[17]

Chitosan forms complexes with metal ions through adsorption, ionic exchange, or chelation. The type of reaction depends on metal ions involved and chemical nature and pH of chitosan.^[18,19] Kamble *et al.* reported that 0.2% chitosan removed the smear layer with to greater extent than 17% EDTA.^[20] Silva *et al.* reported that that 15% EDTA, 0.2% chitosan, and 10% citric acid removed the smear layer effectively from apical thirds of the root canal.^[6]

Penumaka, *et al.*: Retrograde smear layer removal capacity of chitosan and CMC

Compared with chitosan, the solubility of CMC in aqueous solution was improved remarkably because of the introduction of the carboxymethyl group. CMC can dissolve in acidic, neutral, or basic aqueous solution when the degree of substitution of carboxymethylation for chitosan is $>60\%$.^[21]

In the current study, the efficacy of chitosan and CMC were investigated using a normal saline solution as negative control and 17%EDTA as positive control. Statistically, no significant difference was found between 0.2% chitosan and 0.2% carboxyl methyl chitosan and EDTA. Low Hussmann scores were obtained for carboxyl methyl chitosan (mean score 1.35) compared to that of chitosan (mean score 1.6), indicating the improved ability of carboxyl methyl chitosan over chitosan. Improved efficiency of CMC over chitosan could be attributed to the increased solubility of carboxymethylation of the chitosan molecules.

Similar to the EDTA molecule, the chitin dimer shows two nitrogen atoms with pairs of free electrons responsible for the ionic interaction between the metal and the chelating agent. In an acid medium, the amino groups present in the bipolymer are protonated, resulting in an overall positive charge ($-NH^{3+}$). This form is responsible for the attraction of other molecules for adsorption to occur. The formation of complexes between chitosan and metal ions most probably is due to the mechanisms of adsorption, ion exchange, and chelation.^[22]

Mathew *et al.* reported from quantitative analysis of root canal surface treated with EDTA and chitosan using atomic force microscopic that EDTA-treated teeth was associated with significantly higher surface alteration than chitosan.^[23]

Mittal *et al.* reported that 0.2% chitosan was very effective in removing smear and smear plug with less peritubular dentin erosion compared to apple cider vinegar and 15% EDTA.^[24]

It is known that the efficiency of a chelating agent depends on several factors such as application time, pH, concentration of the solution, and amount of solution. Thus, in the present study, the volume of chitosan (pH 3.2) used as the final irrigant was standardized at 5 ml for 3 min. Chitosan exhibits many advantages over EDTA, such as antibacterial property, healing of wounds, bioadhesive nature, chelating action, and can be used as an irrigating solution.^[25] From the results of this study, 0.2% chitosan and 0.2% CMC at pH 3.2 can be a better alternative to EDTA as a smear layer removing solution because of more biological advantages of chitosan and CMC over EDTA.

CONCLUSIONS

Within the limitations of this *in vitro* study, 0.2% chitosan, 0.2% CMC, and 17% EDTA effectively removed the smear

layer from the retrograde root canal preparation. Considering adverse effects associated with 17% EDTA as an irrigant, it was concluded that 0.2% chitosan and 0.2% CMC are deemed to be a better alternative for the removal of the smear layer.

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Nil.

Conflicts of interest

There are no conflicts of interest.

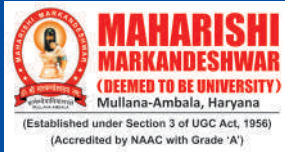
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Correlation of Mother and Child's Taste Perception and their Caries Experience

Abstract

Aim and Background: Nowadays, clinical assessment of caries susceptibility is evaluated by the individuals taste perception. As food habits of the child are mostly influenced by the parents (mothers), it would be beneficial to assess the relation between mother and child's taste status and their caries experience to predict the caries susceptibility of the child. **Methodology:** A convenience sample of 310 mother-child dyads of both the sexes were selected. After obtaining the data on the taste preferences, dietary habits, and oral hygiene practices, caries experience of both the mother and child were recorded using the DMFT and defs indices, followed by the taste assessment using 6-n propylthiouracil (PROP) tester strips. **Results:** Irrespective of the taste status, the majority of the mother and child dyads showed preference to sweet foods. A statistically significant relationship between taste status and caries experience was noticed among mothers and children individually. However, there was no significant association between mother and child taste status. Whereas, a weak-positive correlation is observed between the mother and child's caries experience. The children of supertaster mothers have relatively less caries experience compared to children of moderate and nontaster mothers, which was not statistically significant. **Conclusion:** Mother's taste perception or caries experience may not always be a risk predictor for their child's caries experience, but the PROP tester strips were very effective in predicting the caries risk of an individual.

Keywords: 6-n propylthiouracil tester strips, 6-n propylthiouracil, caries experience, taste sensitivity, taste status, taste threshold

Introduction

Oral health is an important aspect of general health and well-being of any individual. In spite of increasing dental awareness, the high prevalence of dental caries among children still poses a significant health problem. For children, taste is the main driving force behind food consumption. A significant proportion of children's daily energy comes from highly palatable foods, soft drinks, and discretionary fats. Whereas intake of foods such as fruits, vegetables, and whole grains are much lower than recommended, thereby clearly reflecting a child's preference to cariogenic diet.^[1]

Till date, attempts such as evaluating the *Streptococcus mutans* and lactobacilli counts, socioeconomic factors, past caries experience, frequency, and amount of sugar intake have been carried out to identify children with high caries risk. However, it is difficult to identify "at-risk" population with currently available

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caries screening methods.^[2] The role of taste status as a powerful predictor of food selection, which might lead to dental caries, has not been given its due consideration. The knowledge of an individual's taste threshold facilitates the identification of children who are at risk of developing dental caries. Hence, recent concepts such as genetic taste sensitivity and taste thresholds have been evolved to assess the caries risk at an early stage.^[3]

Inherited behavior and taste threshold play an important role in the frequency of carbohydrate intake. Genetic sensitivity to taste may be associated with the preference or rejection of some foods by children. In 1991, a genetic variation in the ability to taste the bitterness of the chemical 6-n-propylthiouracil (PROP) was found to be associated with variation in food preferences in children. In clinical practice, the bitter taste of PROP was found to be a consistent factor for assessing genetic taste sensitivity levels, which is influenced by TAS2R38 gene.^[4]

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Parents modeling of food consumption can have a powerful effect on their child's consumption.^[5] Johannsen *et al.*^[6] confirmed the influence of parents eating behavior of their children. In addition, using sugary snacks as reward have shown to increase the child's preference for that food. It is possible that a mother's taste preference may influence what the child is fed with, which in turn reflects the dental caries risk of the child.^[4] Hence, the present study is aimed at testing the hypothesis that nontasters have a higher caries experience when compared to super and medium tasters and also to assess the correlation between mother's taste perception and the child's caries experience.

Methodology

After obtaining the clearance from the Institutional Ethical Committee, the sample size (294) was determined using the formula $n = (Z\alpha + Z\beta)^2 pq/d^2$. In this cross-sectional study, a sample of 400 children of both sexes aged between 6 and 12 years, who were accompanied by their mothers were considered. Children who fall under the American Society of Anesthesiologists Physical Status I, children and/or mothers without any systemic disorders, and stable mental condition were included in the study. Pregnant mothers and lactating mothers, participants who have nasal congestion or olfactory dysfunction, medical/hereditary condition or long-term/current regimen of medication that can affect the salivary flow, and children who are undergoing orthodontic treatment were excluded from the study. The purpose of the study was clearly explained to the child and the parent before seeking their consent. Out of the selected samples, only 310 children/mother pairs agreed to participate voluntarily.

The Whatman filter paper was cut into 2 cm × 2 cm size and sterilized in an autoclave at 121°C for 15 min. The sterilized strips were weighed and stored in the desiccator till further use. The drug PROP (Swapnroop Drugs and Pharmaceuticals, Maharashtra) 10 mg/ml was dissolved in 5 ml of ethyl alcohol. Then, ten paper strips were soaked in the prepared solution for 1 h so that the drug absorbs completely. Further, the strips were allowed to air dry at room temperature, weighed in electronic weighing machine, and the values were recorded. The difference between the pre- and postimmersion values will give the actual amount of drug impregnated in each strip, which is approximately 1.6 mg/strip [Figure 1a].^[1]

Meanwhile, the mothers of the selected sample were asked to fill the self-designed questionnaire which constituted general information, their past medical and dental history, dietary preferences, dietary habits, and oral hygiene practices.

The caries experience of both mother and child were recorded following the WHO criteria.^[7] Both primary and permanent dentitions were included and the participants with a total decayed-missing-filled teeth (DMFT)/deft score of >5 were considered as a high caries group.



Figure 1: (a) Armamentarium (b) Assessing taste perception

The taste status/threshold was assessed by placing a PROP tester strip on the dorsal surface of the individual's tongue for 30 s; to determine the inherent genetic ability to taste a bitter or sweet substance [Figure 1b]. Based on their ability to rate the intensity of bitter taste on a Labeled Magnitude Scale (LMS) given by Green *et al.*,^[8] they were classified into supertasters, medium tasters, and nontasters. Facial expressions were also observed during the tasting to support the verbal response. To eliminate any ambiguous and conflicting responses, two plain filter paper strips were used intermittently, thereby blinding the patient.

The entire clinical procedure of recording caries experience and determining the taste perception was carried out by a single examiner. However, to avoid bias and test the validity of the results, samples were randomly reevaluated by another examiner who were unaware of the prior results. As the interexaminer variability was not statistically significant ($P > 0.05$), the results obtained by principal investigator were considered. The values, thus obtained were tabulated and subjected to statistical analysis using Microsoft Excel and SPSS version 20 software (IBM SPSS Statistics for windows version 20, Armonk NY, USA)

Results

Irrespective of the taste status, both mothers and children showed preference for sweet food. However, no significant correlation was noticed when the association between taste status and food preferences in both mothers and children were compared.

Table 1 demonstrates the relationship between taste status and caries experience among mother and children. Kruskal–Wallis test showed statistically significant difference ($P < 0.0001$) in the mean DMFT scores among the groups in both mothers and children. Supertasters had the least mean DMFT/def score compared to medium tasters and nontasters. Irrespective of the mother's taste status, majority of the children are supertasters. Mantel–Haenszel Chi-square test ($P = 0.2$) failed to

demonstrate the association between mother and child taste status [Table 2].

Graph 1 shows the relationship between the mother and child caries experience. The histograms are a pictorial representation of DMFT and def scores, and the curve is a normal distribution curve. From the Graph 1a and b, it can be inferred that both DMFT scores (mothers' caries experience) and def scores (children's caries experience) are right skewed. Scatter plot [Graph 1c] shows the correlation between mother and child caries experience. This scatter plot shows how much one variable is affected by the other (DMFT and def). The dotted line in the graph is moving upward as the DMFT score increases, which means that there is a positive correlation between DMFT scores and def scores. However, there is a weak-positive correlation with a Pearson correlation coefficient of 0.16 [Table 3].

The correlation between mother's taste status and children's caries experience was illustrated in Table 4. The

mean DMFT/def score (caries experience) of supertaster mothers' children is 2.20, whereas for children of moderate taster mothers and nontaster mothers are 2.61 and 2.87, respectively. Analysis shows that although mean def score in children of supertaster mothers is low compared to the children of moderate and nontaster mothers, it did not reach statistical significance level ($P = 0.10$).

Discussion

Recent concepts such as genetic taste sensitivity and taste thresholds have been evolved to identify the caries risk at an early stage. It has been extensively documented in the literature that the drug PROP is widely used to test the genetic sensitivity of the individuals in eliciting response to sweet taste on a hedonic scale. Sensitivity of PROP strip is known to be a reliable test in assessing this genetic sensitivity to bitter taste, which is an inherent genetic feature.^[9] Genetic sensitivity to bitter taste may be associated with the preference for or rejection of some foods by children.^[10]

PROP is a medication used in the treatment of Grave's disease (hyperthyroidism).^[11] The therapeutic safety and efficacy of propylthiouracil are well established and are not considered as a human carcinogen or teratogen. Thus, the PROP-impregnated filter paper used for this research contains a very low concentration of 1.6 mg approximately. It is unlikely that casual exposure to PROP in a taste study poses a foreseeable risk to human subjects.^[12]

PROP tasters are more sensitive to many oral sensations, including bitter and sweet taste and the sensation of fats.^[13] When the connection between PROP taste sensitivity and sweet preference were explored, it was found that PROP tasters were more likely to dislike the taste of sweet solutions, whereas nontasters were almost always those

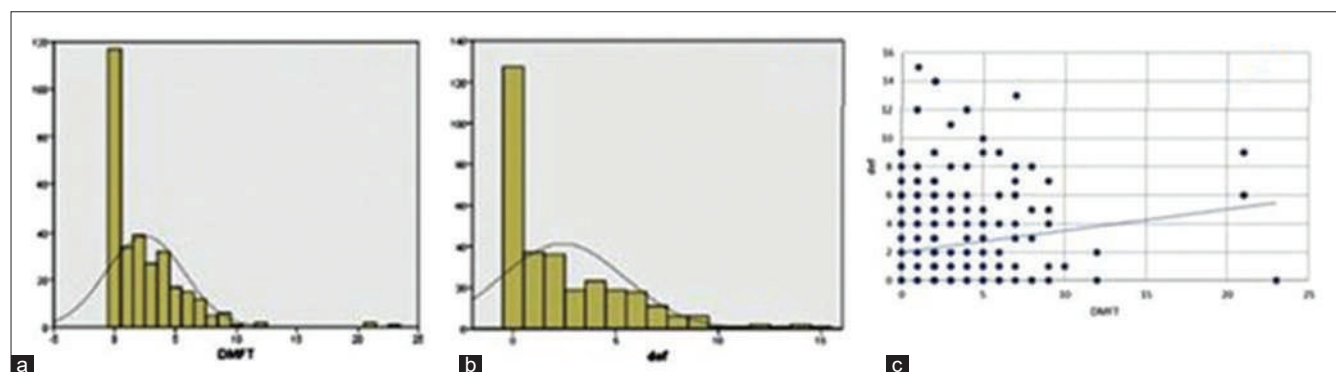
Table 1: Correlation between taste status and caries experience of mother and child

	Mean DMFT	SD	Kruskal-Wallis (χ^2)	P
Mother taste status				
Nontasters	4.14	3.99	42.8	<0.0001*
Moderate tasters	3.83	2.64		
Supertasters	1.77	2.75		
Child taste status				
Nontasters	4.16	3.88	34.6	<0.0001*
Moderate tasters	3.24	2.89		
Supertasters	1.55	2.17		

*Denotes statistical significance, SD: Standard deviation; DMFT: Decayed-missing-filled teeth

Table 2: Association between mother and child's taste status

Mother taste status (n)	Child taste status			Test statistic χ^2 (Mantel-Haenszel)	P
	Nontaster	Moderate tasters	Supertasters		
Nontasters (63)	19	6	38	1.53	0.2
Moderate tasters (36)	11	4	21		
Supertasters (211)	48	23	140		



Graph 1: (a-c) Correlation between mother and child's caries experience

Table 3: Correlation between mother and child's caries experience

Variable	n	Mean	Pearson correlation	P
Def	310	2.39	0.16	0.004*
DMFT	310	2.49		

*Denotes statistical significance, DMFT: Decayed-missing-filled teeth

Table 4: Correlation between mother's taste status and children's caries experience

Mother taste status	n	Mean (def)	SD	Kruskal-Wallis (χ^2)	P
Nontasters	63	2.87	3.24	4.48	0.10
Moderate tasters	36	2.61	2.70		
Supertasters	211	2.20	2.96		

SD: Standard deviation

with a sweet preference. Tasters are more sensitive to sweetness, and they perceive lower concentrations of sugars to be sweeter and they also tend to dislike intensely sweet foods.^[3]

A study by Weintraub *et al.*^[14] found that maternal-untreated dental caries almost doubled the odds of children's untreated dental caries and significantly increased the child's dental caries severity by approximately three surfaces. These findings indicate that mothers oral health status is a good risk indicator for their children. Studies by Ersin *et al.*,^[15] Smith *et al.*,^[16] and Manna *et al.*^[17] found that the mothers' dental caries status has been related to preschool children's dental caries status. Alanzi *et al.*^[4] stated that mothers who are PROP nontasters may consume higher amounts of sugars and have a high frequency of sugar intake compared to supertasters, and also, choose to give more sweets to their children. Thus, a PROP screening of mothers may facilitate the identification of caries risk for their children.

Numerous studies have evaluated the association between taste perception/thresholds and caries experience among children or adults. As most of the studies showed a significant positive correlation, the present study is aimed with a hypothesis that the nontasters are highly susceptible to dental caries when compared to medium- and supertasters and to determine if there is any similar correlation between mother and child's taste status and their caries experience.

In the present study, the taste status of mothers and children were assessed by using the PROP tester strips. Zhao *et al.*^[12] stated that use of the paper-disc method is a valid and reliable screening tool for classifying individuals by PROP taster status.

For the participants' convenience, to express the intensity of bitterness of the PROP tester strip, a LMS was used. The LMS is a semantically labeled scale of the sensation intensity that was developed for the study of oral

somatosensation and gestation. LMS is a quasi-logarithmic, 100 mm scale, in which labeled descriptors are placed along the length of the scale, with the strongest imaginable on the high end of the scale.^[3] LMS provided not only an absolute lower bound at which there is no sensation but also an absolute upper bound labeled "strongly imaginable of any kind." It was believed that preparing the scale in this manner puts different people's responses on the same metric scale creating a "universal ruler."^[18]

The LMS which was used in this study measures the intensity of bitter taste of PROP in six verbal labels which were arranged according to the geometric means of their rated magnitudes. The magnitude increases as the intensity increases.^[19] Children younger than 6 years of age were not included in our study group due to their inability to understand the LMS Scale. Based on the LMS ratings, participants were classified as: (a) supertasters (>60), (b) medium tasters (>12–<60), and (c) nontasters (<12).^[8]

Based on the LMS ratings, out of the 310 mother-child pairs, 211 mothers and 199 children rated the PROP tester strip as intensely bitter and were subsequently categorized as "supertasters." 63 mothers and 78 children who rated the strip as weak or barely bitter in taste were categorized as "nontasters." The remaining 36 mothers and 33 children rated the PROP tester strip as moderately bitter to taste and hence categorized as "medium tasters." Out of the 620 participants (both mother and child), majority of them cumulatively (410) were supertasters, while only 69 were moderate tasters and 141 were bad tasters. These findings were in accordance with the study conducted by Lin^[2] where the number of nontasters was found to be significantly lower (11%) than supertasters.

When food preferences are compared with taste status, 63.5% of nontaster mothers were sweet likers and 78.2% of nontaster children preferred sweets, emphasizing that majority of nontasters prefer sweets. However, when the food preference among supertasters was evaluated, only 50% of supertaster mothers and 29% of supertaster children disliked sweet food which is contradicting with this hypothesis. Thus, the inference is that a majority of the participants in the study (53.8% of the mothers and 74.2% of the children) were sweet likers irrespective of their taste status. This can be attributed to the fact that individuals prefer highly palatable foods such as sweet-fat snacks and soft drinks over less palatable choices such as fruits, vegetables, and whole grains.^[13] Drewnowski *et al.*^[20] stated that the fondness for sweet substances gradually decreases as the age increases, which clearly explains the reason for more sweet likers in children compared to the mothers.

Till date, studies conducted on the taste status, compared it with many parameters such as the caries activity of an individual, taste preferences, obesity, and alcohol intake. Among them, studies comparing the taste status and caries activity in an individual outnumbered. All the

studies done comparing these two parameters showed a significant inverse relationship between the ability to taste PROP tester strip and caries activity. Among them, some landmark studies were those done by Lin,^[2] Verma *et al.*,^[21] and Rupesh and Nayak.^[11] Hence, this led to another hypothesis that a supertaster will have a low caries activity when compared to that of a moderate and nontaster. In the present study [Table 1], the mean caries experience of both the supertaster mothers and children is very less compared to that of the moderate and nontaster mothers and children. The difference in the mean caries experience among the three taste groups of mothers and children were statistically significant, thereby supporting the above-mentioned hypothesis.

Drewnowski *et al.*^[20] stated that many factors influence the development of food preferences in children, the first of which occur via intrauterine experience with flavors from the maternal diet and after birth with flavors of human milk. According to Alanzi *et al.*,^[4] mothers might influence their children via their own food preferences, which may limit the foods offered to their children. Both these studies indirectly indicate that there might be a similarity in mother and child's taste status. However, studies comparing the mother and child's taste status are sparse [Table 2]. Out of the 211 supertaster mothers in the present study, 140 mothers had children with same taste status. However, the moderate and nontaster mothers also had a majority of their children with supertaster status, thereby clearly showing an insignificant relation between mother and child's taste status. However, further research should be carried out along with gene mapping to evaluate the relation.

Smith *et al.*^[16] found a strong association between child's caries experience and their mothers *S. mutans* levels, maternal active caries, and maternal sugar consumption. They concluded that these three maternal risk indicators together can predict child's caries risk. It has indicated the fact that mothers oral health status is a good risk indicator for their children. Similarly, in the present study, when the mother and child's caries experience were correlated [Table 3 and Graph 1] a weak positive linear relationship was observed ($P = 0.004$), which means if one variable changes, the other variable will also change in the same direction.

Alanzi *et al.*^[4] stated that it is possible that a mother's taste preference may influence what the child is fed and therefore may influence the caries risk of children. They found that the prevalence of dental caries in 2–3 years children was significantly greater in children of mothers who could not taste the PROP tester strips. Similarly, in the present study, when the correlation between mother's taste status and children's caries experience was evaluated [Table 4], the mean caries experience (def scores) of the children of supertaster mothers was less when compared to children of

moderate taster mothers and nontaster mothers. However, the difference between the scores was not statistically significant. Thus, the present study could successfully prove the hypothesis pertaining to taste perception and caries experience in both mother and children.

The variables such as racial, socioeconomic, geographic distribution of the participating subjects, dental caries risk, and protective factors (e.g., fluoridated drinking water, saliva flow rate) are certain limitations that could not be controlled and might influence the results. Furthermore, children included in this investigation were from low socioeconomic background and restricted to a small geographic area, thus the results cannot be generalized. Thus, future studies with a larger sample size and a diverse ethnicity/racial background are recommended.

Conclusion

The conclusions drawn from the present study are:

1. The hypothesis which was tested regarding taste perception and caries experience was found to be true, with a strong inverse correlation between the ability to taste the bitterness of PROP tester strip and the caries experience
2. There was no correlation between the taste perception of the mother and their child
3. A weak-positive correlation was noticed with regard to caries experience among mothers and children
4. There is a positive correlation between mother's taste perception and their child's caries experience which was not statistically significant.

The results ascertained that the PROP strips can be used as a risk indicator. The PROP sensitivity test can be a valuable tool in the future to assess the inherent genetic sensitivity of a person for dietary preferences.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Indigenous irrigants as potent antimicrobials in endodontic treatment: An *in vitro* study

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ABSTRACT

Background: The success of an endodontic treatment depends on effective disinfection and complete sealing of root canal. The irrigants that are currently used in the field of endodontics have certain limitations, so the quest for an ideal root canal irrigant continues. Nowadays, the use of herbal extracts such as Triphala and liquorice are used for their potent antimicrobial activity and less side effects. **Aim:** This study aims (1) to evaluate the antimicrobial efficacy of Triphala and liquorice against *Enterococcus faecalis*. (2) To determine any cytotoxic effect on isolated human periodontal ligament (PDL) fibroblasts. **Materials and Methods:** The antimicrobial efficacy of Triphala and liquorice extracts was analyzed at different concentrations (12.5, 25, 50, 75, and 100 mg/ml) using the well-diffusion method. Three percent sodium hypochlorite (NaOCl) and distilled water were taken as positive and negative controls. Minimum inhibitory concentration of the active extract was determined by the broth dilution assay. Human PDL fibroblast tissue culture was used to assess the cytotoxicity of the preparations. The data thus obtained were subjected to statistical analysis. **Results:** The results showed that the mean antimicrobial efficacy of Triphala and liquorice at 50 mg/ml is 20.33 and 9.33, respectively, which are statistically significant ($P < 0.0001$) as compared with a concentration 12.5 and 25 mg/ml. 50 mg/ml showed significant results ($P < 0.001$) on comparing with hypochlorite. Triphala and liquorice showed no cytotoxic effect as compared to NaOCl on human PDL fibroblasts. **Conclusion:** Among the three tested materials Triphala showed the highest antimicrobial efficacy followed by NaOCl and liquorice.

KEYWORDS: *Enterococcus faecalis*, liquorice extract, MTT reagent, periodontal ligament fibroblasts, sodium hypochlorite, Triphala extract

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Introduction

Endodontic infections are polymicrobial in nature dominated by obligate anaerobic bacteria. *Enterococcus faecalis* is such a persistent organism that plays a major role in the etiology of periradicular lesions after root canal treatment. It can even survive in restricted conditions due to biofilm formation and physicochemical properties of the organism that helps it to modify the prevailing environmental and nutritional conditions. Therefore, the choice of instrumentation and irrigating solutions that permit bacterial neutralization and toxin inactivation without

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Satti, *et al.*: Antimicrobial efficacy of herbal extracts and their cytotoxic effect

negative interference with the healing process is fundamental for the success of endodontic treatment.^[1]

Thus, irrigation is considered imperative as it assists in cleaning the root canal system areas that are inaccessible by instruments, which in turn reduces the number of bacteria and control the periapical disease.^[2,3]

Routinely used endodontic irrigants include sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine (CHX). Even though NaOCl is more commonly used, it has certain detrimental effects such as unpleasant taste, toxicity, and its inability to remove the smear layer by itself, as it dissolves only the organic material. EDTA also effectively demineralizes the smear layer but has to be used along with NaOCl to remove the organic part and does not have disinfective ability. CHX is also frequently used to irrigate the canals due to its wide spectrum antimicrobial activity, biocompatibility, and ability to disinfect the infected root canals, but it does not have a tissue dissolving capability.^[2] Due to the constant increase in antibiotic-resistant strains and the side effects caused by irrigants, it has promoted the researchers to explore for herbal alternatives which gained greater attention owing to their beneficial effects.^[1]

Ayurveda is a traditional, natural system of medicine which uses herbal remedies. It is gaining popularity as these herbs used are free of side effects and also have antimicrobial activity and medicinal value.^[4] One such herbs which have numerous medicinal values are Triphala and liquorice.^[5]

Triphala is comprised of fruits of three medicinal trees, Amalaki-*Embllica officinalis*, Vibhitaki-*Terminalia bellirica*, and Haritaki-*Terminalia chebula*. *T. belerica* was the most active antioxidant followed by *E. officinalis* and *T. chebula*. The major ingredients of *T. belerica* are ellagic and gallic acid and in *T. chebula*, gallic acid is the major ingredient. Tannic acid represents the major constituent of the ripe fruit of *T. chebula*, *T. bellirica*, and *E. officinalis*. The phenolic nature of these active ingredients may be responsible to scavenge the free radicals generated by the bacteria.^[1] It possesses analgesic, antiarthritic, antiseptic, hypoglycemic, anti-aging, antiviral, anti-inflammatory, and antibacterial effects. Antibacterial activity of Triphala is complex yet it inhibits the cell division or damage to the cell walls of the bacterium. When used as a mouth rinse, Triphala showed no evidence of staining of teeth. All these properties along with biological activities make it a prospective Ayurvedic drug for the treatment of dental diseases.^[6]

Glycyrrhiza glabra commonly known as liquorice (mulethi) is generally regarded as safe by the Food and Drug Administration when used as food flavoring and sweetening agent. Recent research suggests that

the anti-adherence, antimicrobial, anti-inflammatory properties of liquorice and its various bioactive ingredients provided the potential benefits to treat oral diseases, including dental caries.^[7,8]

Thus, considering the beneficial effects of Triphala and liquorice, the present *in vitro* study was undertaken to evaluate the antimicrobial efficacy of Triphala and liquorice at various concentrations against *E. faecalis* in comparison to NaOCl and also assess the cytotoxicity against human periodontal ligament (PDL) fibroblasts.

Materials and Methods

The *in vitro* microbiological study was conducted where Triphala and liquorice were selected as experimental groups at different concentrations 12.5, 25, 50, 75, and 100 mg/ml, NaOCl and distilled water as positive and negative controls, respectively, and their antimicrobial activity against *E. faecalis* was carried out using agar well-diffusion method.

Extract preparation

Triphala and liquorice extracts available in powder form were procured and mixed at different concentrations of 12.5, 25, 50, 75, and 100 mg with 10 ml of distilled water in Jiffy's centrifuge tubes. These five concentrations of extracts were later immersed in a thermostatic water bath at a temperature of 60°C for 20 min, then allowed to cool and centrifuged at 2500 rpm for 10 min. Thus, the obtained supernatants were used to analyze the antimicrobial efficacy.^[9]

Agar diffusion test

A pure culture of *E. faecalis* was grown on Mueller-Hinton agar, which was inoculated into Mueller-Hinton broth (HiMedia, Mumbai, India), incubated at 37°C overnight and then streaked onto the agar plates. These extracts were then loaded into the wells (6-mm diameter) along with 3% NaOCl and distilled water at different concentrations. All the plates were then incubated in anaerobic jar at 37°C for 24 h, and the diameter of the growth inhibition zone was measured using inhibition zone reader template [Figure 1a-c].^[10]

Determination of minimal inhibitory concentration

Broth dilution method was adopted to determine minimum inhibitory concentration (MIC) of the active extract. The inoculum of the test bacterium was prepared by diluting the overnight culture of bacterium in Mueller-Hinton broth to a level of 1.5×10^7 CFU/ml. 2 ml of extracts diluted in two-fold concentration were added to a sterile glass tube containing 0.5 ml Mueller-Hinton broth. The tubes were then incubated at 37°C for 18–24 h. Due to the turbidity and dark color of the extracts, 0.1 ml of mixture in the tubes were spread onto the surface of Mueller-Hinton agar plates and then they were incubated at 37°C for 18–24 h.^[10]

Bacterial colonies were not detected for Triphala and liquorice extracts at 50, 75, 100 mg/ml. Thus, the lowest concentration, i.e., 50 mg/ml was taken as MIC, which was further used to test the cytotoxicity of the extracts on human PDL fibroblasts.

Culturing of periodontal ligament fibroblasts

The cytotoxic effect of the four groups against human PDL cells was analyzed using MTT ASSAY-3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. After obtaining the institutional ethical committee approval and informed patient consent, sound teeth extracted for orthodontic purpose were selected for the study with the exclusion of carious and periodontally compromised teeth. Human PDL cells were cultured using an explant technique.^[11] The extracted teeth were placed in Hanks' balanced salt solution to maintain the viability of PDL cells. Teeth were rinsed in sterile phosphate-buffered saline (PBS), and PDL tissue was scraped from the middle-third of the roots. The explants were cultured on a 60 mm culture dishes and were grown in α Methyl Modified Eagles Medium (α MEM) supplemented with 10% fetal calf serum and antibiotics (streptomycin and amphotericin). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. After confluence (1 week), the cells were passaged using 0.125% trypsin ethylenediaminetetraacetic. Passages numbers 3–6 were used in this study.^[12]

Cell culture and cytotoxicity testing (MTT assay)

The fibroblasts were plated separately using 96 well plates with the concentration of 1×10^4 cells/well in an α MEM with 10% fetal bovine serum, 50 μ g/ml streptomycin and 3 μ g/ml amphotericin in carbon dioxide incubator at 37°C with 5% CO₂. The cells were later washed with 200 μ L of PBS and then treated with various test concentrations in serum-free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5 mg/ml MTT prepared in PBS was added and incubated at 37°C for 4 h using CO₂ incubator in a dark environment. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 μ l of PBS. Thus, the formed crystals were dissolved with 100 μ l of dimethyl sulfoxide and mixed thoroughly. The viable cells with active metabolism convert MTT into a

purple-colored formazan product with an absorbance maximum near 570 nm, and this color formation serves as a useful and convenient marker of the viable cells. The absorbance was measured at 570 nm using microplate reader.^[13] Thus, the data obtained were subjected to statistical analysis using Kruskal-Wallis test, *post hoc* Mann-Whitney U-test, and independent samples *t*-test.

Results

In the present study, the antimicrobial efficacy of herbal extracts against *E. faecalis* and their cytotoxic effect against human isolated PDL cells were evaluated. Triplicates were performed for each test solutions, and the averages of the three values were taken. $P < 0.05$ was considered as statistically significant.

The mean difference in the zones of inhibition on comparing Triphala and liquorice at different concentrations with 50 mg/ml was mentioned in Table 1, respectively. This represents that Triphala and liquorice at 50 mg/ml concentration have higher antimicrobial efficacy when compared to 12.5 and 25 mg/ml concentrations of each extracts and have

Table 1: Comparison of zone of inhibition at different concentrations

	Zone of inhibition	
	Mean (mm)±SD	P
Triphala (mg/ml)		
12.5	15.67±0.58	<0.0001**
25	17.67±0.58	
50	20.33±0.58	
75	20.67±1.15	
100	22.33±0.58	
Liquorice (mg/ml)		
12.5	0.00±0.00	<0.0001**
25	0.00±0.00	
50	9.33±1.15	
75	9.67±0.58	
100	11.00±1.00	
Sodium hypochlorite (3%)	14.67±0.57	
Distilled water (100%)	0.00±0.00	

Kruskal-Wallis test. **Highly significant ($P < 0.001$). SD=Standard deviation

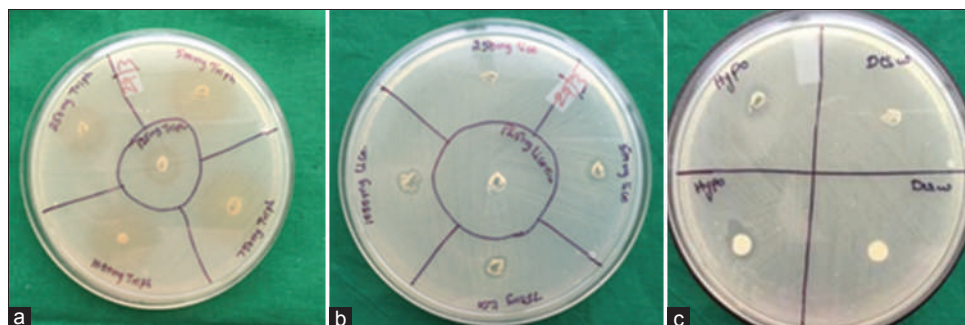


Figure 1: (a-c) The zone of inhibition with Triphala, liquorice, sodium hypochlorite, and distilled water after 24 h against *E. faecalis*


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Satti, *et al.*: Antimicrobial efficacy of herbal extracts and their cytotoxic effect

slightly lower antimicrobial efficacy when compared to 75 and 100 mg/ml concentration.

Intergroup comparison values of Triphala with liquorice, hypochlorite, and distilled water were 11, 5.66, and 20.33, respectively, and the mean difference of zone of inhibition on comparing liquorice with hypochlorite and distilled water was 5.34 and 9.33, respectively [Table 2]. This illustrates that Triphala has superior antimicrobial efficacy than other experimental groups and liquorice have less antimicrobial efficacy when compared to hypochlorite and more when compared with distilled water.

The mean difference of cell viability on comparing Triphala, liquorice and distilled water with hypochlorite was 89.67 and 90.53, respectively [Tables 3 and 4]. This shows that hypochlorite is highly toxic, and Triphala and liquorice are nontoxic to the PDL fibroblasts.

Discussion

NaOCl is used as the popular irrigant till date, due to its strong proteolytic potential as it results in sufficient tissue lysis. The use of higher concentrations increase its ability to dissolve the necrotic tissue and shorten the time needed for the inhibition of bacterial growth, but it causes damage to periapical tissues. The main disadvantages of NaOCl are high toxicity, corrosive to instruments, inability to remove smear layer and reduction in elastic modulus, and flexural strength of dentin.^[14-16] As irrigation with NaOCl is still not predictably effective, researchers have focused on searching for an alternative solution.^[12]

In endodontics, because of the cytotoxic reactions of the most of the commercial available irrigants used and their inability to eliminate bacteria from dentinal tubules, the trend of recent medicine attends to use biologic medication extracted from natural plants.^[17] Medicinal plants such as Triphala and liquorice are such herbal extracts with more pronounced therapeutic values.^[18]

E. faecalis, a saprophytic component of enteric flora is selected for the present study as it is the most common bacterium isolated from endodontic retreatment of apical periodontitis, either as a single organism or as a major component of the flora.^[1,19] *E. faecalis* has the capacity to produce biofilms, where they aggregate and coaggregate with other microorganisms and are embedded in the extracellular matrix.^[19] Distel *et al.*^[20] have revealed that *E. faecalis* can colonize medicated root canals with biofilm formation. That is the reason for selection of *E. faecalis* in studies concerning to the evaluation of endodontic irrigation solutions for cleaning the root canal system.

The antimicrobial efficacy of any natural or synthetic agent can be evaluated using broth dilution method,

agar dilution method, disc diffusion method, cup plate method or agar well-diffusion method, and

Table 2: Comparison of antimicrobial efficacy between Triphala (50 mg/ml), liquorice (50 mg/ml), sodium hypochlorite, distilled water

Zone of inhibition		
	Mean difference (mm)	P
Triphala (50 mg/ml)		
Liquorice	11	<0.0001**
Hypochlorite	5.66	0.000**
Distilled water	20.33	0.000**
Liquorice (50 mg/ml)		
Hypochlorite	-5.34	0.002*
Distilled water	9.33	0.000**
Sodium hypochlorite (3%)		
Distilled water	14.66	0.000**

Independent samples *t*-test. *Statistical significance ($p < 0.01$), ** Highly significant ($p < 0.001$)

Table 3: Cytotoxicity at different concentrations on periodontal ligament fibroblasts

Cytotoxicity Number of viable cells		
	Mean±SD	P
Triphala (mg/ml)		
12.5	98.931±0.974	0.917
25	97.412±5.31	
50	99.047±3.57	
75	99.31±1.19	
100	99.785±3.05	
Liquorice (mg/ml)		
12.5	101.269±1.642	0.348
25	98.85±1.247	
50	99.903±3.54	
75	97.633±3.76	
100	101.792±1.95	
Hypochlorite (3%)	22.784±1.188	
Distilled water	100.53±3.963	

Kruskal-Wallis test. SD=Standard deviation

Table 4: Comparing the cytotoxicity between Triphala (50 mg/ml), liquorice (50 mg/ml), sodium hypochlorite, distilled water

Cytotoxicity Number of viable cells		
	Mean difference	P
Triphala (50 mg/ml)		
Liquorice	-0.856	0.783
Hypochlorite	89.67	0.000**
Distilled water	-1.49	0.645
Liquorice (50 mg/ml)		
Hypochlorite	90.53	0.000**
Distilled water	-0.63	0.847
Sodium hypochlorite (3%)		
Distilled water	-91.16	0.000**

Multiple pairwise comparisons using Tukey's *post hoc* tests; **High statistical significance ($p < 0.001$)

ditch-plate method. However, agar well-diffusion method was used in the present study as it depends on the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing the test material. Similarly, Kriplani *et al.*^[21] also employed agar well-diffusion method to evaluate the antimicrobial efficacy of different groups as it is more reliable, acceptable, and easy to perform.

In the present study, the mean zone of inhibition diameter using Triphala at a concentration of 50 mg/ml was 20.33 mm. Similarly, in the study conducted by Bag *et al.*^[22] hot aqueous extract of Triphala was found to be more potent against *E. coli* strains with an inhibitory zone diameter ranged from 20 to 21 mm. Thus, a hot aqueous extract of Triphala was used in the study as water is a high polarity solvent, and almost all compounds in the plant would be soluble in water. On the contrary, the mean zone of inhibition diameter using liquorice at 50 mg/ml concentration was 9.33 mm. In a study conducted by Badr *et al.*^[18] Liquorice extract showed the largest zone of inhibition (3.97 ± 0.24) when compared to mixture of liquorice and calcium hydroxide and CaOH alone. Similarly, Chittrarasu *et al.*^[23] determined that liquorice extract has higher activity than calcium hydroxide against enterococci and better activity on biofilms. The antimicrobial effect of liquorice extract against *E. faecalis* may be related to the glycyrrhizin.^[24] The mode of action of antibacterial effects of saponins seems to involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium, thus being influenced by microbial population density.^[25,26]

Minimal inhibitory concentration (MIC) of Triphala was found to be 50 mg/ml. However, this finding was found to be disagreement to a study conducted by Prabhakar *et al.*^[27] where MIC of Triphala is 3.125 mg/ml. On the other hand, the MIC of liquorice was found to be 50 mg/ml in the present study. This finding was also found in disparity to a previous study conducted by Ajagannanavar *et al.*^[5] comparing the effect of aqueous and alcoholic liquorice root extract with CHX against *Streptococcus mutans* and *Lactobacillus acidophilus* where MIC of aqueous liquorice root extract for *S. mutans* was established at 25 mg/ml and *L. acidophilus* at 50 mg/ml and the MIC of alcoholic liquorice root extract for *S. mutans* was established at 12.5 mg/ml and *L. acidophilus* at 6.25 mg/ml. This difference in the results may be attributed to the method of aqueous extract preparation.

Triphala is found to have a superior antimicrobial efficacy that might be due to the presence of Tannic acid which is bacteriostatic and bactericidal. These findings are similar to the studies conducted by Shakouie *et al.*^[28] revealing that Triphala exhibited better antibacterial activity than NaOCl as an irrigant

solution against *E. faecalis* and Prabhakar *et al.*^[29] who conducted a study to evaluate the antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD (Mixture of Tetracycline, Acid and Detergent) and 5% NaOCl against *E. faecalis* biofilm formed on the tooth substrate in which, Triphala achieved 100% killing of *E. faecalis* at 6 min. However, liquorice showed less antimicrobial efficacy than NaOCl which could be due to the less solubility of liquorice in distilled water which was similar to the study conducted by Ajagannanavar *et al.*^[5]

In the present study, fibroblast cell primary cultures isolated from human periodontal tissue of the extracted teeth were used. According to Hernández-Sierra *et al.*^[30] primary cultures offer the advantage of having the same characteristics as the original tissue. The cytotoxicity can be determined using methods such as trypan blue dye exclusion assay and MTT assay. However, Avelar-Freitas *et al.* reported that trypan blue dye exclusion assay has certain limitations like dye can be incorporated by live cells after a short exposure time and personal reliability, related to the expertise of the analyst can affect the results.^[31] Thus, MTT an enzyme-based method which relies on a reductive coloring reagent and dehydrogenase in a viable cell was used to determine the cell viability with a colorimetric method in this study. This method is found superior because of its ease in usage, safety, and high reproducibility.^[32] The MTT assay determines the functional state of mitochondria that indicates the cell viability. A mitochondrial dehydrogenase enzyme in living cells reduces the yellow tetrazolium salt MTT to blue MTT formazan, which is precipitated in uninjured cells.^[33] Similarly, Zhang *et al.*^[34] used MTT assay method to determine the cytotoxicity of MTAD irrigant.

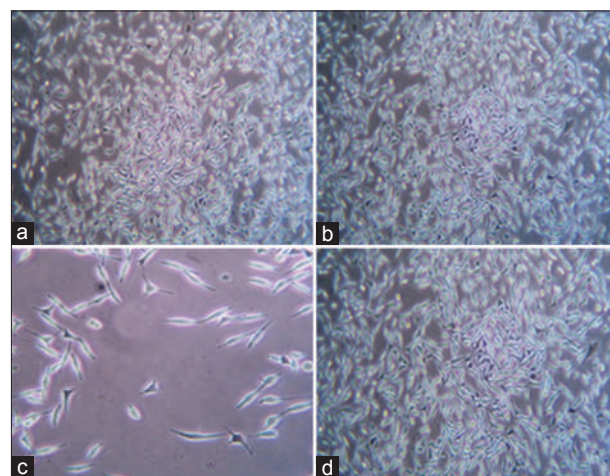


Figure 2: (a, b, and d) Representative phase micrograph of human periodontal ligament fibroblasts which are arranged irregularly with different size and shape and they have stellate reticulum like morphological appearance (c) illustrates few fibroblast which are plump in nature, and there is no stellate reticulum like shape

Satti, *et al.*: Antimicrobial efficacy of herbal extracts and their cytotoxic effect

The mean cell viability of Triphala, liquorice, and NaOCl is 99.047, 99.903, and 22.784, respectively. This represents that Triphala and liquorice at 50 mg/ml concentration have no cytotoxic effect and NaOCl has a high cytotoxic effect on PDL fibroblasts [Figure 2]. In a study conducted by Badr *et al.*^[18] liquorice has also shown greater biocompatibility with fibroblasts cells compared to calcium hydroxide, which was severely toxic to the cells. Both the herbal extracts, Triphala and liquorice showed the biocompatibility with human PDL cells.

As the aqueous extracts of Triphala and liquorice were used to determine the antimicrobial efficacy at different concentrations against *E. faecalis* and this efficacy might change when we use other solvent systems like alcohol, because herbs might have the better dissolving capacity in alcohol. Even these *in vitro* results cannot be extrapolated completely to a clinical scenario as the efficacy of the herbal extracts might be altered and also other properties such as staining and substantivity of herbal extracts should also be evaluated to recommend these herbal extracts as root canal irrigants. Further clinical trials should be carried out to ascertain the effect of these herbal extracts on periodontal pathogens and other caries causing microorganisms.

Conclusion

Herbal extracts (Triphala and liquorice) exhibited significant inhibitory effects against *E. faecalis* compared to NaOCl and distilled water. These herbal extracts exerted no cytotoxic effects on human PDL cells as compared to NaOCl which showed maximum cytotoxic effect. Hence, indigenous extracts can be promising endodontic irrigants.

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Conflicts of interest

There are no conflicts of interest.


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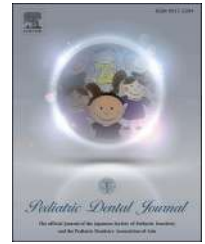
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Research Paper

Association of dental caries with socio-demographic and nutritional factors among school children in Guntur district of Andhra Pradesh, India



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ABSTRACT

Objective: The main objective of the study was to determine the association of dental caries with socio-demographic factors like age, gender and socioeconomic status (SES); second is to explore the relationship between dental caries and nutritional status in school going children of Guntur district, Andhra Pradesh.

Methods: In this cross-sectional study, SES of 1022 children aged between 5 and 11 years were assessed according to modified kuppuswamy SES scale. The body mass index (BMI) values were calculated and then the children were grouped into 3 categories (underweight, normal, overweight + obese) based on revised World Health Organization (WHO) growth charts. Caries index (deft+/DMFT) of each child was recorded using American Dental Association (ADA) Type 3 examination. Data was analysed and the tests employed were Chi-Square (X²); Analysis of Variance (ANOVA) and Logistic regression analysis.

Results: The prevalence of dental caries was 57.14% and underweight was 40.3% among Guntur district school children. A significant association between dental caries and SES was noticed (OR = 1.74). However, no significant association of dental caries experience with other factors like age, gender and BMI was observed.

Conclusion: Nutritional status of children may not show association with dental caries, but SES influences the caries prevalence in children.

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1. Introduction

Dental caries is one of the most prevalent oral ailments of childhood [1]. Globally, 2.4 billion adults and 486 million children had dental caries according to the survey done by Global Burden of Disease Study (2015) [2]. In spite of many scientific advancements in the field of caries prevention, the disease continues to be a major public health problem in most of the developing countries including India [1].

Dental caries is a multifactorial disease with bacteria, fermentable carbohydrates, susceptible host and time as four main etiological factors. Furthermore, certain socio-demographic and nutritional factors also influence the caries prevalence of an individual which include: age, gender, nutritional status and socioeconomic status [3]. Oral health, being a depicter of general health is sturdily influenced by the intake of sweetened foods and increased caries index is associated with uneven dietary patterns [4]. The most commonly used index to assess nutritional status of an individual is body mass index (BMI) [5]. It may be hypothesized that certain socio-demographic and nutritional factors might affect the prevalence of dental caries in children. Reports of different studies on the association of these factors with dental caries in different parts of India show inconsistent results [1,6-8]. Moreover, very few publications were found describing the association of dental caries with socio-demographic and nutritional factors in school children of Guntur district, Andhra Pradesh, India. So, the present study was carried out with two objectives: first, to determine the association of dental caries with socio-demographic factors like age, gender and socioeconomic status; second is to explore the relationship between dental caries and nutritional status in Guntur district school children.

2. Materials and methods

This study was carried out among 5-11 year old school going children in Guntur district, Andhra Pradesh. The study period was for 6 months and the required sample size was estimated based on previous study which showed a prevalence of 45.33% [9]. With this anticipated population proportion of 0.05 and a power of 80%, a sample of 1003 children was needed to be recruited for this study. The sample size was estimated using the formula

$$n = \frac{(Z \alpha + Z \beta)^2 \times pq}{d^2}$$

where p is the prevalence of dental caries; q = 1-p; Z alpha & Z beta are standard normal variants; d = acceptable margin of error (4.4).

For the purpose of the study, Guntur district was arbitrarily divided into four geographical regions, which correspond to the four varying demographic areas of the district i.e., North, south, east and west. Schools from each region were randomly selected to obtain the desired sample size, such that there was an equal representation from each of the four zones. Six government and six private schools were selected by lottery method to meet the sample size of 1003. A two-stage

random sampling method was followed, with schools as the primary sampling unit and individual children were selected proportionate to the number of children in each school by systematic random sampling. All the required and relevant information about the number and location of schools in Guntur district was obtained from the office of the Deputy Director of Public Instruction, Guntur district. The study protocol was approved by the institutional ethical committee. Permission was obtained from the selected school authorities and an informed written prior consent was also obtained from the parents of children who participated in the study.

A total of 1022 children of both the sexes between 5 and 11 years of age group without any known systemic conditions were included. Uncooperative, medically and physically compromised children, children on long term medication, children undergoing orthodontic treatment and children who haven't returned the questionnaires which were used to assess SES were excluded from the study. Semi-structured questionnaires were utilized to obtain information regarding parent education, parent occupation, family income per month. Questionnaires were distributed to the parents of 1070 children. The answered questionnaires were collected on the next two days from the schools to assess the SES of children. Out of 1070, 1022 (95%) questionnaires were returned, so remaining 48 children were excluded from the study.

SES was assessed according to modified Kuppaswamy SES scale where the children were classified into one of the three clusters: upper class score >25, middle class - total score between 11 and 25 and lower class score <10 [10].

Anthropometric measurements of children were noted prior to examination of the oral cavity by a single investigator. Height and weight of each individual were recorded to obtain BMI-for-age. The weight of each child without shoes and dressed in a minimum amount of clothing was measured using a portable analog weighing machine. The height was measured to the nearest 0.5 cm, using a portable height measuring unit with the soles of both feet fully supported on a horizontal surface. BMI was calculated using the following formula, i.e., weight in kilograms divided by height in meter square (weight/height²). These children were classified into three categories using age- and gender-specific criteria recommended by WHO using revised WHO growth charts-2007 [11] as: Underweight group children with BMI for age <5th percentile; Normal group children with BMI for age ≥ 5th percentile and <85th percentile; Overweight + Obese group children with BMI for age ≥ 85th percentile. The participants falling under overweight and obese were clubbed together under one group.

A second investigator, blinded to the anthropometric measurements, proceeded to record the deft+/DMFT index in a separate operatory. Oral examination of school children was carried out visually, under natural light using plane mouth mirrors and CPI probes; no radiographs/transillumination were used (ADA Type 3 examination). A single investigator examined all the children. Diagnosis of dental caries (deft and DMFT) was established according to World Health Organization guidelines (WHO, 1997). Intra-examiner calibration was performed with respect to the dental caries scores. Significant correlation (Kappa value 0.96, P < 0.05) was noticed.

Immediate dental care was given and a referral was made as and when required.

The data obtained was analysed using Statistical Package of Social Sciences (SPSS Version 15; Chicago Inc., USA) software. The tests employed were Chi-Square (X²); Analysis of Variance (ANOVA) and Logistic regression analysis. Regression analysis was performed using dental caries experience as dependent variable and socio-demographic and nutritional factors like age, gender, SES and BMI as independent variables where the significance level was fixed at $P < 0.05$.

3. Results

Out of 1022 children, 508 (49.7%) were males and 514 (50.3%) were females. Assessment of SES in these children showed that 806 (78.9%) were from the low income group and the rest 216 (21.1%) from the middle income group. The higher income group in this study included none due to non availability. BMI scores showed that 412 children (40.3%) were undernourished and 38 children (3.7%) were overweight/obese. Remaining 572 children (56%) showed normal BMI [Table 1].

Table 2 shows the distribution of caries by age, gender, BMI and SES within the sample. Significantly higher prevalence of dental caries in lower SES group than middle SES group. However, no significant difference in caries prevalence by age, gender and BMI was noticed.

When the mean deft+/DMFT scores were compared among the subgroups of BMI and SES, highly significant increase in mean deft+/DMFT score was elicited in lower SES (1.50) group than the middle SES (1.12) group. Further, the mean deft+/DMFT score was significantly lower in overweight (0.98) group than normal (1.49) and underweight (1.34) groups [Table 3].

Univariate logistic regression analysis showed significant association of SES with dental caries. Conversely, no

Table 1 – Demographic variables of the study population.

Age	Frequency	Percent
5	54	5.3
6	106	10.4
7	124	12.1
8	164	16
9	218	21.3
10	184	18
11	172	16.8
Sex	Frequency	Percent
Female	514	50.3
Male	508	49.7
SES	Frequency	Percent
Middle	216	21.1
Low	806	78.9
BMI	Frequency	Percent
Normal	572	56
Overweight	38	3.7
Underweight	412	40.3
Total	1022	100

Table 2 – Distribution of dental caries by age, gender, BMI and SES.

Age	No Caries	Caries	P value
5–7	45.10%	54.90%	0.55
8–11	42.00%	58.00%	
Sex			
Female	42.40%	57.60%	0.86
Male	43.30%	56.70%	
SES			
Middle	53.70%	46.30%	0.012 ^a
Low	40.00%	60.00%	
BMI			
Normal	41.80%	58.20%	0.39
Overweight	47.70%	52.30%	
Underweight	46.30%	53.70%	

^a Significant.

Table 3 – Mean deft+/DMFT scores among the subgroups of BMI and SES.

BMI	deft+/DMFT		P-Value
	Mean	SD	
Normal	1.49	1.76	0.03 ^a
Overweight	0.98	1.06	
Underweight	1.34	1.74	
SES			
Middle	1.12	1.65	0.004 ^b
Low	1.50	1.73	

^a Significant.
^b Highly significant.

significant association of dental caries with other factors like age, gender and BMI was observed [Table 4].

Findings from the final multiple logistic regression model after adjusting for age and gender showed that SES was significantly related to dental caries, with the adjusted odds ratio (95% CI) being 1.743 (1.287, 2.360). Nevertheless, no significant association of BMI with dental caries was found [Table 5].

Table 4 – Factors associated with dental caries in the univariate logistic regression analysis.

Factors	Odds ratio	95% CI	P
Age			
5–7 years	1		
8–11 years	0.87	0.65–1.15	0.32
Sex			
Female	1		
Male	0.98	0.76–1.25	0.84
SES			
Middle	1		
Low	1.74	1.28–2.36	<0.001 ^a
BMI Status			
Normal	1		
Overweight	0.79	0.55–1.15	0.23
Underweight	0.79	0.50–1.24	0.30

^a Highly significant.

Table 5 – Factors associated with dental caries in the final multiple logistic regression model.

Factors	Odds ratio	95% CI	P
SES			
Middle	1		
Low	1.743	1.287–2.360	<0.001 ^a
BMI			
Normal	1		
Underweight	0.811	0.519–1.265	0.355
Overweight	0.816	0.564–1.181	0.281

^a Significant.

4. Discussion

Currently, Dental caries and Malnutrition in children constitute two major health problems in many developing countries, including India, and present great challenges for public health. Malnutrition is a silent emergency. Globally, more than one third of child deaths were attributable to undernutrition [12]. Across the world, eighty percent of the undernourished children live in 20 countries, with India being centre to nearly 60 million underweight children [13]. National Family Health Survey-3 data revealed that 43% of children below 5 years of age were undernourished and overweight prevalence to be 1.6% [14]. In the present study, 40.3% children were underweight and 3.7% children were overweight. According to the report of Vaz M et al. (2005), approximately 4.1% of school going children in the Asia-Pacific region were overweight [15]. However, in contrary to this results, the reported prevalence of overweight (40.1%) is higher and underweight (4.1%) is lower according to the study conducted by Begum M et al. (2014) [7].

The prevalence of dental caries in the present study is 57.14%, which was similar to the reported caries prevalence (57.5%) in a study conducted by Reddy KV et al. (2018) [6]. However, it is less than the reported caries prevalence (79.72%) by National Oral Health Survey (2002–2003) in 12 year old children. This decrease in caries prevalence may be attributed to differences in the study sample selection and sampling techniques used and differences in socio-demographic factors among these children. In this study, a wide range in age group (5–11 years) was selected as it is an easily available sampling frame for health surveys and as the individuals grow, their dietary habits and the amount of body fat changes which might influence the growth and development of the child.

The association between BMI and dental caries in children was more complex, which cannot be explicated by carbohydrate consumption alone [16]. It is likely that not a single common risk factor plays a role in the relationship between dental caries and BMI but rather a complex interface of dietary pattern like snacking between meals, intake of drinks containing sucrose, SES, oral hygiene practices and genetic aspects that determine alterations in both caries and BMI. To overcome the shortfalls of the previous classification put forth by Indian Academy of Paediatrics (IAP) regarding the estimation of malnutrition, a new classification of child growth standards by WHO was considered in this study as it

was accepted by the government of India to assess BMI of children [17].

In the present study, no significant association of caries prevalence to one particular BMI category was noticed. However, the mean caries score (deft+/DMFT) was significantly lower in the overweight group than the normal and underweight groups which is in agreement with the study conducted by Sanchez-Perez L et al. (2010) who found that overweight children probably came from families that provided fluoridated dentifrices more often, and with better access to dental services compared to other children [18]. Other possible explanation might be the adiposity of body tissues and increased intake of dietary fats can provide a level of protection against dental caries [19]. This is in contrary to the other studies which reported significantly higher dental caries in the obese children [6,20].

Social status was reported to be a powerful determinant to affect child's general and oral health [16]. Previous studies on the relationship between SES and dental caries reported quite different results. In high societies, increased caries prevalence was noticed in spite of good oral hygiene, whereas in low social class, caries rate was not high but oral hygiene was very poor. Thus, it can be substantiated that people with better SES use refined sugars more often when compared to low income people, resulting in increased caries, but on the other hand, they have improved oral hygiene in order to have good appearance and smile [21].


There is no doubt that dietary and oral hygiene habits are affected by income, education and social environment. Soares et al. (2016) reported that there was a decline in caries experience in developed economies in the past decade, but an upsurge in developing economies [22]. Asli PM et al. (2017) had reported that children with low SES tend to have more dental caries [16]. Similarly, in this contemporary study, caries prevalence was highest among lower SES children compared to that of middle SES, that was in accordance with various studies, which depicted that decreasing SES was associated with increasing development of caries [23,24].

When logistic regression analysis was applied among observed risk factors for dental caries like age, sex, SES and BMI, SES alone was a significant factor associated with dental caries with increased caries risk in low SES children which is consistent with the previous studies [6,16]. The most probable reasons for increased caries experience include unsupervised oral hygiene practices, dietary habits and poor living standards make them more vulnerable to dental caries.

Being a cross sectional study, it may not establish the cause effect relationship which is the main limitation of the study. Other limitations include the presence of sampling bias and no data was recorded on key etiological confounding factors for dental caries like dietary habits and oral hygiene practices. Moreover, the results cannot be generalized to the entire population as the study was performed in a small geographic location with less number of participants.

5. Conclusions

Though the nutritional status of an individual is not associated with dental caries, but socioeconomic status influences


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the caries prevalence of children in this population. Further, multi-factorial etiology and complexity of interactions for dental caries need more profound research adopting a longitudinal pattern with more study variables to derive definite conclusions.

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Nil.

Conflict of interest

Nil.

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Effect of Fluoride-releasing Elastomers on Mutans Streptococci in Dental Plaque: An *In Vivo* Study

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ABSTRACT

Aims: The purpose of this study was to evaluate the effect of fluoride-releasing elastomeric modules in the control of *Streptococcus mutans* levels in the oral cavity.

Materials and methods: The study consisted of 30 patients, with two experimental periods of three weeks and a three-week washout period between experimental periods. At the first visit, fluoridated modules were placed around brackets on 12 11 33 and non-fluoridated ones on 21 22 43. During the 2nd visit, the modules were removed and sent for a microbiological analysis. Non-fluoridated modules were placed on all brackets for one visit to allow for a washout period. At the 3rd visit, fluoridated elastomeric modules were placed around brackets on 21 22 43 and non-fluoridated on 12 11 33. At the 4th visit, the procedures at the 2nd visit were repeated.

Results: A mean comparison between bacterial counts of fluoride-releasing and non-fluoridated elastomeric modules in both the trials were done by the Mann–Whitney *U* test, which showed the result to be significant ($p < 0.001$). The mean comparison of bacterial counts between fluoride-releasing and non-fluoridated elastomeric modules in a specific area was done by the Wilcoxon signed rank test, which showed the result to be significant ($p < 0.001$). A comparison between bacterial counts of fluoride-releasing elastomeric modules in both trials were done by the Mann–Whitney *U* test, which also showed a significant result ($p < 0.001$).

Conclusion: The sustained-release fluoridated elastomeric modules are effective in reducing the levels of *Streptococcus mutans* in dental plaque around the brackets for a time period of 21 days.

Clinical significance: The sustained-release fluoridated modules were effective in reducing the CFU of *S. mutans* and are also stable at the end of 21 days of the experimental period. But the action of fluoride released from the modules is localized, temporary, and requires constant maintenance of these modules over the brackets throughout the treatment period.

Keywords: Demineralization, Elastomeric modules, Fluoride, *Streptococcus mutans*, Sustained release.

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INTRODUCTION

Orthodontic treatment with fixed appliances predispose to accumulation of plaque, resulting in carious lesions around brackets because of the intense demineralization around the brackets.^{1,2} There will be also a significant increase in the number of *Streptococcus mutans* (*S. mutans*), which is associated with the development of white spots and a later development of carious lesions.¹

During orthodontic treatment, preventive measures such as giving instructions for maintenance of oral hygiene, mechanical removal of plaque, encouraging the daily usage of fluoride mouth wash rely on participation of the patient.^{3,4} Hence, methods that do not require patient cooperation such as application of sealing material, fluoride collagen materials, fluoride cements, and fluoride varnishes around the bracket can be employed to prevent demineralization. But these methods are technique-sensitive and time-consuming.^{1,4,5}

Elastomeric modules are being used regularly as a part of routine orthodontic therapy for ligating the arch wire to the brackets. Hence, the use of fluoride-releasing elastomeric modules aids in the local delivery of fluoride and is more beneficial.⁵ But, one of the main disadvantages is that the fluoride is released only for a shorter duration; however, it can be resolved by using the sustained fluoride-releasing system, which has an advantage of releasing constant levels of fluoride for a longer duration.¹

Some studies^{6,7} reported contradictory results stating that no anticariogenic effect has been found for fluoride-releasing

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elastomeric ligatures because of inconsistencies in the release of fluoride. In contrast, controlled delivery systems permit the release of fluoride consistently over a time frame. For this reason, a biocompatible and non-inflammatory polymer, polyethylene co-vinyl acetic acid derivation (PEVA), is utilized as delivery vehicles.⁸ A new method is used to fuse fluoride into PEVA to give a controlled release of fluoride ions, which would be helpful to prevent the advancement of white spot lesions in orthodontic patients.⁹

Hence the present study was conducted to evaluate the efficacy of fluoride-releasing elastomeric modules in the control of *S. mutans* levels in the oral cavity and to quantify the reduction in the amount of *S. mutans* levels in the dental plaque.


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MATERIALS AND METHODS

The study was carried out on 30 patients (14 males and 16 females) who were undergoing orthodontic treatment. Ethical clearance was obtained from the institutional ethical committee (Reference No: 19/CIR/13) and the informed consent was obtained from all the participants before the onset of the study. All information is kept confidential and can only be accessed by researchers.

The sampling frame was based on the following inclusion and exclusion criteria. Both male and female patients of any age group undergoing an orthodontic treatment with good general health were included in the study. In contrast, patients with any systemic medical condition that could interfere our planned treatment (e.g., uncontrolled diabetes and current pregnancy at the time of treatment), under antibiotics or using any antimicrobial mouthwash, or under treatment with self-ligating brackets were excluded from the study.

Materials Used (Fig. 1)

For this study, sustained fluoride-releasing elastomeric modules (colored and custom-made) were used as experimental group and conventional (non-fluoridated) elastomeric modules (Leone Orthodontics, Sesto Fiorentino, Firenze, Italy) as control group. A ligature gun is used for engaging these modules to the brackets; after the experimental period, these modules were collected in microcentrifuge tubes. The selective medium for *S. mutans* is prepared by adding 0.2 units/mL bacitracin to mitis-salivarius agar base and poured into a petri dish.

Preparation of Custom-made Sustained Fluoride-releasing Elastomeric Modules

Polyethylene co-vinyl acetate (PEVA) at an amount of 4.2 grams was taken and dissolved in about 20 mL of methylene chloride along with 0.40 grams of Naf powder for effective and safer release of fluoride concentration within therapeutic levels—i.e., about 1.43 mg F2/ring/day as given by Baturina et al.⁹ This mixture was shaken vigorously for about 2–3 minutes on a vortex machine and later followed by a 10 minutes treatment in the sonicator for a homogenous distribution. This mixture was poured in to the moulds to create the ring shape of the elastomeric modules. It was then benchdried at room temperature for a night.

Another solution of PEVA/methylene chloride was prepared as above and was poured into another mould containing previously prepared fluoride module so that this freshly prepared solution forms an overcoat on the previously prepared fluoride modules.

Methodology

The study design was a randomized clinical trial, employing a split mouth, crossover design. It consisted of two experimental periods of three weeks with a washout period of 3 weeks in between. During the first visit, fluoride-releasing elastomeric modules were placed around brackets on 12 11 33, and non-fluoridated elastomeric modules were placed around brackets on 21 22 43 (Fig. 2). Non-fluoridated toothpaste was also advised.

During second visit, sterile microcentrifuge tubes containing 2 mL of reduced transport fluid (RTF) were prepared. The fluoride-releasing and the non-fluoridated elastomeric modules were removed and placed aseptically in two separate sterile microcentrifuge tubes containing 2 mL of reduced transport fluid (RTF) and were labelled. A total of 90 fluoride-releasing modules were collected in this first trial from 30 patients. These samples were then sent for microbiologic analysis. After finishing these procedures, Non-fluoridated elastomeric modules were placed around all brackets for one visit (i.e., for 3 weeks) to allow for a washout period (Fig. 3).

During the third visit, i.e., the next experimental visit, the conventional elastomers that were placed around brackets on 12 11 21 22 33 43 during the second visit were removed and discarded by the same operator. Later elastomeric modules were applied to the teeth that are exactly contralateral to those that received elastomeric modules during the first visit—i.e., fluoride-releasing elastomeric modules were placed around the brackets on 21 22 43 and nonfluoridated elastomeric modules around brackets on 12 11 33 (Fig. 4). On the fourth visit, procedures during visit 2 were repeated. A total no. of 90 fluoride-releasing modules was collected in this second trial from 30 patients. These samples were then sent for microbiologic analysis.

Microbiologic Process

The samples were vortexed for 30 seconds using a Vortex mixer (Cyclo Mixer, Remi Equipments PVT LTD., India) and serially diluted up to 10^{-2} dilution in distilled water. From the diluted sample,



Fig. 1: Materials used



Fig. 2: Fluoridated modules engaged on 12 11 33 and conventional modules engaged on 21 22 43 during the 1st visit



Fig. 3: Conventional modules engaged on 12 11 21 and 22 33 43 during the washout period



Fig. 4: Fluoridated modules engaged on 21 22 43 and conventional modules engaged on 12 11 33 during the 3rd visit

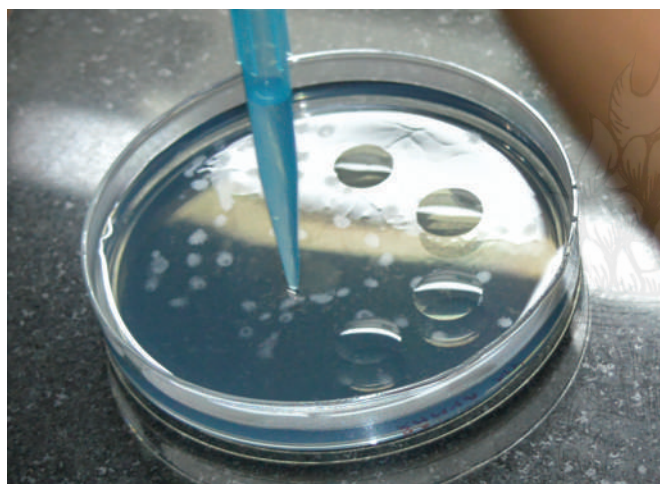


Fig. 5: Plating of sample onto the MSSB agar

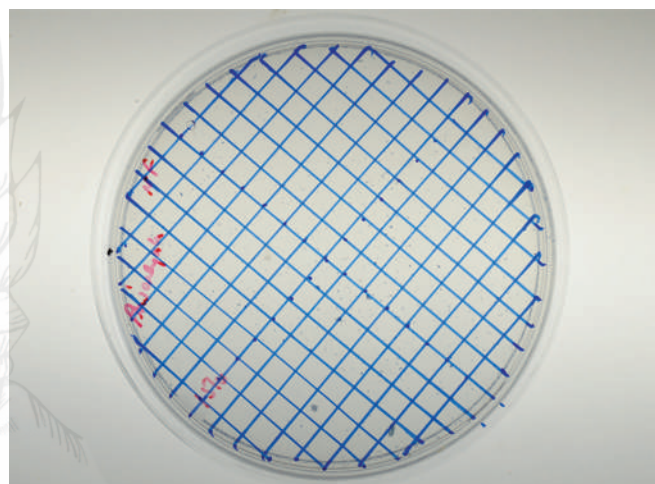


Fig. 6: Colony count

100 μ L of sample was plated onto the mitis salivarius sucrose bacitracin (MSSB) agar (Fig. 5). All petri dishes were then incubated in a 5%-CO₂-enriched anaerobic incubator at 37°C for 48 hours (B.O.D Incubator, Inco instruments and Chemicals PVT. LTD, India). After 48 hours, the Petri dishes were removed from the incubator and total numbers of colony forming units (CFU) were counted to estimate the growth of *S. mutans* (Fig. 6).

All the statistical analysis was done in Statistical Package for Social Sciences software (version 15, SPSS Inc., Chicago, USA). Means and standard deviations for bacterial count in fluoride-releasing and non-fluoridated elastomeric modules for trials 1 and 2 were done and the associated *p* values were listed in Tables 1 to 4.

The mean comparison between the effects of fluoride-releasing and non-fluoridated modules on the *S. mutans* count in both the trials and the mean comparison between bacterial counts of fluoride-releasing elastomeric modules in different trials were analysed by the Mann-Whitney *U* test, and a comparison of bacterial counts between fluoride and non-fluoride modules in a specific area (i.e., fluoride modules on 12 11 33 in first trial and non-fluoride modules on 12 11 33 in second trial, and also non-fluoride modules

on 21 22 43 in first trial and fluoride modules on 21 22 43 in second trial) to evaluate the efficacy of the fluoride modules were analysed by the Mann-Whitney *U* test.

RESULTS

The results were depicted in Tables 1 to 4 and Figures 7 to 10. The results and statistics showed that the fluoride-releasing elastomeric modules are more efficient in reducing the *S. mutans* count when compared with the conventional modules for a time period of 21 days. Even though the individual sample results showed not much reduction when compared to the control results; the obtained result was statistically significant ($p < 0.001$).

DISCUSSION

During the orthodontic treatment by the fixed appliance therapy, there would be an increase in the plaque accumulation around the brackets, resulting in the development of white spots and later development of carious lesions.² Thus, prevention of white spot formation is important for the orthodontist to consider. Although previously published reports^{10,11} have indicated that the molars are

Table 1: A mean comparison between bacterial counts (CFU/mL) in fluoride-releasing and non-fluoride elastomeric modules in trial 1 and trial 2

<i>Trials</i>	<i>Clinical parameters</i>	<i>Mean</i>	<i>SD</i>	<i>Mean ± SD</i>	<i>Z value</i>	<i>p value</i>
1st trial	Fluoride	408.73	86.68	171.24 ± 33.45	5.131	<0.001 Significant
	Non-fluoride	579.97	120.13			
2nd trial	Fluoride	392.73	82.07	174.10 ± 53.60	5.317	<0.001 Significant
	Non-fluoride	566.83	135.67			

Statistical analysis: Mann–Whitney *U* test. Statistically significant if $p < 0.05$

Table 2: A mean comparison between bacterial counts (CFU/mL) of specific area in fluoride-releasing and non-fluoride elastomeric modules in different trials

<i>Variables</i>	<i>Trials</i>	<i>Mean</i>	<i>SD</i>	<i>Mean ± SD</i>	<i>Z value</i>	<i>p value</i>
Fluoride	1st trial	408.73	86.68	158.10 ± 48.99	4.618	<0.001 Significant
Non-fluoride	2nd trial	566.83	135.67			

Statistical analysis: Wilcoxon signed rank test. Statistically significant if $p < 0.05$

Table 3: A mean comparison between bacterial counts (CFU/mL) of specific area in fluoride-releasing and non-fluoride elastomeric modules in different trials

<i>Variables</i>	<i>Trials</i>	<i>Mean</i>	<i>SD</i>	<i>Mean ± SD</i>	<i>Z value</i>	<i>p value</i>
Fluoride	2nd trial	392.73	82.07	187.24 ± 38.06	4.762	<0.001 Significant
Non-fluoride	1st trial	579.97	120.13			

Statistical analysis: Wilcoxon signed rank test. Statistically significant if $p < 0.05$

Table 4: A mean comparison between bacterial counts (CFU/mL) in fluoride-releasing elastomeric modules in different trials

<i>Variables</i>	<i>Trials</i>	<i>Mean</i>	<i>SD</i>	<i>Mean ± SD</i>	<i>Z value</i>	<i>p value</i>
Fluoride	1st trial	408.73	86.68	16 ± 4.61	0.5777	0.564
Fluoride	2nd trial	392.73	82.07			

Statistical analysis: Mann–Whitney *U* test. Statistically significant if $p < 0.05$

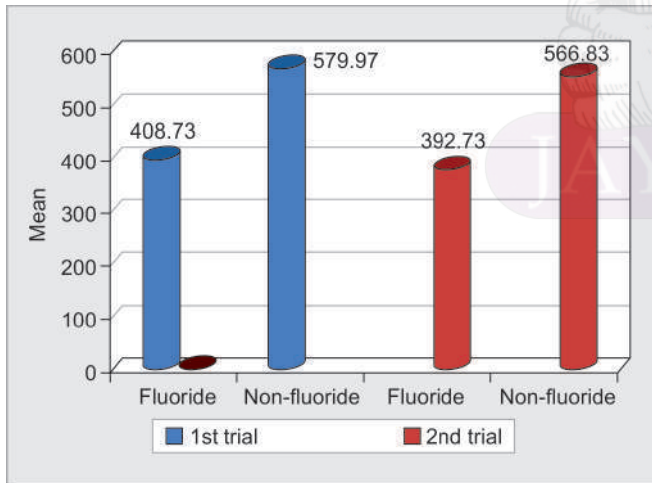


Fig. 7: A mean comparison between fluoride and non-fluoride bacterial counts in the 1st trial and 2nd trial

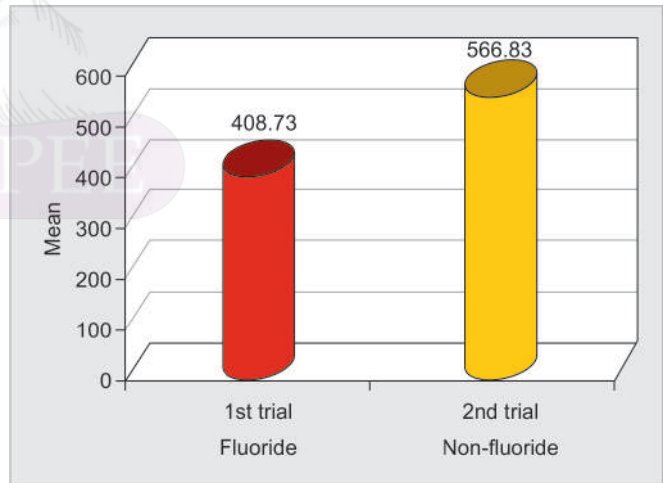


Fig. 8: A mean comparison between fluoride and non-fluoride bacterial counts in different trials

more susceptible to white-spot formation because of difficulties in maintaining plaque control, the 6 maxillary anterior teeth were considered independently for two reasons: the frequency of white-spot formation has been accounted to be high in this area¹² and the orthodontic patients are, to a great degree, careful about the appearance of the front teeth.

During the 1st trial, the comparison of *S. mutans* levels between fluoride-releasing and non-fluoridated elastomeric modules showed a significant reduction in the number of CFU of *S. mutans* with the fluoride-releasing modules and was statistically significant ($p < 0.001$). In the same way, when the levels of *S. mutans* between the fluoride and non-fluoride modules after the second trial were

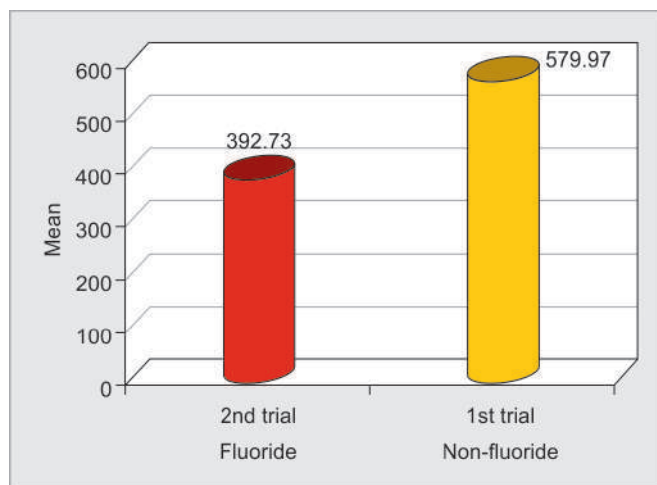


Fig. 9: A mean comparison between fluoride and non-fluoride bacterial counts in different trials

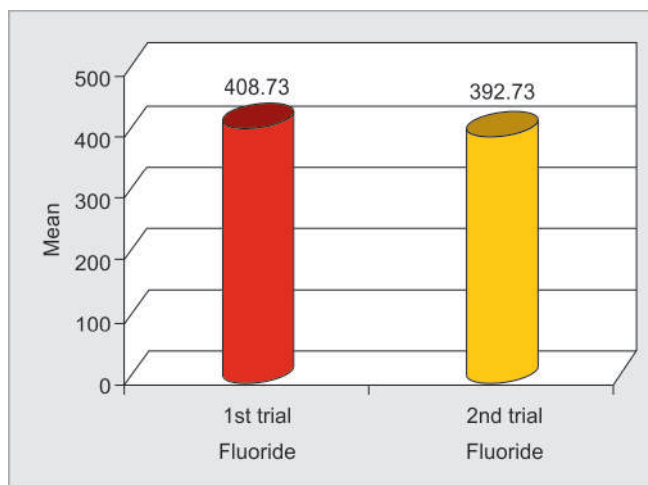


Fig. 10: A mean comparison between fluoride bacterial counts in different trials

analysed, there was also a significant reduction in the CFU of *S. mutans* in the dental plaque around fluoride-releasing modules.

These findings are in agreement with the study done by Wilson and Gregory¹, where there was a significant reduction in the *S. mutans* levels after a time period of 1 week. But this effect was not significant by the 2nd week through week 13 in the same study. Previous research^{13,14} states that concentrations of less than 0.05 ppm are reported to be beneficial in reduction of caries.

The past studies^{3,12,15,16} propose that the clinical utilization of fluoride-releasing elastomeric modules might be compelling in anticariogenic prophylaxis from the principal day of bonding and past the main week, even up to 6 months. It has been prompted that for ideal clinical advantage, elastomeric ligature ties ought to be replaced month to month.³

One report,¹⁷ however, indicates that the elastomeric modules would need to be replaced even sooner. Replenishment of fluoride-releasing elastomeric ties would reintroduce a monthly high dose of fluoride release that would once again benefit calcium fluoride formation in the enamel surrounding the brackets. Moreover, it has been reported in the studies^{13,14,18,19} that the frequency of fluoride application (not merely the level of concentration), is important in anticariogenicity, highlighting the importance of slow-release properties of fluoride from materials.

Some studies^{7,10,15,16} showed that the fluoride-releasing elastomeric modules released the fluoride with an initial burst effect, and later the release was negligible. It was also suggested that replenishing the fluoride supply in the oral cavity at regular intervals helps maintaining the fluoride concentrations in the oral cavity and helps in the prevention of white spot lesions.^{3,17} In order to achieve this, apart from these modules, additional fluoride supplements have to be prescribed, which may again depend on compliance. Replacing the fluoride modules at regular appointments may help achieve this goal.

As per the studies^{8,9} polyethylene co-vinyl acetic acid derivation (PEVA) could discharge the safe therapeutic fluoride concentration. PEVA incorporated with NaF powder coated with a thin layer of pure PEVA polymer were able to release fluoride into the surrounding medium in a favorable profile. These sustained-release fluoride modules were placed for about 21 days in each patient in each trial, respectively.

A mean comparison between fluoride and non-fluoride modules of different trials was also done in this study to see the efficacy of these sustained-release fluoride modules. When the bacterial counts of fluoride-releasing elastomeric modules in the first trial were compared with bacterial counts in non-fluoridated modules of the second trial, there was a mean difference of 158.10 CFU/mL with a standard deviation of 48.99 CFU/mL.

This result suggests that even though the CFU of *S. mutans* were reduced in number by the effect of fluoride released from the fluoridated modules, the number get increased when non-fluoride modules were placed in the place of fluoridated modules during the 2nd trial. This proves that the effect of the fluoride from the fluoride-releasing elastomeric modules is localized and is for a short period of time until these fluoride modules are in place or even though if some of the fluoride may be present in those areas after the removal of fluoridated modules in trial 1, during the washout period of 21 days, the remnant fluoride may have been washed out by the salivary flow and new microorganisms may have been accumulated owing to the presence of conventional elastomeric modules on those teeth throughout the washout period.

When the teeth with non-fluoridated modules of the first trial were replaced with fluoridated modules in the 2nd trial, the mean difference observed was 187.24 CFU/mL with a standard deviation of 38.06 CFU/mL. It shows that as soon as the fluoridated modules have been placed, there was a reduction in the number of CFU of *S. mutans*. The above two comparisons prove that the action of fluoride released from the fluoride-releasing elastomeric modules is localized, temporary, and requires constant maintenance of these modules over the brackets throughout the treatment period.

Another comparison was also done between the fluoride-releasing elastomeric modules of both the trials to see whether any external factors such as the dominant-side tooth brushing had an influence on the number of CFU of *S. mutans*. The fluoride-releasing elastomeric modules of the 2nd trial showed less number of CFU of *S. mutans* when compared to the fluoride-releasing elastomeric modules of the 1st trial, with a mean difference of 16 CFU/mL and standard deviation of 4.61 CFU/mL, which is statistically not very significant ($p = 0.564$).

Hence, these sustained-release fluoridated modules were effective in reducing the CFU of *S. mutans* and were also stable at

the end of 21 days of the experimental period without any swollen, unesthetic appearance intraorally.

As orthodontic patients are seen every 21–45 days for their routine appointments, it would be better to have a continuous fluoride release between the appointments.⁹ In the present study, each trial was done only for 21 days. Hence, further studies are required to prove the *in vivo* efficacy of this sustained fluoride-release system for a time period of more than 21 days.

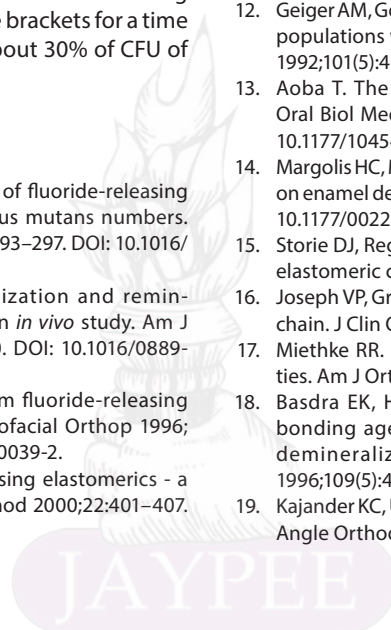
The daily recommended supplemental fluoride intake to prevent the demineralization of enamel is 0.024–0.05 ppm, which corresponds to a fluoride release rate of 1.2–2.8 µg F/ring/day, assuming 28 elastomeric rings per patient.⁹ But in the present study, each trial was done with only three sustained fluoride-release modules in a time period of 21 days. Hence, further *in vivo* studies are required to measure whether there is a constant fluoride release rate at therapeutic levels.

CONCLUSION

The sustained-release fluoride modules are effective in reducing the *S. mutans* levels in dental plaque around the brackets for a time period of 21 days. There was a reduction of about 30% of CFU of *S. mutans* levels.

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Bilateral Sagittal Split Osteotomy for Mandibular Retrognathism: Two Case Reports

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Abstract

Adult patients with severe skeletal malocclusions require orthognathic surgeries. Two case reports with skeletal Class II malocclusion due to retrognathic mandible discussed in this article were treated by bilateral sagittal split osteotomy mandibular advancement. Treatment results showed good improvement in facial profile, Angle Class I molar and canine relation, good occlusion after surgery for both the cases.

Keywords: Bilateral sagittal split osteotomy, mandibular advancement surgery, orthognathic surgery, skeletal Class II

INTRODUCTION

Skeletal Class II malocclusion with mandibular deficiency is one of the most common problems that patients seek treatment. Adult patients with severe skeletal Class II malocclusion need orthognathic surgery for successful treatment.^[1] Bilateral sagittal split osteotomy (BSSO) is the most often preferred technique for these patients. This procedure includes three phases: (1) presurgical orthodontic phase, (2) surgical phase, and (3) postsurgical orthodontic phase. BSSO along with orthodontic treatment improves hard-tissue relationship along with soft-tissue profile improvement.^[2-5]

Two cases treated with BSSO and orthodontic treatments are presented in this article.

CASE REPORTS

Case 1

An 18-year-old female patient reported to the Department of Orthodontics with a chief complaint of forwardly placed upper front teeth. Clinical examination revealed Angle Class II div 1 malocclusion with brachyfacial pattern with 6 mm of interlabial gap at rest, a convex profile, an acute nasolabial angle, and a deep mentolabial sulcus. There were interdental spacings in the upper anterior region, Class II molar and canine relationship, 13 mm of overjet and 60% overbite with exaggerated curve of Spee [Figure 1].

The cephalometric analysis revealed a skeletal Class II due to mandibular retrognathism, hypodivergent growth pattern, reduced lower anterior facial height, proclined upper and lower anterior teeth [Figure 2 and Table 1].

Treatment plan

Treatment goals were set to improve the facial profile by reducing facial convexity, to increase lower facial height, and to achieve a Class I molar and canine relationship with normal overjet and overbite. Because of the presence of large interdental spaces in the upper anterior region, to achieve the above goals, a nonextraction treatment plan and BSSO with mandibular advancement was planned and was suggested both to the patient and her parents, and a written informed consent was obtained.

Treatment progress

Both the upper and lower arches were banded and bonded with 0.022" slot preadjusted MBT bracket prescription (McLaughlin, Bennett, and Trevisi). Upper and lower arches were leveled and aligned using the nickel-titanium (NiTi) wires. Wire sequence was 0.016" NiTi, 0.018" NiTi, 0.016" × 0.022" NiTi, and 0.019" × 0.025" NiTi. Space closure was done on 0.019" × 0.025" stainless

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steel wires, and 0.020" × 0.028" stainless steel stabilizing wires were placed in upper and lower arches. Presurgical records were taken [Figures 3 and 4], models were mounted, mock surgery

was done, and a surgical splint was fabricated. Mandibular advancement of 7 mm with BSSO was performed, and osteotomy cuts were secured with titanium plates. Finishing and detailing was done for 4 months, and debonding was done after achieving the preset treatment goals [Figures 5 and 6]. An upper wraparound retainer and a lower fixed bonded lingual retainer were given.

Table 1: Pre- and posttreatment cephalometric values

Measurement	Pretreatment	Posttreatment
SNA	81°	81°
SNB	72°	79°
ANB	9°	2°
N-A-Pog	24°	18°
Pn -A (mm)	1	-1
SN-Go-Gn	18°	23°
FMA	12°	18°
Max1-SN	116°	110°
Max1- NA (mm)	35°	26°
	7	4
IMPA	113°	101°
LI - NB (mm)	33°	28°
	8	5
Nasolabial angle	99°	99°
Upper lip to E line (mm)	4	2
Lower lip to E line (mm)	5	2
Upper lip to S line (mm)	7	2
Lower lip to S line (mm)	6	3
Mentolabial sulcus (mm)	-9	-5

Case 2

A 17-year-old male patient reported to the Department of Orthodontics with a chief complaint of forwardly placed upper front teeth. Clinical examination revealed Angle Class I malocclusion with brachyfacial pattern with 6 mm of interlabial gap at rest, a convex profile, an acute nasolabial angle, and a deep mentolabial fold. The patient had 10 mm of overjet, 40% overbite and crowding in the lower anterior region [Figure 7].

The cephalometric analysis showed skeletal Class II due to mandibular retrognathism, hypodivergent growth pattern, reduced lower anterior facial height, proclined upper and lower anterior teeth [Figure 8 and Table 2].

Treatment plan

Treatment goals were set to improve the facial profile by reducing facial convexity, to increase lower facial height, and to achieve normal overjet and overbite. To achieve these goals, extraction of upper 5's and lower 4's along with BSSO with mandibular advancement was planned and was suggested to the patient and his parents. Written informed consent was obtained.

Treatment progress

Both upper and lower arches were banded and bonded with 0.022" slot preadjusted MBT bracket system. Upper and



Figure 1: Pretreatment intraoral and extraoral photographs of case report 1

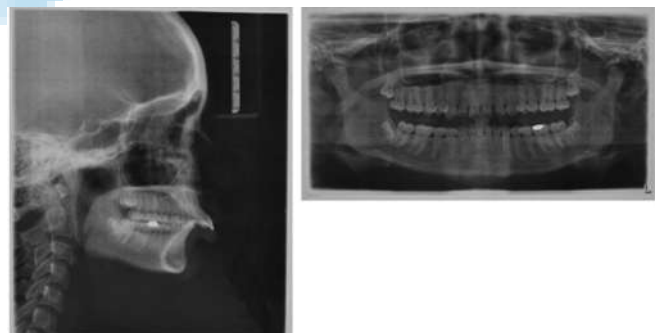


Figure 2: Pretreatment radiographs of case report 1



Figure 3: Presurgical intraoral and extraoral photographs of case report 1

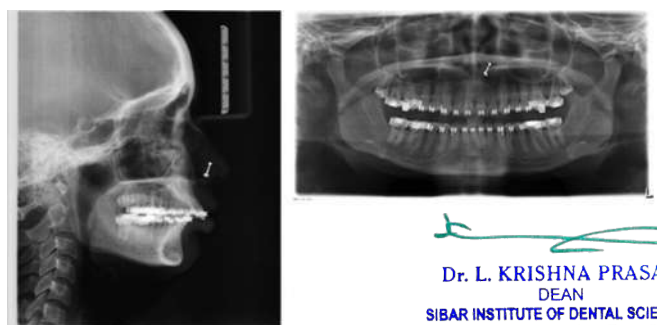


Figure 4: Presurgical radiographs of case report 1

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lower arch leveling and aligning was done using NiTi wires with 0.016" NiTi, 0.018" NiTi, 0.016" × 0.022" NiTi, and 0.019" × 0.025" NiTi wire sequence. Space closure was done on 0.019" × 0.025" stainless steel wires by lower anterior retraction and protraction of upper molars. In the upper arch, mini-implants were used to reinforce the anchorage during molar protraction. Following space closure, presurgical records were made [Figures 9 and 10], models were mounted, a mock surgery was done, and a surgical splint was fabricated. Mandibular advancement of 7 mm with BSSO was performed, and osteotomy cuts were secured with titanium

plates. Finishing and detailing was done for 6 months, and the appliance was debonded [Figures 11 and 12]. Upper wraparound retainer and lower fixed bonded lingual retainers were given.

DISCUSSION

Clinical and cephalometric findings of the two patients in this case report had skeletal Class II bases due to retrognathic mandible and orthognathic maxilla, with proclination of the upper and lower anterior teeth. Different extraction patterns were followed in the two cases presented here. As clinical examination revealed a Class I molar relationship in case 2, upper second premolars were extracted and upper molars were protracted to achieve a Class II molar relationship before the surgery. Upper molars were protracted using temporary anchorage devices. Lower first premolar extraction space was utilized for decrowding



Figure 5: Posttreatment intraoral and extraoral photographs of case report 1

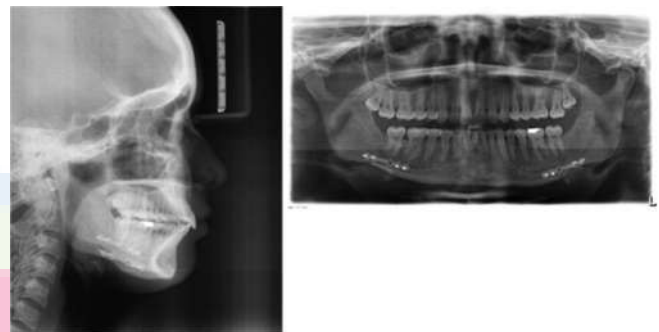


Figure 6: Posttreatment radiographs of case report 1

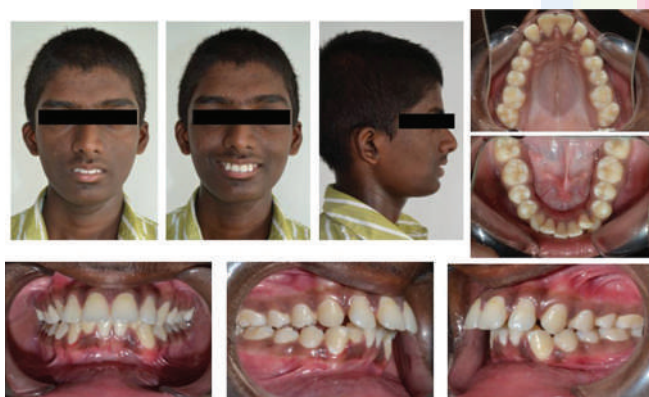


Figure 7: Pretreatment intraoral and extraoral photographs of case report 2



Figure 8: Pretreatment radiographs of case report 2

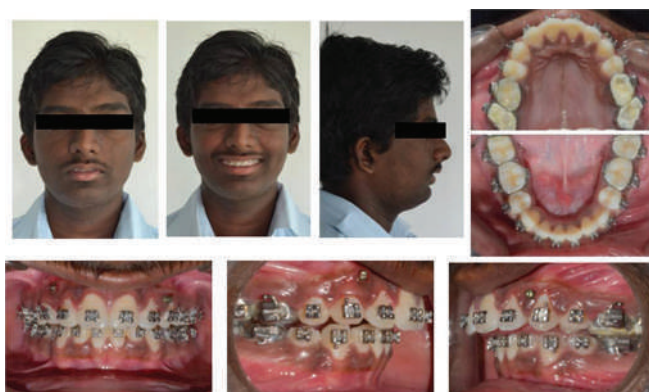


Figure 9: Presurgical intraoral and extraoral photographs of case report 2



Figure 10: Presurgical radiographs of case report 2


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the lower anterior teeth which were crowded. However, Case 1 was treated by the nonextraction method because molars were in full cusp Class II relationship to start with and sufficient interdental anterior spacings were present which can be used for alignment and retraction. After the completion of presurgical orthodontic phase in both the cases, mock surgery was done [Figure 13]. Later, BSSO advancement was done.

Superimposition of pretreatment and posttreatment lateral cephalometric tracings was done for both the cases [Figures 14 and 15]. There was a change of 7° for SNB and ANB for Case 1 and 4° for Case 2 with no change in maxillary position and an increase in mandibular plane angle in both the cases, demonstrating both sagittal and vertical skeletal changes, which translated into a reduced facial convexity and an increased lower facial height [Tables 1 and 2] and a pleasing soft-tissue facial profile. Molars and canines were finished in Class I with ideal overjet and overbite.

Table 2: Pre- and posttreatment cephalometric values

Measurement	Pretreatment	Posttreatment
SNA	81°	81°
SNB	75°	79°
ANB	6°	2°
N-A-Pog	7°	-1°
Pn -A (mm)	1	1
SN-Go-Gn	21°	26°
FMA	19°	23°
Max1-SN	124°	114°
Max1-NA (mm)	42°	30°
	10	4
IMPA	108°	100°
LI - NB (mm)	29°	23°
	7	4
Nasolabial angle	106°	107°
Upper lip to E line (mm)	2	-2
Lower lip to E line (mm)	3	-2
Upper lip to S line (mm)	3	0
Lower lip to S line (mm)	3	0
Mentolabial sulcus (mm)	-6	-3



Figure 11: Posttreatment intraoral and extraoral photographs of case report 2

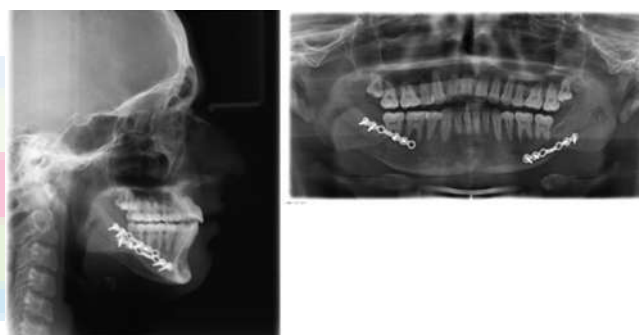


Figure 12: Posttreatment radiographs of case report 2

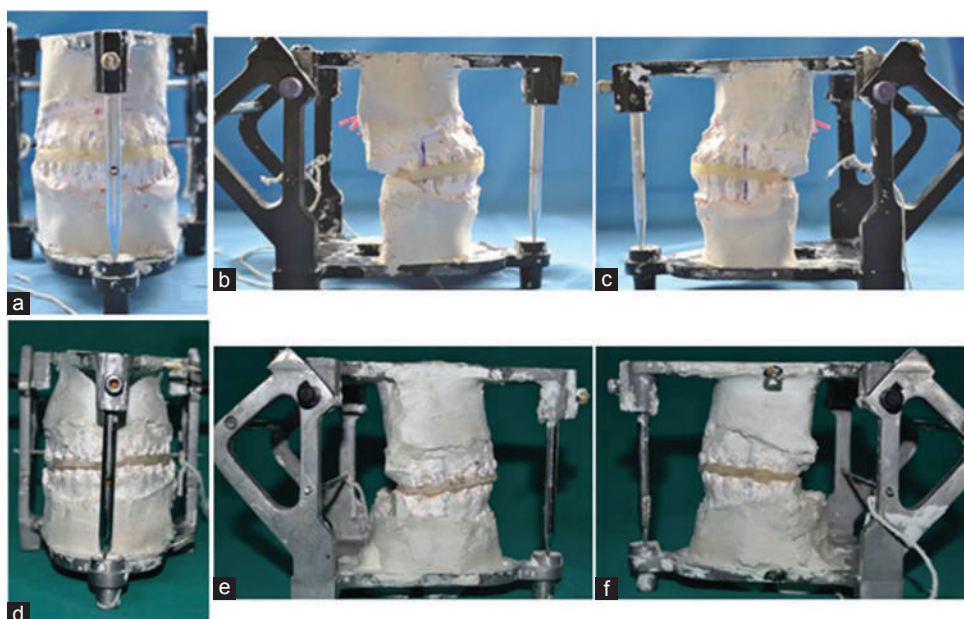



Figure 13: (a-c) Mock surgery for case report 1 and (d-f) mock surgery for case report 2


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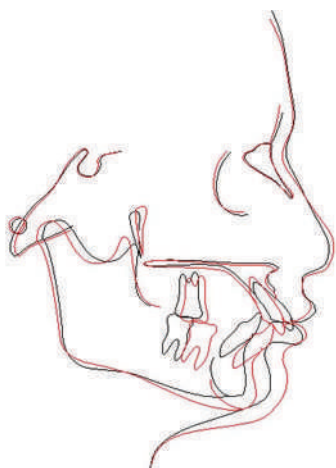


Figure 14: Cephalometric superimposition of case report 1

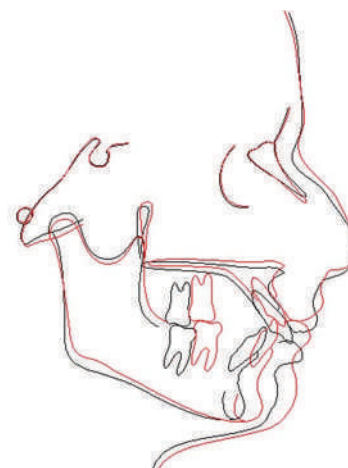


Figure 15: Cephalometric superimposition of case report 2

CONCLUSION

A skeletal Class II malocclusion treated with proper diagnosis and treatment planning improves the esthetic value of the patient. In the present article, two skeletal Class II cases with orthognathic maxilla, retrognathic mandible, and reduced lower anterior facial height treated with BSSO were presented which not only improved the overall facial esthetics but also resulted in good occlusion.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Dental x-ray exposure is not associated with risk of meningioma: a 2019 meta-analysis

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Steven R. Singer, DDS

Objective: To determine the risk of developing meningiomas after exposure to various types of dental ionizing radiation at diagnostic levels. **Method and materials:** A literature review was conducted using databases such as PubMed, Scopus, Google Scholar, CINAHL, Clinical Key, and Web of Science. Data extracted from the studies were tabulated and a meta-analysis was performed using all relevant data. **Results:** Twelve articles related to the topic that met the selection criteria were initially included in the systematic review. After evaluating the methodology and statistical analysis of each study, six were ultimately included in the meta-analysis. A forest plot created using RevMan v5.3 software (Cochrane Collaboration) pro-

duced a weighted odds ratio of 1.13 (95% confidence interval, 1.06 to 1.19) for meningioma risk. **Conclusions:** Studies in the recent past have found a positive association between exposure to ionizing radiation and risk of developing meningiomas. In this study, no association was found between dental x-ray exposure and the development of meningioma. The data were weighted heavily for one of the subgroups due to a larger patient pool as noted via the forest plot. Regardless of the weighting, the majority of the subgroups showed no significant association, further strengthening this conclusion. (*Quintessence Int* 2019;50:822–829; doi: 10.3290/j.qi.a42575)

Key words: brain tumor, dental x-rays, ionizing radiation, meningioma, meta-analysis, risk assessment

Since the introduction of computed tomography (CT) scanners in the early 1970s,¹ the general public has been exposed to an increasing dose of ionizing radiation, especially as a result of increased diagnostic radiation.² In the United States, 74% of medical radiation-related procedures in 2006 were either conventional diagnostic x-rays or fluoroscopy-based procedures. In particular, dental x-rays fall under the most frequent diagnostic radiation source, yet account for less than 1% of the global annual radiation dose.² Different types of dental radiography, such as bitewing (BW) radiographs and full-mouth radiographic series (FMS), involve varying degrees of exposure.³

It has been known for some time that exposure to ionizing radiation, particularly x-rays, can contribute to tissue damage and genetic mutation.⁴ These risks depend heavily on the amount of radiation to which a patient is exposed.⁵ Some stud-

ies that warn of increased risk of developing cancer due to ionizing radiation include instances where there are very high doses of ionizing radiation, such as atomic bombs or therapeutic sources.⁶ However, it is not known if this increased risk of developing cancers also applies to lower doses of ionizing radiation.

The effect of diagnostic ionizing radiation on a patient's risk of developing benign brain tumors has not been satisfactorily determined. Some studies have reported significantly increased meningioma risk after exposure to certain types of diagnostic radiation.^{7,8} Others have found no increased risk of developing meningiomas after exposure.⁹ Some have even reported unexplained lowered risks of developing meningiomas after exposure to dental diagnostic x-rays.¹⁰ A recent meta-analysis concluded that there was no association between dental radiographs and meningioma.¹¹

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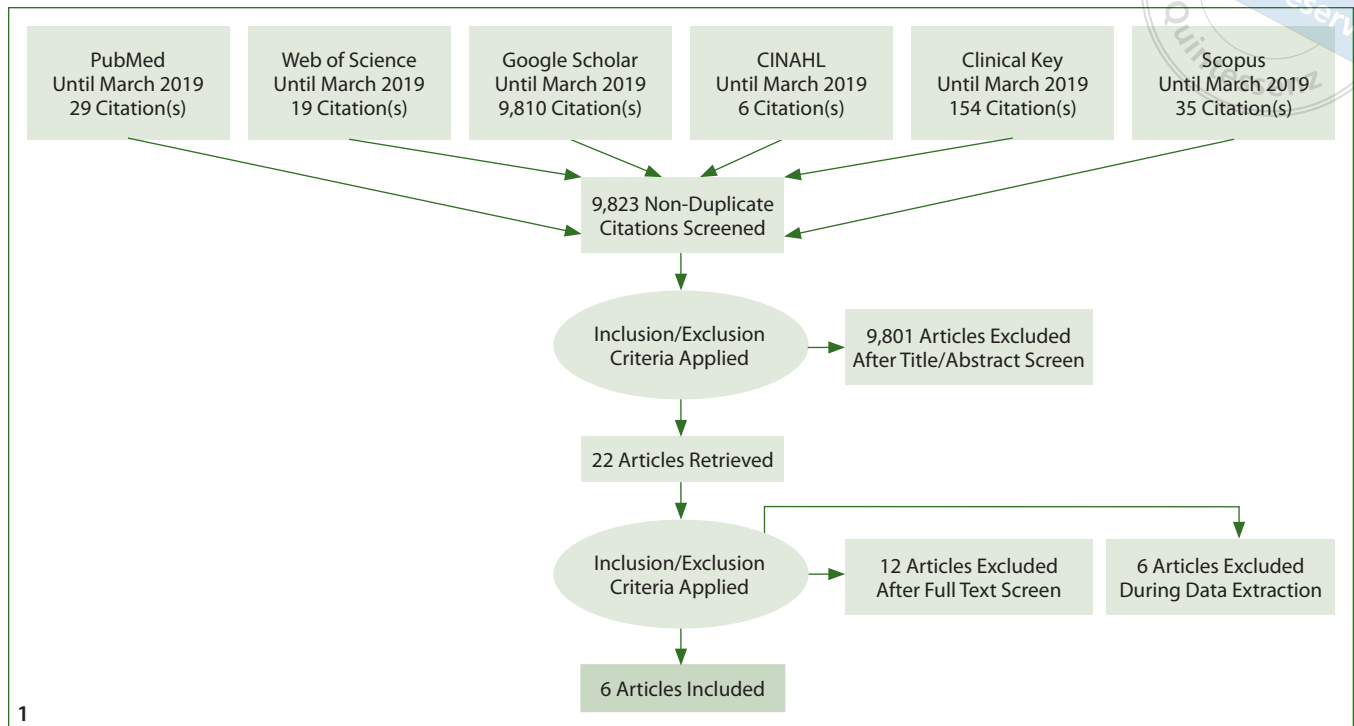


Fig 1 PRISMA flow chart showing the total numbers of citations that were screened and the application of inclusion and exclusion criteria resulting in the final six articles that were included for the meta-analysis.

Due to the global increase in the dose of ionizing radiation, any indication of a medical risk associated with diagnostic ionizing radiation would be of great importance.

In order to conduct this systematic review, a focused research question was developed to aid the literature search: "Does exposure to low levels of dental diagnostic ionizing radiation (x-rays) influence the development of meningiomas in the future?"

Method and materials

A literature review was conducted using the English language databases PubMed, Scopus, Google Scholar, CINAHL, Clinical Key, and Web of Science. The initial search included all articles studying the effects of ionizing radiation on the risk of developing meningiomas. Included initially were case-control studies, cohort studies, systematic reviews, and meta-analyses. Each article was evaluated by two authors (MM, SRS) to determine its eligibility. For the meta-analysis stage, systematic reviews and other articles that were not eligible for data extraction (such as those that did not present the numbers of study patients) were excluded. From a total of 9,823 non-duplicate citations, 9,801

citations were eliminated after title and abstract search, leaving 22 citations. After full-text search, 10 articles were excluded using the exclusion criteria. Out of the remaining 12 articles, six articles remained after data extraction. There were two systematic reviews and one study with incompatible statistics among the six that were excluded, leaving six studies for the final review. The PRISMA flow chart shows the selection criteria used for the six articles that eventually remained for this analysis (Fig 1).

Search strategy

A literature search on the electronic databases was conducted using key words relating to the relevant topic. The PRISMA statement and checklist were followed for uniformity in reporting the systematic review.¹² To ensure thoroughness, different combinations of key words were used. Significant terms included [ionizing radiation], [diagnostic], [medical radiation], [meningioma], and [benign brain tumors]. For supplementary articles, references of accepted systematic reviews were used as suggestions for additional sources. Other accepted study types were cohort studies, case-control studies, and meta-analyses.

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Table 1 The selection of the articles based on the application of exclusion and inclusion criteria (the analysis of the selected studies was used to create the Forest plot shown in Fig 2)

Study	Year of publication	Publication	Article type/use	Procedures tested	Included in meta-analysis?
Preston-Martin et al ¹⁴	1980	J Natl Cancer Inst	Case-control	FMS	No
Ryan et al ⁹	1992	Eur J Cancer B Oral Oncol	Case-control	FMS, panoramic	Yes
Rodvall et al ¹⁶	1998	Oral Oncol	Case-control	Diagnostic dental radiographs	Yes
Longstreth et al ¹⁷	2004	Cancer	Case-control	BW, FMS, panoramic, lateral cephalometric	Yes
Blettner et al ⁸	2007	Eur J Cancer	Case-control	Panoramic, CT	Yes
Umansky et al ¹⁰	2008	Neurosurg Focus	Systematic review	FMS	No
CADTH ³	2012	CADTH	Systematic review	BW, FMS, panoramic, CT	No
Claus et al ⁴	2012	Cancer	Case-control	BW, FMS, Panoramic	Yes
Lin et al ⁷	2013	Ann Oncol	Case-control	Panoramic, CT, lateral cephalometric	Yes

BW, bitewing; CADTH, Canadian Agency for Drugs and Technologies in Health; CT, computed tomography; FMS, full-mouth series.

After reviewing the accepted articles, it was necessary to include articles for meta-analysis. A summary of each article was created detailing the author, type of study, variables tested, and conclusions (Table 1). Articles were excluded if they did not report patients' history of dental or medical diagnostic radiation. Articles dealing with patients receiving higher doses of ionizing radiation such as radiotherapy patients or atomic bomb survivors were excluded, as this study attempted to focus on lower doses. Included articles dealt with dental procedures such as FMS and panoramic radiographs, and medical procedures such as computed tomography (CT) scans. Exclusion criteria included those studies that only reported odds ratios (ORs) without presenting the total numbers of cases and controls surveyed in the study. Studies that used data prior to 1945 were excluded due to unreliable radiation dose reporting. A PRISMA flow chart-generator¹³ was used to create the flow chart (Fig 1). Because each paper used different calculations to determine their ORs and 95% confidence intervals (CIs), the raw numbers of cases and controls were necessary to calculate new ORs.

Selection criteria

Initial criteria included articles relating to meningioma risk after exposure to diagnostic dental or medical radiation. A case-control or cohort study or systematic review was eligible for systematic review if the history of exposure to ionizing radiation was presented in detail in the patient (meningioma) history.

Papers were excluded based on the types of diagnostic radiation recorded. BW radiographs (exclusively) were not incorporated into the meta-analysis, as the radiation exposure dose for this procedure was considered too low in comparison to other techniques. Included methods were FMS (BWs as part of the FMS), panoramic radiographs, and CT scans of the head and neck. Other studies were excluded due to incomplete or incompatible data and unknown type of diagnostic radiation examined in the study.

Data analysis

Each article found from the initial database search was analyzed based on the inclusion criteria. Information was then extracted from all selected articles and summarized. Information screened included author, year of publication, experiment type, dose of exposure tested, total number of cases and controls, methods used to recruit participants, and history of exposure to ionizing radiation and the types of radiation. Each article was then analyzed for data to determine if the given statistics were eligible for data analysis. If this was the case, the data were then evaluated to determine how many subgroups were available for study based on different ionizing radiation doses administered by different procedures.

After compiling available data for meta-analysis, in order to standardize ORs and 95% CIs of each study, these statistics were recalculated based on the reported number of event



Dr. L. KRISHNA PRASAD

DEAN

cases (meningioma patients who had been exposed to diagnostic x-ray radiation) and event controls. New ORs were calculated using the formula:

$$OR = \frac{([event\ cases]/[non-event\ cases])}{([event\ non-cases]/[non-event\ non-cases])}$$

Calculations of 95% CIs were based on each OR using RevMan v5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Recalculating the ORs made statistics from different papers comparable with each other for a meta-analysis. A forest plot and a cumulative OR (95% CI) were then generated. In the cumulative statistics, each study was weighted based on sample size.

Results

After searching the online databases, 12 articles were accepted for systematic review initially. Three articles were removed for inconsistent data. Three systematic reviews and six case-control studies met the inclusion criteria. No cohort studies met the inclusion criteria. All included articles were published between 1992 and 2013. Articles studied the effects of panoramic radiographs, FMS, and CT scans.

Six studies reported statistics acceptable for meta-analysis. Some articles also reported patients' histories of exposure to different sources of diagnostic ionizing radiation, producing multiple subgroups for a single study. Eight subgroups were created and a total of 8,103 cases and 21,514 controls were pooled from accepted studies. In the meta-analysis, the ORs of four subgroups were weighted below 5% due to lower patient totals, and one subgroup consisting of significantly larger groups of cases and controls was weighted above 50%, producing a cumulative OR of 1.13 (95% CI, 1.06 to 1.19). Regardless of the weighted OR, a majority of the subgroups reported showed no increased risk of developing meningioma with exposure to ionizing radiation. Some other subgroups had an OR greater than 1.00, but their calculated 95% CI was below 1.00.

Discussion

Preston-Martin and colleagues^{14,15} published results of their case control studies performed on men and women who received medical and dental diagnostic radiation, among other factors. Both studies correlated the occurrence of meningiomas to radiation exposure. In the study where women were the subjects, the strongest association was with early exposure to diagnostic radiation (within the previous 20 years). A total of 101 case-control pairs of women and 105 pairs of men filled out

questionnaires to help the researchers make their conclusions. They also studied factors such as smoking, dietary habits, and history of therapeutic x-ray treatment in both of their studies. These two studies were excluded due to the fact that there was no clear distinction between the subjects who received diagnostic radiation as opposed to those who received therapeutic radiation. The questionnaires analyzed patient histories of x-ray exposure to radiation therapy (RT), which carried a much higher radiation dose. It was reported that some of the patients reported x-ray treatment for acne and tinea capitis. Because it was not clear as to who received radiotherapy and who received lower-dose diagnostic radiation, it was decided to exclude this patient pool. Another reason for exclusion was the time of exposure. Some control subjects were questioned about diagnostic radiation prior to 1945. The meningioma patients were not questioned regarding their diagnostic radiation exposure prior to 1945.

Ryan et al⁹ studied the effects of diagnostic dental x-rays on the risk of developing gliomas or meningiomas. Subjects pooled from South Australia were interviewed and given a questionnaire to determine their histories of exposure to panoramic radiography or FMS. Controls were matched by characteristics such as age and sex. Sixty meningioma cases, 110 glioma cases, and 417 controls were studied in this article. Researchers reported an increased risk of developing meningioma for men after exposure to diagnostic dental radiation. They also reported that choosing the unexposed control group greatly impacted the results of the study. Both the panoramic and FMS subgroups were included in the meta-analysis.

Rodvall et al¹⁶ studied the effects of dental radiography on the risk of developing tumors of the central nervous system. Subjects were pooled from a neurosurgery clinic or by telephone and given a questionnaire examining their history of dental radiography. In total, 236 cases with gliomas, meningiomas, and acoustic neurinomas were pooled, and 343 meningioma controls were found. The results suggested an increased risk of developing meningiomas after exposure to dental radiography. This study was included in the meta-analysis.

A case-control study conducted by Longstreth et al¹⁷ studied the risk of developing meningiomas after exposure to BWs, FMS, panoramic radiography, and lateral cephalometric radiography. Patients were residents of Washington State, United States of America. Random digit-dialing and Medicare lists were used to pair each case with two controls based on age and gender. Patients were interviewed to determine their histories of exposure to ionizing radiation. A total of 200 cases and 400 controls were interviewed. Only the FMS group was found





to increase the risk of developing meningiomas, although the data did not indicate a dose-response relationship. The subgroups for FMS and panoramic radiographs were included in the meta-analysis.

A study by Neglia et al⁶ examined a group of cancer survivors who underwent radiotherapy. The study included patients who had developed gliomas as well as meningiomas. A total of 66 meningioma patients were interviewed and matched with 464 control subjects based on their personal characteristics, such as age and sex, and time since the original diagnosis. It was determined that a patient's risk of developing meningioma was higher after exposure to radiation above 30 Gy. This study was not included in the meta-analysis because of the type of radiation exposure studied. Inclusion criteria required that the article discuss the risk of meningioma development after lower doses of ionizing radiation from diagnostic dental radiographs. The study conducted by Neglia et al,⁶ however, referred to sources of higher doses, such as cancer treatment. Because this study did not address the level of radiation being studied, it was excluded after review.

A case-control study by Blettner et al⁸ studied the effects of medical diagnostic radiation on the risk of developing meningiomas, gliomas, or acoustic neuromas. Studied exposures included panoramic x-rays and CT scans, among other potential factors such as mobile phone use. Patients in Germany were interviewed, and prior exposure to ionizing radiation was self-reported. A total of 381 meningioma cases and 762 controls participated in the study. The study reported no increased risk of developing meningiomas after exposure to x-rays of the head or neck; however, it did produce a slightly increased risk after exposure to CT scans. This study⁸ was included in the meta-analysis.

A systematic review conducted by Umansky et al¹⁰ studied the effects of various sources of ionizing radiation on the risk of developing meningiomas. The researchers studied the risks of developing meningiomas after childhood cancer or tinea capitis treatment, exposure to dental x-rays, or exposure to atomic explosions. They discussed five articles relating to low-dose exposure to ionizing radiation and concluded that there may be an increased risk of developing meningiomas after exposure to diagnostic dental radiation. This article¹⁰ was not included in the meta-analysis, since exposure to ionizing radiation from non-dental sources may have produced confounding results.

Claus et al⁴ studied the effects of various dental diagnostic procedures on the risk of developing meningiomas. They used questionnaires to meningioma patients from across the United States to evaluate the history of dental diagnostic radiation. A total of 1,433 cases and 1,350 controls were gathered. These

participants reported having BW, FMS, and panoramic radiography. The study reported a potentially increased risk of developing meningiomas for higher-dose procedures such as FMS and for patients who were exposed to diagnostic radiation at a young age. This study was included for the present meta-analysis; however, although the FMS and panoramic radiographic results were included from Claus et al,⁴ BW radiographs were excluded. The rationale for this was that there were no data on whether there were two BW radiographs or four BW radiographs and that memory recall was used for determining whether or not BW radiographs were taken. Natural background radiation is approximately 6.2 μ Sv per day¹⁸ and the exposure from a set of four BW radiographs is approximately 3.4 μ Sv,¹⁹ far less than the daily background radiation. Therefore, that subgroup was excluded.

The Canadian Agency for Drugs and Technologies in Health (CADTH)³ conducted a review of literature concerning the relationship between dental x-rays and CT scans and the risk of developing meningiomas. A literature search was conducted on databases such as PubMed and the Cochrane Library. Articles were then evaluated by a reviewer who included articles relating dental x-ray exposure to the risk of developing any type of cancer. Each article was then summarized and its results were evaluated. The researchers found three articles relating to meningioma risk after exposure to ionizing radiation. The paper³ concluded that there may be a connection between dental x-rays and the risk of developing meningiomas.

A study conducted by Lin et al⁷ considered the effects of diagnostic dental radiation on the risk of developing benign brain tumors (BBTs) such as meningiomas and malignant brain tumors (MBTs) such as gliomas. Cases and controls were pooled from Taiwan National Health Insurance care data. In total, 4,123 BBT cases and 16,492 controls were gathered. The health insurance data were also used to determine if the patient was ever exposed to diagnostic dental radiography. The researchers concluded that there was an increased risk of developing MBTs after exposure, but there was no increased risk of BBTs. This article⁷ was included in the meta-analysis.

Xu and associates¹¹ conducted a systematic review and published their results in 2015. In their study, they used seven case-controlled studies. Included in their analysis were the two studies conducted by Preston-Martin and colleagues (1980 and 1989). The reasons behind the exclusion of these two studies were stated earlier in this discussion. The inclusion of the studies that exclusively had diagnostic dental radiation was the primary goal in the present study as including those associated with radiation therapy would yield different results.

Dr. L. KRISHNA PRASAD
DEAN

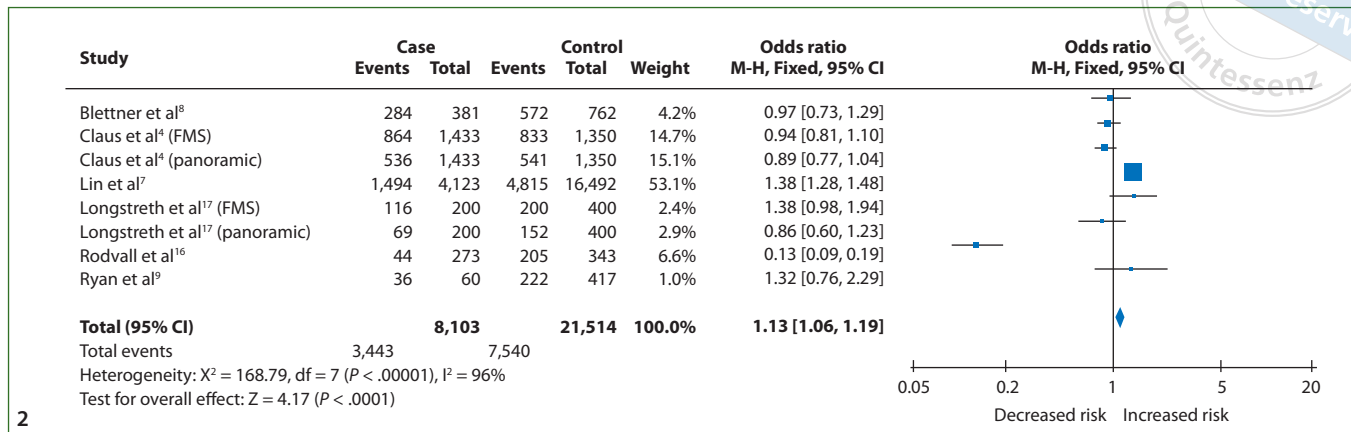


Fig 2 Forest plot of studies with data on diagnostic dental x-ray exposure and events of meningioma occurrence recorded after excluding studies with exposure to therapeutic radiation. The analysis included data from six studies with a total of 8,103 cases and 21,514 controls. The P value for heterogeneity was .00001.

Forest plot interpretation

As shown in Fig 2, the OR and 95% CI calculated from the subgroups included in the meta-analysis was 1.13 (1.06 to 1.19). Five out of eight subgroups presented ORs equal to or less than 1.00. Five out of the eight subgroups had ORs lower than 1.00, while only one subgroup, which was weighted heavily due to its large patient pool, produced ORs and 95% CI greater than 1.00. Although there are inconsistencies in the ORs calculated for each subgroup, a majority of them suggest that prior exposure to low doses of ionizing radiation, such as FMS and panoramic radiography, do not result in a significantly increased risk of developing meningiomas. Although some studies have reported increased risks, a majority of the subgroups do not show any correlation between diagnostic dental radiation and the risk of developing meningiomas.

Included studies measured the effects of many sources of diagnostic dental radiation, such as FMS, panoramic radiography, CT scans, and other procedures, and found no relationship between these sources and the patients' risks of developing meningiomas. Many studies have found that the risk of developing meningiomas has significantly increased with exposure to higher doses of radiation, such as radiotherapy.⁶ However, the overall OR, 1.13 (95% CI, 1.06 to 1.19), indicates that this increased risk is not established for lower doses of diagnostic radiation.

Some limitations of the meta-analysis include the validity of included studies and the limitations of case-control studies. Some studies reported uncertain conclusions, such as a lack of

a dose-response relationship between ionizing radiation and risk of developing meningioma.⁴ It is believed that some form of bias exists in these studies, particularly recall bias for older patients in studies utilizing self-reported radiation history.²⁰⁻²²

The study by Claus et al⁴ was analyzed by several authors including Dirksen et al,²³ who questioned the role of low-dose exposure to ionizing radiation leading to detectable increase in the risk of tumors. They came to the conclusion that the study by Claus et al⁴ provided very little or no evidence of an increased risk of meningioma for individuals exposed to low doses of dental radiation. They recommended that future studies should take into consideration dosimetry as well as error analysis beyond standard CIs. The role of questionnaires and the use of recalled information from the past, in some cases spanning decades, is controversial, especially if used for calculation of risk. Another common source of bias in case-control studies is selection bias. For some studies, the response rate differed considerably between cases and controls. One study reported a response rate of 84.4% for cases and only 60.6% for controls.³ The effect of this type of bias on the results of the studies remains unclear. However, these sources of variation may explain the inconsistency in ORs produced by the subgroups.

The Department of Health and Human Services included both x-rays and gamma radiation in their list of potential carcinogens in 2005.²⁴ Perhaps the perception of this risk along with the diagnosis of malignancy influences the response to specific questions on malignancy-related topics. The bias in these situations is more concerning for research purposes, as



there will ultimately be an effect on the data collection, especially if a recall questionnaire has been used. Over time, better diagnostic studies that use better methods of data collection and more predictable outcomes will lead to a better assessment of the diagnostic dental radiation risk. ■

Summary and conclusions

No evidence for correlation between diagnostic dental radiation and occurrence of new meningiomas was found based on this meta-analysis.

No cause and effect relationship can be derived from these data between dental x-rays and meningioma occurrence.

This study in no way implies that low dose is safe. As Low As Reasonably Achievable (ALARA) and As Low As Diagnostically Acceptable (ALADA) principles must be followed for any diagnostic dental radiation procedure.²⁵

Surveys requiring recollection from past decades are of questionable reliability, especially when participants are aware of their current diagnosis and the role of radiation as a potential cause of malignancy.

An ideal study for risk estimates would be a prospective design that follows radiation-exposed patients over a period of time to see if the incidence of new meningiomas is greater than in non-radiation exposed patients. Unfortunately, this form of prospective study may not be feasible in the United States due

to time constraints and difficulty in locating subjects, as people move away and generally are lost to follow-up after some time.

If the current trends of ALARA, technological innovation, including wellness initiatives, and overall reduction in the use of diagnostic dental radiation continue, the risks associated with the use of ionizing radiation can be substantially mitigated.

A reduction in dose from diagnostic dental imaging may be achieved by stricter implementation of NCRP/ICRP (National Council on Radiation Protection and Measurements/International Commission on Radiological Protection) guidelines including appropriate selection and justification of examinations by following published guidelines, utilization of direct current (DC) x-ray machines, rectangular collimation, and appropriate radiation protection for the patient.

Although the present study can conclude that, based on the included research, no association could be found between ionizing radiation from dental sources and development of meningiomas, it is still incumbent upon the dental profession to use appropriate selection, dose-sparing technologies, and meticulous clinical practices to mitigate risk to patients. Adherence to ALARA and ALADA principles, and sound radiographic selection criteria^{26,27} are recommended. It is hoped that information from the present study, as well as others to come, can provide targeted risk information that can assist clinical decision making in specific potential benefit versus potential risk analysis.

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Dr. L. KRISHNA PRASAD
DEAN

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Comparing Caries Experience between *Azadirachta indica* Chewing Stick Users and Toothbrush Users among 35-44-Year-Old Rural Population of Southern India

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ABSTRACT

Objectives: To compare the caries experience between *Azadirachta indica* chewing stick users and toothbrush users among 35-44-year-old rural population in Southern India. **Materials and Methods:** This *ex post facto* research was conducted in the rural parts of two sub-administrative areas of a district in the Southern Indian state of Andhra Pradesh. The sample size for the study was determined to be 400, with 200 subjects in each group. Subjects following indigenous oral hygiene methods were identified using an interviewer-administered questionnaire. After obtaining 200 subjects using *A. indica* chewing sticks, age, gender, and socioeconomic status matched controls using toothbrush were identified. American Dental Association type III examination was carried out to record caries experience (decayed missing filled teeth (DMFT) Index) after obtaining informed consent and thus obtained data were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS) software, version 20. **Results:** It was observed that the caries experience was more in toothbrush users compared to subjects following indigenous methods (DMFT, 4.38 ± 1.93 vs. 3.54 ± 1.02). Similar results were obtained when the decay component of DMFT index was exclusively compared. No significant difference in the plaque scores and the mean number of filled, missing teeth was observed between the two groups. **Conclusion:** Though conclusive results cannot be drawn from this study about the positive influence of indigenous methods on caries experience, the results emphasize the cardinal need to more thoroughly understand the potential benefits of indigenous methods before dismissing them as retrogressive approaches.

KEYWORDS: Caries experience, indigenous, oral hygiene, tooth brushing

INTRODUCTION

Despite enormous scientific progress in the past few decades, there are people who follow health-care practices based on the knowledge they gained by virtue of practical engagement in day-to-day life. This knowledge, though not often backed up by scientific evidence, conventionally is a result of rational reasoning over generations and continues to exist in the cultures of its origin.^[1] India being a country with large ethnic society and huge biodiversity, it is not uncommon to find indigenous health-care practices and the magnitude of these primordial practices only

increases with regard to oral health care. Although such oral health-care practices possess strong cultural and economic justifications, it is more important to determine whether these indigenous methods of oral health care have positive oral health outcomes.

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India has an ancient history of using traditional oral hygiene methods. *Charaka Samhita*, an Ayurvedic medicine treatise, describes two types of mouthwashes, namely *Gandoosha* and *Kavalagra*, the use of which were common.^[2] Various forms of indigenous oral hygiene practices include the use of salt, charcoal, ash, cool tea leaves, lotus leaves, tea polyphenols, coconut leaves, sunflower oil, and a variety of chewing sticks.^[3] History suggests that Babylonians used chewing sticks 7000 years ago. The use of this oral hygiene method is still prevalent in some countries.^[4-6] Use of chewing sticks was also a routine practice in rural India since ancient times. Conventionally used chewing sticks are obtained from *Acacia arabica* (babul), *Psidium guajava* (guava), *Azadirachta indica* (neem), *Mangifera indica* (mango), and *Salvadora persica* (miswak). These sticks are reckoned to be effective in increasing the salivation and assisting in the expulsion of oral microorganisms. The affordable nature of the traditional oral hygiene practices, locally discussed benefits such as exercise for masticatory complex led to the increased use of these practices, especially in rural areas. In case of *A. indica*, nimbodin, azadirachtin, and nimbinin are active compounds responsible for antibacterial activity. *A. indica* also shows antifungal, anti-ulcer, and antinociceptive activity. The leaves and the bark extract of the tree are good sources of antioxidants and boosts the immune response in gingiva and oral tissues.^[7] There are 300 structurally distinct constituents in neem, majority of which are limonoids that manifest their effects by modulating multiple cell signaling pathways.^[8] The phytochemicals of *A. indica* have multiple advantages including prevention of noncommunicable diseases such as cancer, cardiovascular disease, and diabetes.^[9] Neem seed oil is reported to be used in cosmetics, toothpastes, and soaps.^[10] The World Health Organization (WHO) has suggested the use of these chewing sticks as effective tools for oral hygiene, and the influence of chewing sticks on aerobic and anaerobic microorganisms has been studied. However, it is said that the traditional preferences determine the choice of these sticks than clinical effectiveness.^[11] In this context, it becomes important to know the effectiveness of indigenous oral hygiene practices in reducing plaque accumulation and caries incidence as compared to that of toothbrush users.

With this background, the objective of this study was to document the differences in oral hygiene and caries experience between subjects using *A. indica* chewing sticks and modern oral hygiene practices.

MATERIALS AND METHODS

This *ex post facto* research was conducted in the rural parts of two sub-administrative areas of a district

in the Southern Indian state of Andhra Pradesh having access to oral health care at a teaching dental institution located within 15 km distance from these areas. Transect walks were conducted in the rural parts of the aforementioned administrative divisions before drawing the study protocol. It was observed that those people who regularly use toothbrush resort to use of chewing sticks occasionally during agricultural labor. It was concluded that occasional users of chewing sticks would not be included in the study. No other forms of indigenous oral hygiene practices were considered in this study as *A. indica* chewing stick was the most exclusive indigenous oral hygiene practice in the study area.

The institutional review board of the teaching dental institution with protocol number 180/IEC/SIBAR/2018 approved the study. Sample size was determined based on the results of a pilot study conducted in the study area with caries experience as the dichotomous dependent variable. Sample size to be obtained was 400 with 200 participants each from the indigenous and toothbrush groups. House-to-house survey was conducted in the study region during the months of January to February 2018, to inquire about the oral hygiene practices of the people aged 35–44 years. The basis for the selection of the age group was that the age group was recommended as the standard monitoring group for health conditions of adults and the full effect of dental caries and the level of severe periodontal involvement. Subjects with severe medically compromising conditions and those who were not local to the study area were excluded from the study. Informed consent was obtained from all the participants who satisfied the inclusion criteria before clinical examination. An interviewer-administered questionnaire was used to obtain the demographic data and the details regarding oral hygiene practices being followed. Socioeconomic status of the subjects was determined according to BG Prasad scale.^[12]

American Dental Association type III clinical examination was carried out by a single calibrated investigator to rule out the possibility of inter-examiner variability. Plaque index and decayed missing filled teeth (DMFT) index/WHO 1987 modification were recorded as the primary outcome variables. Caries experience scores were dichotomized with the target suggested by WHO/Federation Dentaire Internationale (World Dental Federation) by 2000 as reference (DMFT score, 3).

STATISTICAL ANALYSIS

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM, Armonk, NY) and Mann–Whitney *U* test, chi-square

test, and multiple linear regression were carried out to analyze the data.

RESULTS

Of the 400 subjects involved in the study, 184 (46%) were males. The mean age of the study participants was 39.42 ± 2.41 years and no significant differences were observed in the mean age between participants from *A. indica* chewing stick using group and toothbrush using group. No significant differences were observed in the socioeconomic status of the study subjects with 59.25% of people belonging to lower middle class (n = 237). Most of the study participants were agricultural laborers and share similar dietary habits.

No significant difference was found in the mean number of teeth present between the two groups. Table 1 shows the differences in plaque scores and caries experience scores between the two groups. Higher mean plaque scores were observed among toothbrush users compared to that among chewing stick users, though the difference was not statistically significant. A significant difference was observed in the mean caries experience between the study groups, with toothbrush users showing higher caries experience. When solitary components of DMFT were compared, no significant differences were observed between the groups except in mean number of decayed teeth.

Table 2 shows the difference in proportion of subjects having DMFT scores of more than 3 between the two

Table 1: Comparison of mean plaque scores, mean number of decayed, missing, and filled teeth as solitary scores and composite caries experience (DMFT) scores between toothbrush users and subjects using *Azadirachta indica* chewing stick

Variable	Type of oral hygiene practice		P value
	Toothbrush	<i>Azadirachta indica</i> chewing stick	
Mean plaque score	1.09 ± 0.26	0.87 ± 0.14	0.07
Mean no. of decayed teeth	2.28 ± 0.62	1.63 ± 0.49	0.013*
Mean no. of missing teeth	0.46 ± 0.18	0.39 ± 0.13	0.078
Mean no. of filled teeth	1.64 ± 0.32	1.52 ± 0.41	0.092
DMFT Score	4.38 ± 1.93	3.54 ± 1.02	0.001*

*P ≤ 0.05 considered statistically significant
Mann-Whitney U test
DMFT = decayed missing filled teeth

Table 2: Association between type of oral hygiene aid and caries experience

Group	DMFT score		Prevalence odds ratio	95% confidence interval (CI)	P value
	≤3 (%)	>3 (%)			
Toothbrush	81 (40.5)	119 (59.5)	1.908	1.21–3.46	0.002*
<i>Azadirachta indica</i> chewing stick	113 (56.5)	87 (43.5)			

*P ≤ 0.05 considered statistically significant
Chi-square test

Table 3: Influence of oral hygiene practices on caries experience, mean number of decayed, missing, filled teeth, and composite caries experience (DMFT)

Dependent variable	Oral hygiene practice	Exponent [β]	95% confidence interval	P value
DMFT	Toothbrush (with <i>Azadirachta indica</i> chewing stick as reference)	3.18	2.4–3.96	0.01*
	Plaque score	0.89	0.78–0.96	0.072
Decayed teeth	Toothbrush (with <i>Azadirachta indica</i> chewing stick as reference)	3.74	2.81–4.63	0.0012*
	Plaque score	1.82	1.26–2.38	0.046*
Missing teeth	Toothbrush (with <i>Azadirachta indica</i> chewing stick as reference)	1.46	0.81–2.02	0.08
	Plaque score	0.64	0.49–0.78	0.12
Filled teeth	Toothbrush (with <i>Azadirachta indica</i> chewing stick as reference)	1.302	0.63–1.93	0.094
	Plaque score	0.83	0.75–0.91	0.231

Multiple linear regression analysis
*P ≤ 0.05 considered statistically significant
DMFT = decayed missing filled teeth


Dr. L. KRISHNA PRASAD
DEAN
SIBAR INSTITUTE OF DENTAL SCIENCES
GUNTUR-522509, A.P., India.

groups. Table 3 shows the results of multiple linear regression with caries experience and components of caries experience as the continuous dependent variables, and type of oral hygiene practice and plaque scores as the independent variables. Subjects using toothbrush were found to be having higher incidence of decayed teeth and composite caries experience compared to those following indigenous oral hygiene measures.

DISCUSSION

It has been long acknowledged that dental caries has multi-factorial etiology. An attempt was made in this study to compare the exclusive influence of oral hygiene aid on caries experience by offsetting the other potential influential factors as people belonging to the same culture, geographical region, age group, equal access to oral health-care services, and who follow similar dietary practices were selected. *Ex post facto* research provides an opportunity to gain insights into the differences in outcomes between two naturally divided groups that have been observing a different routine, which could influence the outcome, for considerably longer period. This could be seen as a pragmatic alternative for experimental design in the determination of causal association, where conduct of experimental studies is not possible for ethical or practical reasons.

There is enough evidence in literature putting forth the antibacterial properties of *A. indica*. The antibacterial properties of *A. indica* against *Streptococcus mutans* and *Streptococcus feacalis* were reported by Siswomihardjo et al.^[13] Packia Lekshmi et al.^[14] advocated that chloroform extract of *A. indica* leaf inhibited *S. mutans* and *S. salivarius* and is useful against dental caries. Bhuiyan et al.^[15] discussed the bactericidal nature of acetone extract from *A. indica* bark against *S. sobrinus* and purported the anticariogenic properties of *A. indica*. It was observed that a mucoadhesive gel containing *A. indica* was superior to chlorhexidine gluconate mouthwash in reducing plaque index and salivary bacterial counts.^[16] A tree, which is extensively used in Ayurvedic, homeopathic, and Unani medicine, *A. indica* is now being described as a wonder tree of modern medicine as well in view of the aforementioned antibactericidal properties together with its antihyperglycemic, anti-inflammatory, antioxidant, and immunomodulatory properties.^[17]

Findings of this study highlight the nonsignificant differences between toothbrush users and those using *A. indica* chewing sticks in the primary outcome of plaque scores. Even the caries experience evaluated using dichotomized DMFT scores, the other outcome

was slightly less among indigenous oral hygiene practices. Chewing sticks are commonly used oral hygiene aids across the globe. There are ranges of trees from which chewing sticks are routinely obtained based on the geographic region. In the study area, *A. indica* trees are ubiquitous, and though their chewing sticks are commercially available, subjects in this study procured chewing sticks from their surroundings. Use of chewing sticks is very prevalent in the rural areas and among tribal communities. Kadanakuppe and Bhat,^[18] in 2013, conducted a study in the Iruliga tribal community, Karnataka, India, and found that 79.8% of the Iruligas use chewing sticks. Only 0.03% reported using toothbrush in the aforementioned study, whereas 14.9% used finger with either rangoli powder, salt, or charcoal.

No differences were observed in this study in the proportion of males and females between chewing stick users and the toothbrush users. Kahar et al.,^[19] in 2016, reported that males resort to indigenous oral hygiene practices with increased frequency compared to females. Occupation of the subject may play a role in the choice of oral hygiene aid, with subjects from those occupations demanding early morning work away from home, resorting to indigenous oral hygiene practices more commonly. The contrasting finding observed in this study could be due to the fact that most of the study subjects were agricultural laborers regardless of gender.

No significant difference was observed in the plaque scores between the two groups. These findings were consistent with those reported by Bhambal et al.,^[20] 2011. Contrary to the alleged problems with chewing sticks that it may not be possible to access all the surfaces of whole dentition, studies suggest that chewing sticks are as effective as toothbrushes with regard to maintenance of oral hygiene.^[21]

In this study, caries experience was more among the subjects using toothbrush compared to those using *A. indica* chewing sticks. Ezoddini-Ardakani,^[22] in 2010, reported that the risk for dental caries was almost 10 times more among the subjects using toothbrush compared to those using chewing sticks. The low incidence of dental caries among chewing stick users had been attributed to the improved mechanical cleansing action of these sticks and the antimicrobial properties. Islam et al.^[23] published similar observations in 2007 attributing the low incidence of dental caries to less plaque deposits among chewing stick users. Norton and Addy^[24] reported that the plaque scores and caries incidence were less among chewing stick users in their study conducted in Ghana, West Africa. Literature


Dr. L. KRISHNA PRASAD
DEAN

SIBAR INSTITUTE OF DENTAL SCIENCES
GUNTUR-522509, A.P., India.

suggests that chewing sticks from *A. indica*, miswak, babul, and so on, contain fluoride and could have the benefits of topical fluoride in remineralization of the teeth.^[25]

Though an abundance of literature comparing the mechanical cleansing properties of toothbrush and chewing sticks was available, differences in the incidence of dental caries between modern toothbrush users and those using chewing sticks was seldom reported. In this study, an attempt was made to compare the caries experience between the two oral hygiene methods. Plaque index was taken to rule out the confounding effect plaque scores could have on caries experience. DMFT index was used to record the caries experience as elimination of “F” component may lead to spurious results, especially if the rate of utilization of dental services is different between these groups. Moreover, care was taken only to include those teeth that were filled as a consequence of decay under “F” component. The debate on inclusion of “M” component in DMFT owing to the unclear reasons for tooth loss is still open without consensus.^[26] A study by Mustafa *et al.*^[27] among participants from Saudi Arabia reported less caries incidence among miswak users compared to non-miswak (toothbrush) users. Another study conducted by Shetty *et al.*^[28] comparing the effect of commercially available tooth paste and herbal tooth paste (Munident) on *Streptococcus mutans* counts reported comparable findings in both the groups. However, toothbrush was used by participants in both the groups in the aforementioned study.

A study conducted by Sirisha *et al.*,^[29] in 2014, in the Guntur area on low socioeconomic adults revealed that 40.4% are using twig as an oral hygiene aid and the overall mean DMFT score was 3.69 ± 2.71 , which is on par with this study. Owing to the fact that subjects in either group belong to the same geographical area and have similar dietary habits, analysis of diet was not carried out.

Over the centuries, Indians have used indigenous methods of oral hygiene practices. If there is no harm due to these practices on oral tissues, such practices can be continued as they are well accepted by the community. However, randomized controlled trials are to be undertaken to establish both the clinical efficacy and to rule out the possible adverse effects with the use of indigenous oral hygiene aids. Though the type of toothpaste whether fluoridated or non-fluoridated was not documented in this study, a pilot study in the study area revealed that almost all the toothpastes being used among the study population are fluoridated. However, this potential moderating effect of fluoride in toothpaste

would be offset by the fluoride- and calcium-releasing properties of the chewing sticks. The limitations of the study are that periodontal status was not considered and examination for dental caries was limited to the coronal portions of teeth using DMFT index. Future research needs to focus on differences in periodontal health and the occurrence of wasting diseases of teeth between chewing stick users and toothbrush users.

CONCLUSION

The use of indigenous oral hygiene methods, though might have reduced over the years, continues to be observed by considerable number of people in the rural areas. Although conclusive results cannot be drawn from this study about the positive influence of indigenous methods on caries experience, the results emphasize the cardinal need to more thoroughly understand the potential benefits of indigenous methods before dismissing them as retrogressive approaches.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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Factors Affecting Stress among Students in Dental Colleges of Neo-Capital State in India

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Abstract

Background: Stress in dental students may be multifactorial, arising from the academic and sociocultural environment. Identifying the sources of stress helps in advocating policy changes and strategies to alleviate the stress. **Aim:** To evaluate the factors affecting stress among undergraduate and postgraduate dental students of Andhra Pradesh, India. **Materials and Methods:** A self-administered Modified Dental Environmental Stress questionnaire was distributed among 390 dental students who participated voluntarily. Data were analyzed using IBM SPSS Statistics, version 20, and ANOVA and Student's *t*-test were conducted where $P \leq 0.05$ was considered statistically significant. **Results:** Students perceived more stress from domains related to the future prospects (2.53 ± 0.97) and personal factors (2.35 ± 0.66) followed by stress associated with faculty (2.12 ± 0.78) and clinical/academic factors (2.12 ± 0.55), with a statistically significant difference ($P = 0.001$). Third- and final-year undergraduate students perceived higher levels of stress regarding future, clinical/academic factors, and personal factors. Students who chose dentistry as their first choice of admission experienced less stress compared to the students whose choice of admission was medicine or other courses. **Conclusion:** Overall stress levels were slight to moderate and were significantly higher among the third- and final-year undergraduate students.

Keywords: Dental education, dental students, India, occupational stress, professional burnout

INTRODUCTION

According to a report of the Global Congress in Dental Education (2008), "Dental Education is regarded as a complex, demanding and often stressful pedagogical exposure. It involves acquisition of required academic, clinical and interpersonal skills during the course of learning." Practicing dentistry requires clinical and patient management skills, characteristics that also add to the stress perceived by the students.^[1]

The term STRESS describes external demands (physical or mental) on an individual's physical and psychological well-being, leading to a deleterious effect on academic performance.^[2] Stressors associated with dentistry include time and scheduling pressures, highly technical and intensive nature of work, and managing uncooperative patients. This resulting stress can lead to depression, anxiety, substance misuse, absenteeism, diminished work efficiency, and burnout. The

roots of this occupational stress may have their origin in the educational process as dental students experience high levels of stress during training.^[3] Lovallo defined "Occupational stress" as a state of physical and mental tension resulting from excessive demands or lack of resources.^[4]

In chronic or extreme circumstances, occupational stress can precipitate a state of "burnout" in the susceptible practitioner. Maslach and Jackson^[5] elucidated "burnout" as a unique response to frequent and intense clinician-patient contacts consisting of the following three components: emotional exhaustion (mental fatigue), depersonalization (psychological distancing from others), and reduced personal accomplishment.^[4] Professional burnout shows symptoms such as fatigue, sleeplessness, risk of infectious

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diseases (such as hepatitis B, hepatitis C, and human immunodeficiency virus), headaches, low resistance, irritation, suspicion, overconfidence, drug abuse, negative attitude, boredom, and lack of challenge.^[6]

In India, the duration of the Bachelor of Dental Surgery program is of 4 calendar years with progression examination at the end of each year, followed by 1-year rotatory internship in the dental colleges which is governed by the Dental Council of India. During the first 2 years of graduation, students are taught the basic sciences and preclinical dentistry, whereas the later years are dedicated for imparting clinical education. Several studies identified the major academic stressors among dental college students to be examinations, competition for grades, fear of failing the year, and inadequate time for relaxation.^[7]

Cooper *et al.* reported that the dental profession was ranked the most stressful when compared to other health-care professions.^[8] Previous investigators have found that 10% of dental students suffered from severe emotional exhaustion, 17% complained about a severe lack of accomplishment, and 28% reported severe depersonalization symptoms.^[9]

The ability to cope well with stress is important because stress can result in health-damaging behaviors and psychological morbidity. Recent research indicates that psychological morbidity, pathological anxiety, and emotional exhaustion in dental students are not uncommon.^[10] Hence, the purpose of this study was to identify the factors affecting stress among dental students of Andhra Pradesh.

MATERIALS AND METHODS

Study design

A cross-sectional questionnaire-based survey was conducted among undergraduate and postgraduate dental students of two dental colleges residing in the neo-capital city of Andhra Pradesh, of which one is a government dental college and the other one is a private dental college. After obtaining ethical approval, permission was sought to survey the students at the end of the whole class lectures for each of the academic years. Students were briefed on the objectives of the study and ensured that participation was voluntary and anonymous, which might help in getting their true perceptions.

Ethical approval

A detailed review of the multipronged aspect of the research and about the surrogate consent of the participants was elaborated for acceptance and approval from the Institutional Ethical Committee (Pr. 14/IEC-SIBAR/2016). The anonymity of the participants was secured by following the ethical principles of the World Medical Association Declaration of Helsinki.^[11]

Participants

Overall, 390 undergraduate and postgraduate students including 89 students of 1st-year BDS, 89 students of 2nd-year BDS, 35 students of 3rd-year BDS, 78 students of 4th-year BDS, 49 interns, and 50 postgraduates participated willingly.

Consent

Prior to the study, an informed written consent was obtained from all the individuals willing to participate and also enacted from parents or legal guardians of students aged below 18 years followed by the assent from students.

Inclusion criteria

All willing students who were present on the day of the survey were included, and on every alternative day, an effort was made to trace the students who remained absent on the survey day.

Questionnaire design

Pretested, self-administrated, modified Dental Environmental Stress (DES) Questionnaire was used in the study to measure the sources of stress. This 34-item questionnaire was further divided into four domains: (1) stress due to clinical and academic factors, (2) stress due to faculty, (3) stress about career, and (4) personal stress. The investigated items were based on those examined previously by several researchers in studies of stress among dental students.^[12-14]

The questionnaire consists of two sections; in the first section, demographic characteristics such as age, gender, and year of the study were included. The second section included 34 questions related to possible sources of stress which are divided into four domains. Each item was scored using a 5-point Likert scale of severity where 1 = not stressful, 2 = slightly stressful, 3 = moderately stressful, 4 = severely stressful, and 5 = not applicable. In addition to this questionnaire, two more questions were asked about their choice of joining dentistry, that is, whether dentistry is their own choice or due to parental pressure, and the second question was about their first choice of course, that is, whether it is medicine, dental, or other courses [Appendix I]. A pilot study was conducted to test the internal consistency and reliability of the questionnaire, by considering a convenience sample of 50 from the same population. Cronbach's alpha was computed to measure the modified DES scale's internal consistency and had an alpha of 0.928.

Statistical analysis

Data were entered and analysis was performed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.^[15] Descriptive statistics, Student's *t*-test, and one-way ANOVA were employed for data presentation and analysis. Descriptive statistics were used to describe the samples' covariate characteristics (gender, age, and year of the study) and the responses to the stress items. $P \leq 0.05$ was considered statistically significant.

RESULTS

Demographic profile

A total of 390 dental students participated in the study, out of which 24.4% were male and 75.6% were female, and majority of the participants belong to 17–21 years' (62.6%) age group, with percentage of participation as 22.8%, 22.8%, 19.1%, 20%, 12.6%, and 12.8% for 1st-, 2nd-, 3rd-, and 4th-year BDS students, interns, and postgraduates, respectively [Table 1].

Table 1: Demographics and academic details of the participants

Variables	n (%)
Gender	
Female	295 (75.6)
Male	95 (24.4)
Age (years)	
17-21	244 (62.6)
22-26	128 (32.8)
27-31	14 (3.6)
32-36	2 (0.5)
>36	2 (0.5)
Year of the study	
1 st BDS	89 (22.8)
2 nd BDS	89 (22.8)
3 rd BDS	35 (9.0)
4 th BDS	78 (20.0)
Intern	49 (12.6)
Postgraduates	50 (12.8)
Joining dentistry	
Parent's choice	167 (42.8)
Own choice	223 (57.2)
First choice of admission	
Medicine	304 (77.9)
Dental	51 (13.1)
Other courses	35 (9.0)
Institute	
Private	291 (74.6)
Government	99 (25.3)

Overall 57.2% ($n = 223$) of students opted dentistry out of his or her own will and 42.8% ($n = 167$) opted dentistry due to parental pressure; 78% ($n = 304$) of students' first choice of admission was medicine, whereas only 13% ($n = 51$) of students' first choice was dentistry.

Stressors in dental students

The mean DES score perceived by the dental students was high for the stress domain of stress about future (2.53 ± 0.55) followed by the domains, namely personal stress (2.35 ± 0.55), stress associated with faculty (2.12 ± 0.78), and clinical/academic stress (2.12 ± 0.55) [Figure 1], and there was no statistically significant difference in various stress domains with respect to gender ($P = 0.907$).

Students who opted dentistry due to parental pressure and students whose first choice of admission was medicine and other courses showed higher level of stress for all the domains [Table 2].

Table 3 shows the comparison of means of stressors according to academic classes where 3rd-year BDS students perceived higher levels of stress for domains such as stress due to clinical/academic factors (2.46 ± 0.51 , $P < 0.001$), stress about future (2.89 ± 1.04 , $P < 0.001$), and personal stress (2.53 ± 0.81 , $P = 0.169$), where 4th-year BDS students perceived higher stress for domains such as stress associated with faculty

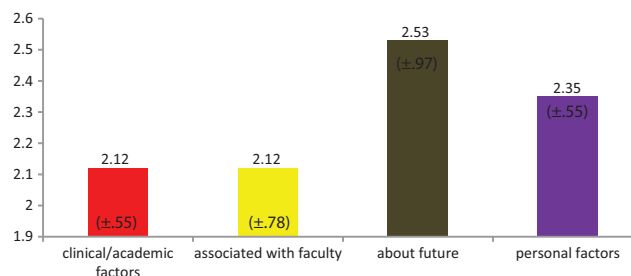


Figure 1: Mean (standard deviation) Dental Environmental Stress scores according to the stress domains

(2.51 ± 0.80 , $P < 0.001$) when compared to their counterparts. Private college dental students expressed higher level of stress for domains such as stress associated with faculty (2.16 ± 0.79) and personal stress (2.35 ± 0.66), whereas government college dental students expressed higher level of stress for domains such as stress due to clinical/academic factors (2.16 ± 0.50) and stress about future (2.72 ± 1.03) [Table 4].

Tukey's *post hoc* test was done to find out the difference between all possible pairs of academic years within the domains of stress due to clinical/academic factors and stress associated with faculty. It was identified that the students from the preclinical years were significantly different from their counterparts with regard to their mean score associated with stress due to clinical/academic factors. No significant differences were found in this regard between students from different clinical years. However, no clear pattern was observed in the mean scores associated with stress from faculty [Table 5].

DISCUSSION

Today, India has the largest dental education system in the world with 310 dental colleges, of which 268 are private and 42 are government colleges, with 26,000 undergraduate and 5400 postgraduate dentists graduating every year. It is likely that, by 2020, India might have a surplus of about 1 lakh dentists in the country.^[16] The rapid growth in the production of dentists has not helped to address the problems of the public health system further; instead, it has led to the imbalance in distribution and unemployment, indicating the public health dentist's role in planning dental health workers' availability and addressing the existing inequality.^[17] This inequality leads to an increase in insecurity toward settlement and opportunities, which could be the reason for the high stress levels toward career/future prospect in dental students in this study.

Stress levels were notably least in students who joined dentistry by their own choice and in students whose first choice of admission was dentistry, as the increased likelihood for the dentistry allows the committed students work more comfortably and positively accepting challenges and stress. However, students who joined dentistry unwillingly showed high levels of stress which could be due to their low opinion and pessimistic outlook towards the dental course and its future. These findings were in accordance with the

Table 2: Mean scores of perceived sources of stress according to student's reason of joining dentistry and their first choice of admission

Stressors	Joining dentistry			First choice of admission			
	Parent's choice (mean±SD)	Own choice (mean±SD)	P	Medicine (mean±SD)	Dentistry (mean±SD)	Other courses (mean±SD)	P
Stress due to clinical/academic factors	2.15±0.55	2.11±0.56	0.607	2.15±0.55	2.11±0.61	2.12±0.55	0.158
Stress associated with faculty	2.20±0.78	2.07±0.78	0.871	2.16±0.78	1.91±0.82	2.15±0.73	0.117
Stress about the future	2.64±1.0	2.46±0.94	0.438	2.63±0.97	1.97±0.83	2.51±0.88	0.001*
Personal stress	2.38±0.69	2.32±0.65	0.471	2.36±0.66	2.20±0.74	2.43±0.54	0.192

Student's *t*-test, one-way ANOVA, *Statistically significant. SD – Standard deviation

Table 3: Comparison of different stressors' mean according to academic years of the participants

Stressors	Academic year	n	Mean±SD	P
Stress due to clinical/academic factors	1 st -year BDS	89	1.71±0.42	0.001*
	2 nd -year BDS	89	1.94±0.42	
	3 rd -year BDS	35	2.46±0.51	
	4 th -year BDS	78	2.44±0.56	
	Intern	49	2.33±0.44	
	MDS students	50	2.26±0.54	
Stress associated with faculty	1 st -year BDS	89	1.84±0.57	0.001*
	2 nd -year BDS	89	1.80±0.68	
	3 rd -year BDS	35	2.47±0.82	
	4 th -year BDS	78	2.51±0.80	
	Intern	49	2.50±0.86	
	MDS students	50	2.01±0.68	
Stress about the future	1 st -year BDS	89	2.53±0.86	0.132
	2 nd -year BDS	89	2.50±1.02	
	3 rd -year BDS	35	2.89±1.04	
	4 th -year BDS	78	2.57±0.99	
	Intern	49	2.55±1.02	
	MDS students	50	2.27±0.87	
Personal stress	1 st -year BDS	89	2.27±0.56	0.169
	2 nd -year BDS	89	2.25±0.63	
	3 rd -year BDS	35	2.53±0.81	
	4 th -year BDS	78	2.45±0.62	
	Intern	49	2.33±0.83	
	MDS students	50	2.36±0.64	

One-way ANOVA, *Statistically significant. SD – Standard deviation

Table 4: Comparison of stressors' mean according to the study institutes of the participants

Stressors	Institute	n	Mean±SD	P
Stress due to clinical/academic factors	Private dental college	291	2.11±0.57	0.096
	Government dental college	99	2.16±0.50	
Stress associated with faculty	Private dental college	291	2.16±0.79	0.136
	Government dental college	99	2.01±0.76	
Stress about the future	Private dental college	291	2.47±0.94	0.252
	Government dental college	99	2.72±1.03	
Personal stress	Private dental college	291	2.35±0.66	0.300
	Government dental college	99	2.34±0.68	

Unpaired *t*-test. SD – Standard deviation

study done at Davangere (India),^[18] Lagos (Nigeria),^[19] and Tokyo (Japan).^[20]

Overall mean scores for all the domains were observed to increase through the year of the study, with peak in the third year which is the transition period from preclinical to a clinical acquaintance, and these results are in accordance with those of the studies conducted at Nellore (India),^[7] Riyadh (Saudi Arabia),^[21,22] and Quebec (Canada),^[23] but they are contrary to the longitudinal studies conducted at seven European dental schools (Amsterdam, Belfast, Cork, Greifswald, Helsinki, Liverpool, and Manchester)^[4] and a cross-sectional study conducted in London (UK).^[24] In the present study, there was no gender discrimination in the distribution of stress levels, which is in contrast with a West Indies study^[25] where female students perceived more stress than male students.

Several factors influence the perceived stress levels of students in both government and private dental colleges. These factors vary in a wide range among private and government colleges, where the level of stress related to faculty in government dental students was the least influencing factor for stress as observed in the study and is in contrast with private college dental students.

Many dental students need to be economically independent after the graduation course. The uncertainty of settlement in dentistry makes the student feel more stressed, keeping their future in mind. This was mostly observed in the students of government dental colleges where they receive a decent stipend in their internship and postgraduate program, which was not guaranteed immediately after the completion of course.

The main limitation of this study is the generalizability as the results may differ with varying sociocultural contexts. However, this study identified the potential stressors among undergraduate and postgraduate dental students, which paves way for further research to investigate the impact of stress on personality traits, behavior, performance, and its changing trends. The factors identified are needed to be tackled by incorporating the stress-coping measures, to reduce the stress, as well as to bring out the best performance from students in a healthy and stress-free environment. It is necessary to include subjects such as stress management, practice management, and communication skills. Future research needs to consider the use of a more precise measure to evaluate the stress level to aid in better understanding of dental students' experience and accordingly improving their learning environment.

Table 5: Multiple pair-wise intergroup comparison of stress domains according to the participants' academic year

Stressors	Academic year	P					
		1 st -year BDS	2 nd -year BDS	3 rd -year BDS	4 th -year BDS	Intern	PGs
Stress due to clinical/academic factors	1 st -year BDS	-	0.02*	0.001*	0.001*	0.001*	0.001*
	2 nd -year BDS	0.02*	-	0.001*	0.001*	0.001*	0.001*
	3 rd -year BDS	0.001*	0.001*	-	1.0	0.80	0.40
	4 th -year BDS	0.001*	0.001*	1.0	-	0.81	0.32
	Intern	0.001*	0.001*	0.80	0.81	-	0.98
	PGs	0.001*	0.001*	0.40	0.32	0.98	-
Stress associated with faculty	1 st -year BDS	-	0.99	0.001*	0.001*	0.001*	0.75
	2 nd -year BDS	0.99	-	0.001*	0.001*	0.001*	0.54
	3 rd -year BDS	0.001*	0.001*	-	1.0	1.0	0.52
	4 th -year BDS	0.001*	0.001*	1.0	-	1.0	0.001*
	Intern	0.001*	0.001*	1.0	1.0	-	0.001*
	PGs	0.75	0.54	0.05*	0.001*	0.01*	-

Post hoc Tukey's test, *Statistically significant, PGs: Postgraduates

The dental educational system should deal with the potential stressors of students by the following stress-coping measures:

- Parents should be counseled prior related to the ill effects of pressuring their children in joining an educational program and for academic excellence
- Identifying vulnerable stressful individuals early for improving their emotional and professional well-being
- Measures such as changes in length and type of curriculum, small group assignments, student-centered methodologies, reduction of educational costs, individual counseling, formative assessment of student rather than summative assessment, faculty-incorporated advising systems, and quota reduction should be implemented
- Advocating health promotion policies to ensure a future supply of effective dentists
- A public health dentist's roles are summarized as follows:
 - A public health dentist can be assigned as an academic advisor to each student throughout his or her education
 - Can collect data on what students think impossible and motivate them toward positivity
 - Also, can counsel and mold students psychologically to tackle the stress levels by making them aware of the stress consequences such as psychological disorders and help them to cope up with the problems.

CONCLUSION

Overall stress levels were slight to moderate which were significantly higher among third- and final-year undergraduate students, and the major stressors are stress about career/future and personal stress. Effective assistance from teaching staff, institutional administrators, and families is essential to cope up with the stress in dental students.

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Conflicts of interest

There are no conflicts of interest.

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APPENDIX 1

Sir / Madam,

It is an anonymous study, where the personal and institutional details are not collected. No one, including the investigator can trace the details of respondents. Hence, I request you to kindly provide the most appropriate answers. I thank you in advance for your valuable inputs.

Signature of student/parent/guardian

Age:

Gender:

Class:

Note: Mark the appropriate answers (√)

Joining dentistry was: Parents choice

Own choice

First choice of admission: Medicine

Dental

Other courses

	Not stressful (1)	Slightly stressful (2)	Moderately stressful (3)	Severely stressful (4)	Not applicable (5)
STRESS DUE TO CLINICAL AND ACADEMIC FACTORS:					
Amount of assigned work					
Lack of cooperation by patients					
Full loaded day					
Competition with batch mates					
Patients being late or not showing for their appointments					
Examinations and grades					
Difficulty in learning clinical/ preclinical procedures					
Completion of clinical/ preclinical assignments in time					
Lack of confidence in clinical decision making					
Difficulty in understanding literature					
Difficulty with English language					
Shortage of allocated time to complete clinical/preclinical procedures					
Fear of being unable to catch up if getting behind with work					
Getting an ideal case for clinical examination					



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Shaik, *et al.*: Dental students' stress in India

	Not stressful (1)	Slightly stressful (2)	Moderately stressful (3)	Severely stressful (4)	Not applicable (5)
STRESS ASSOCIATED WITH FACULTY:					
Receiving criticism from staff for academic or clinical work					
Availability of faculty in clinic/preclinical lab					
Atmosphere created by faculty in clinic/preclinical lab					
Amount of unfairness/biased attitude of faculty					
Rules and regulations of the faculty					
Unapproachability of the teaching faculty					
Attitudes of staff towards women dental students					
Differences in opinion between staff members regarding clinical/preclinical procedures					
STRESS ABOUT THE FUTURE:					
Fear of not having the possibility to pursue a post graduate program					
Fear of un employment after graduation					
Lack of confidence to be a successful dentist					
PERSONAL STRESS:					
Relations with members of opposite Gender					
Lack of time for relaxation					
Staying away from family					
Lack of comfortable atmosphere in living quarters/hostels					
Fear of failing a course or year					
Fear of facing parents after failure					
Financial resources					
Personal health					

Any factors that are causing stress (Describe briefly):

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Utilization of health services in Tenali Mandal, Andhra Pradesh- A cross-sectional study

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ABSTRACT

Introduction: India has a plurality of health care with different systems of medicine delivered by government and local bodies in hospitals and clinics. Public hospitals provide 60% of all hospitalizations, while the private sector provides 75% of all routine care. Utilization is the actual attendance by the members of the public at health care facilities, which measures the number of visits per year or the number of people with at least one visit during the previous year, serves as an important tool and acts as a guiding path in understanding disease profile and also helps resource allocation. **Materials and Methods:** A cross-sectional study was done using the National Pathfinder survey (stratified cluster random sampling) to know the health care utilization, profile, and pattern in Tenali Mandal, Guntur, Andhra Pradesh. **Results:** The study sample comprised of 1,500 subjects who were equally divided among 5 age groups. Out of 1,500 participants, 52.8% were females and 47.2% were males. In total, 71.7% of the study participants have utilized health services, with majority of them (44.5%) had availed services within the last 6 months with prime reason of fevers (15.8%), while the main barrier for not seeking care was distance (17.03%), almost an equal proportion of the population sought care for their problems through home remedies and over-the-counter. **Conclusion:** There is an utmost need to minimize barriers of utilizing by making them aware of the health problems, so that they develop a positive attitude toward health care utilization. Therefore, knowledge of utilization of health services and associated factors is important in planning and delivery of interventions by the primary care physicians to improve health services coverage.

Keywords: Barriers, health care utilization, health services, healthcare delivery system

The fundamental principle of equity is equal treatment for all socio-economic groups, irrespective of their ability to pay, as well as the gender with equal needs.^[1,2] Studies have shown two forms of inequity; social inequity is because of poverty, ethnicity, and lack of education, while spatial inequity arises owing to the place of residence, such as rural, urban, hilly, and forest areas, which effect on access to health care.^[3,4] The measure to gauge equity is to assess the distribution of opportunities in health care system suggesting a shift to measure social and spatial barriers of morbidity and mortality,

and health-seeking behavior can be used as indicators to assess equity.^[5]

Health care utilization is the point in health systems where patients need to meet the professional system. It is well known that apart from need-related factors, health care utilization is also supply induced, and thus, strongly dependent on the structures of the health care system, and positive health care seeking behavior of an individual is influenced by the factors such as accessibility, affordability, and availability of health care services. India has a plurality of health care with different systems of medicine delivered by government and local bodies in hospitals and clinics. Public hospitals provide 60% of all hospitalizations, while the private sector provides 75% of all routine care.^[6-8]

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Changing dynamics in the health-care system as well as the role of health care personnel requires an authenticated and well-planned demographic data with reference to health as the demographic characteristics of the population are of fundamental importance because they define who receives care.

Socio-demographic data on health act as a guiding path in understanding disease profile to primary health care physicians i.e., doctors, nurses, Ayush workers, Anganwadi teachers, and emergency caregivers as they are the first persons to come in contact with the public at the local level. Understanding data on health gives an opportunity for primary care physicians to know the prevalence, trends of disease occurrence, care sought by the community, and also the economic impact of health, thereby providing primary health care physicians to establish well-balanced care.^[9]

Utilization is the actual attendance by the members of the public at health care facilities, which measures the number of visits per year or the number of people with at least one visit during the previous year, serves as an important tool for health policy decision-making.^[10] In much of the literature, the terms demand and utilization are used almost synonymously; economists almost invariably use the term demand (meaning utilization) in their studies of the dental care market. Hence, the aim of the present study is mainly focused to determine the utilization of health care services in Tenali Mandal, Guntur (Dist.), Andhra Pradesh.

Objectives

- To document self-reported general health diseases that the population suffered in recent times.
- To know the type of service received, place, avenue, and personnel involved in the delivery of health services.
- To know the factors influencing the pattern of health care utilization.

Materials and Methods

A cross-sectional study was done to know the general health care utilization, profile, and pattern in Tenali mandal, Guntur, Andhra Pradesh.

Study area

According to 2011 census, Tenali mandal of Guntur district has a total population of 2,40,031 with an average sex ratio of 1,024; thus, for every 1,000 men, there are 1,024 females while 68.7% people lives in urban areas, 31.3% in rural areas, and the average literacy rate stands at 79.89% with 1,74,711 literates.^[11] There are 9 villages, 2 peri-urban, and 1 town present in the Tenali mandal and following National Pathfinder survey 4 sites in 4 directions i.e., north, south, east, and west are taken from urban area and one site in any of the directions from each of the 9 villages and 2 peri-urban areas were selected for the present study.

Study population

Both male and females of age groups, 5, 12, 15, 35–44, and 65–74 years were considered as these age groups reflect significant periods of life cycle.

Sample size calculation

All the five age groups were selected from each site of 4 urban, 2 peri-urban, and 9 rural sites where 20 individuals from each group i.e., a total of 100 individuals from each site were selected resulting in sample size of 1,500, and for the age groups of 5 and 12 years, the questionnaire is administered to their parents/guardians.

Each site 5 age groups = 5×20 individuals = 100

15 sites = 100×15 = 1,500 sample size

Sampling technique

National pathfinder survey (Stratified cluster random sampling).

Inclusive criteria

- 1) Both male and female subjects of WHO recommended age groups.
- 2) Subjects who are present on the day of the study.

Exclusive criteria

- 1) Non-co-operative and mentally challenged.
- 2) Who are non-residents of Tenali mandal.

Ethical clearance

Ethical clearance was obtained from the ethical committee of SIBAR institute of dental sciences with IEC protocol no: Pr. 35/IEC-SIBAR/CIR/15 A, and informed consent were obtained from all the study participants prior to the start of the study, where the subjects are well ensured of the confidentiality of their responses.

Scheduling

The study was done between the months of July 2017–September 2017, where 20–25 subjects was interviewed per day while 15 min of duration were taken to gather information, per person.

Formulation of questionnaire

A pretested interviewer-administered questionnaire was used along with BG Prasad's classification for socioeconomic status owing to its applicability in both urban and rural areas, which is mainly depending on per capita monthly income.^[12]

Evaluation of psychometric properties of the questionnaire

Initially, face validity was tested that signified the validity of the question and adequate representation of each and every aspect that totally balance the coverage of the issue. Further, content validity was checked to know whether the questionnaire covers

the domains to be measured and it was done by quantifying seven expert's degree of agreement regarding the content relevance of the questions from the Department of Public Health Dentistry.

It was calculated by the formula:

Calculation of content validity ratio (CVR)^[13]

$$CVR = n - N/2/N/2$$

Where n is the number of experts who gave a rating of essential for an item

N is the total number of experts

In the present study, the content value of the questionnaire is 0.7, which is adequate.

A pilot study was done in the month of March 2016 on a sample of 150 subjects, to check the feasibility of the study using a semi-structured, pretested, validated, interviewer-administered questionnaire. Through pilot test, the internal consistency of the questionnaire was tested and yielded a result of Cronbach's α value (0.81), which is satisfactory.

Statistical analysis

Statistical package for social sciences (IBM SPSS, Chicago) version 20.0 is used for the analysis, and P value ≤ 0.05 is considered as statistically significant. Statistical tests such as descriptives, Chi-square test, Spearman correlation, and binomial logistic regression were used for the analysis.

Results

The study sample comprised of 1,500 subjects who were equally divided among five age groups. Out of 1,500 subjects, 52.8% were females and 47.2% were males [Table 1]; majority of them are unmarried (60.2%) with 60% of the study participants belonging to rural followed by urban (26.7%) and peri-urban (13.3%). In total, 51.3% has 4–5 members in a family and majority of the study population belonged to middle class (40.1%) with primary occupation as clerical, shop owner, and farmer, where 32.1% of them have primary school certificate, while predominant population follow a mixed diet (89%) and majority of them belonged to open category caste (53.1%), while 63.2% of them have below poverty line cards issued by the government of Andhra Pradesh [Table 2].

In total, 71.7% of the study participants have utilized health services [Table 3] with majority of them (44.5%) had availed services within last 6 months with prime reason of fevers (15.8%), followed by gastrointestinal problems (11.5%), metabolic problems (11%), and cardiovascular problems (10.4%) [Table 4]. When asked about nearest available facility its distance and care sought from various places, 76.6% of them said that the nearest place being private hospital with distance less than

Table 1: Distribution of study population according to Demographic profile

Demographic Profile	Frequency	Percent	
Age	5 years	300	20.0
	12 years	300	20.0
	15 years	300	20.0
	35-44 years	300	20.0
	65-74 years	300	20.0
	Total	1500	100.0
Gender	Male	708	47.2
	Female	792	52.8
	Total	1500	100.0
Marital status	Married	600	40
	Unmarried	900	60
	Total	1500	100.0
Place	Urban	400	26.7
	Peri-urban	200	13.3
	Rural	900	60.0
	Total	1500	100.0
No. of family members	1-3 members	663	44.2
	4-5 members	770	51.3
	6-7 and above	67	4.5
	Total	1500	100.0
Socio-economic class	Upper class	308	20.5
	Upper-middle-class	451	30.1
	Middle class	602	40.1
	Lower-middle-class	95	6.3
	Lower class	44	2.9
	Total	1500	100.0

5 km from their residence (67.2%), while majority of them sought care from private clinics (52.84%), followed by primary health centers (11.53%). Among care sought from others, majority of them sought care from (6.05%) registered medical practitioner (RMP) followed by homeopathy (2.33%), and voluntary organizations (1.86%) [Table 5].

The main reason for selection of particular health care provider/center was nearness (36.84%) followed by Doctors reputation (35.35%), whereas 50% of them reported that they were very much relieved from suffering after they sought care. However, 30.33% of the study population has spent 501–2000 INR for their last medical visit, while their primary mode of payment was out of pocket (73.02%) [Table 6].

Majority of them reported that cold and cough (17.77%) followed by knee pain (17.03%) and fevers (14.07%) are the problems that suffered without taking care, while the main barrier for not seeking care was distance (17.03%), equal proportion of the population (16.29%) sought care for their problems through home remedies and over-the-counter (OTC) [Table 7].

With 89.3%, 65–74 years age group subjects were found to be dominant in the utilization of health services followed by 82.7% of the 5 years age group, and the difference between the age group and health services utilization was found to be statistically significant ($p \leq 0.001$, $r = 0.76$).

Table 2: Distribution of subjects according to occupation, education, diet, caste, and ration card

		Frequency	Percent
Occupation	Profession	236	15.7
	Semi-profession	132	8.8
	Clerical, shop owner, farmer	500	33.3
	Skilled	232	15.5
	Semi-skilled	153	10.2
	unskilled	211	14.1
	unemployed	36	2.4
	Total	1500	100.0
Education	Profession/honors	125	8.3
	Graduate/Postgraduate	83	5.5
	Intermediate/Post high school diploma	124	8.3
	High school certificate	236	15.7
	Middle school certificate	357	23.8
	Primary school certificate	482	32.1
	Illiterate	93	6.2
	Total	1500	100.0
Caste	ST	34	2.3
	SC	201	13.4
	BC	468	31.2
	OC	797	53.1
Ration card	White card	948	63.2
	Pink card	354	23.6
	No card	198	13.2

Table 3: Distribution of subjects according to health services utilization

Utilization	Frequency	Percent
No	425	28.3
Yes	1075	71.7
Total	1500	100.0

Utilization of services in a lower class (97.70%) and lower-middle-class (94.70%) was found to be predominant, and the difference between the socio-economic class and health services utilization was found to be statistically significant ($p \leq 0.001$, $r = 0.114$).

In total, 74% of the urban area population was found predominant in utilization of health services, and the difference between the place of residence and health services utilization was found to be not statistically significant ($p \leq 0.422$, $r = -0.32$).

In total, 72.5% of the males were found higher in utilization of health services, and the difference between gender and the health services utilization was found to be not statistically significant ($p \leq 0.279$, $r = -0.017$).

Health services utilization among 5 years age group was 0.755 times less when compared to 65–74 years age group, which is not statistically significant ($p \leq 0.260$). Gender wise utilization revealed that males utilized 0.941 times less compared to females, which is not statistically significant ($p \leq 0.623$); while for the place of residence, urban place was 1.332 times more utilizing

Table 4: Distribution of subjects according to time of last medical visit and reasons for visit

		Frequency	Percent
Last medical visit	Never visited	425	28.3
	1-6 months	668	44.5
	7-12 months	190	12.7
	1-2 years	113	7.5
	2-3 years	39	2.6
	More than 3 years	65	4.3
Reasons for medical visit	No history	425	28.3
	CVS problems	156	10.4
	GIT problems	172	11.5
	Respiratory problems	50	3.3
	General check-up	59	3.9
	Musculoskeletal problems	91	6.1
	Ophthalmic problems	11	0.7
	Accident/trauma	26	1.7
	Allergy	22	1.5
	Fevers	237	15.8
	Neurological problems	6	0.4
	Immunological problems	1	0.1
Metabolic disorders	165	11.0	
Others	65	4.3	
Renal problems	14	0.9	

Table 5: Distribution of subjects according to nearest available medical facility, its distance as perceived by the subjects, and care sought from various centers or places

		Frequency	Percent
Nearest available medical facility	Don't know	2	0.1
	Private hospital	1149	76.6
	PHC	184	12.3
	RMP	87	5.8
	Anganwadi	39	2.6
	Government hospital	39	2.6
Distance for medical facility from your place	Don't know	16	1.1
	Less than 5 km	1008	67.2
	More than 5 km	476	31.7
Care sought from Government centers	Sub center	6	0.56
	PHC	124	11.53
	Area hospital	8	0.74
	District hospital	38	3.53
Care sought from private centers	Private clinic	568	52.84
	Nursing home	96	8.93
	Multispecialty hospital	100	9.30
	Medical college	19	1.77
	Corporate hospital	2	0.19
Care sought from other agencies	Voluntary organizations	20	1.86
Others	RMP	65	6.05
	Over-the-counter (OTC)	2	0.19
	Homeopathy	25	2.33
	Home remedies	2	0.19

the services when compared to rural place, which is statistically significant ($p \leq 0.059$). On the basis of socio-economic status, the lower-middle-class utilized 0.412 times less when compared to lower class, which is not statistically significant ($p \leq 0.427$);

Table 6: Distribution of subjects according to various reasons for selection of particular center, their experience, amount spent, and mode of payment during their last general health care visit

		Frequency	Percent
Reasons for selection of particular health care provider/center	Free	17	1.58
	Doctors reputation	380	35.35
	Near	396	36.84
	Known doctor	85	7.91
	Better treatment/care	120	11.16
	Low cost	37	3.44
	Thought home remedies are sufficient	11	1.02
	Less time	29	2.70
Are you relieved from suffering after you sought care?	Very much	750	69.77
	Somewhat	311	28.93
	Undecided	2	0.19
	Not really	3	0.28
Amount spent for last medical visit	Not at all	9	0.84
	Free	196	18.23
	1-500	248	23.07
	501-2,000	326	30.33
	2,001-10,000	207	19.26
Mode of payment	10,001-100,000	98	9.12
	Insurance	23	2.14
	Reimbursement	71	6.60
	Out of pocket	785	73.02
	Government hospital/schemes	196	18.23

while for utilization among pink card ration, cardholders were 2.130 times more when compared to no cardholders, which is statistically significant ($p \leq 0.001$) [Table 8].

Discussion

Health is a universal human need across all cultures and groups. It has been established beyond doubt that optimal health cannot be attained or maintained independent of oral health.^[14] Health service utilization is a multifactorial phenomenon and depends on various factors such as health conditions, socio-economic conditions, attitude, and financial conditions.^[15]

Utilization of health services

Utilization of health care services in the present study was 71.7%, which was high when compared to other studies done by Saritha Vargese *et al.* (2013).^[16] The utilization of public health services was 36% in rural population, whereas it was only 25% in urban slums. However, 48% households utilized services from public sources in a study done by Sur D *et al.* (2004),^[17] and it was 26% in a study done by Gupte R.K *et al.* (1996)^[18] among the slum dwellers.

Reasons and last time of medical visit

In total, 44.5% of the study population has sought care in the past 6 months. Fevers such as malaria, typhoid, dengue, and

Table 7: Distribution of subjects according to various problems suffered and reasons for refraining from seeking health care

		Frequency	Percent
Various medical problems of the participants who have not utilized the services	Bruises	8	5.92
	Allergies	7	5.18
	Cold and cough	24	17.77
	Headache	12	8.88
	Knee pain	23	17.03
	Chest pain	18	13.33
	Gastritis	12	8.88
	Hypertension	12	8.88
Reasons for refraining from seeking medical care	Fever	19	14.07
	Lack of time (Morning or evening)	10	7.40
	No accompanying person	11	8.14
	Negligence	20	14.81
	Does not affect my work	15	11.11
	I like to try self care and home remedies	23	17.03
	Distance	23	17.03
	Expensive (OTC)	11	8.14
Over-the-counter (OTC)	22	16.29	

chikungunya which are of viral origin being the predominant reason for the medical visit, which occupied 15.8% in the present study, followed by gastrointestinal disorders (11.5%) and metabolic disorders such as diabetes (11%) and cardiovascular problems (10.4%). Fevers as the main health problem followed by the gastrointestinal problem was reported in a study done by Alastair Ager and Katy Pepper (2005).^[19]

Acute febrile illness can occur whenever some type of infectious agent invades the body, but it is especially worrisome in tropical and sub-tropical regions like India, where serious diseases loom. Therefore, the condition being acute has made them to utilize the services more when compared to others.

It was found that 85% of the deaths are related to cardiovascular problems alone in the state of Andhra Pradesh. Among them, 97% being under the below poverty line and only 3% above the poverty line, whereas 57% being males and 43% females. Most persons suffering from cardiovascular problems lie down between 48 and 58 years age group.^[20]

In rural areas, on average, a person spends around 6, 260 rupees for medication; whereas in urban areas, it was around 10,000 rupees, which accounts for 34% of their monthly income in urban areas and 27% among rural areas. It was estimated that by 2040, 13 crores people are going to suffer from diabetes. The reasons could be owing to genetics, lifestyle changes, lack of exercise, more carbohydrate diet, and finally, changes in the environment.^[21]

Table 8: Binomial logistic regression between general health services utilization and independent variables

Variables	OR	95% CI		p
		Lower	Upper	
Age groups				
5 years	0.755	0.462	1.232	0.260
12 years	0.247	0.157	0.389	0.000
15 years	0.178	0.114	0.278	0.000
35-44 years	0.357	0.223	0.572	0.000
Gender				
Males	0.941	0.739	1.199	0.623
Place				
Urban	1.332	0.989	1.793	0.059
Peri-urban	1.194	0.827	1.723	0.344
SES				
Upper class	0.105	0.014	0.791	0.029
Upper-middle-class	0.093	0.012	0.695	0.021
Middle class	0.098	0.013	0.724	0.023
Lower-middle-class	0.412	0.046	3.682	0.427
Ration card				
White card	2.117	1.448	3.095	0.000
Pink card	2.130	1.410	3.218	0.000

List of reference categories for each independent variable: Age groups- 65-74 years; Gender- Females; Place- Rural; SES- Lower class; Ration card- No card

In the present study, there was 0.1% of the population suffering from the immunologic diseases such as HIV, and it was around 4% as per Guntur district statistics comprising of 3.31 lakh HIV patients, with an incidence of 3,672 cases between 2015 March and 2016 April in Guntur district.^[22]

Center/place for care sought

In total, 52.84% of the population sought care from the private clinics followed by primary health centers (11.53%), whereas 54% of the subjects preferred primary health centers for the treatment of leptospirosis in rural areas in a study done by Mathew G (2007).^[23] Astonishingly, 6.05% of the present study population sought care from RMP/informal providers; while in a study done by James Kisia *et al.* (2012),^[24] most participants (63.4%) reported that they utilized community health workers for malaria treatment.

People consult informal providers such as RMPs for a variety of common conditions, which include fevers, diarrhea, and respiratory problems according to studies done by Rohde and Vishwanathan 1995;^[25] Kanjilal *et al.* 2007;^[26] Gautham *et al.* 2011;^[27] George and Iyer 2013;^[28] and Meenakshi Gautam *et al.* 2014.^[29]

The private health sector has grown drastically during the post-independence period, and the contribution of the private sector was less than 8% at the time of independence (1947). By 2012, approximately 80% of healthcare services in India were provided by the private sector and it accounts for 85% of the health care professionals. The public sector currently provides about 20% of outpatient care services and over 40% of inpatient care. The planning commission has reported that in the private health sector incentives are tilted toward curative services and medical education.^[30]

Distance of the nearby health service provider

In the present study, 67.2% of them reported that the distance from the nearest service provider as less than 5 km, whereas rest 31.7% reported being more than 5 km. While in a study done by Pawan Kumar Sharma *et al.* (2008),^[31] 40% of them has reported that the nearest health service provider with proximity from their place was less than 5 km and the rest 60% reported more than 5 km.

Reasons for selection of particular health care provider/center

Totally, 36.84% of the study population has said that accessibility/near was the main reason for selection of the particular center/provider followed by 35.35% of them reported reputation of the doctor as another reason for the choice of the center. In a study done by Saritha Vargese *et al.* (2013),^[16] 64.1% reported lower user fees followed by 18.4% near to house were the main reasons for the selection of a particular center. Reputation in terms of perceived quality—has been found to be one of the main determinants of utilization of a particular health care provider, and “recovery” is one of the most important criteria that patients use to judge the quality of services and choice of health service provision (Haddad *et al.* 1998,^[32] Alastair Ager and Katy Pepper. 2005).^[19] In a study done by Pawan Kumar Sharma *et al.* (2008),^[31] the main reasons for selection of a particular hospital are inexpensive (87.9%) and skilled doctors (65.9%).

Mode of payment during their last medical visit

In addition, 73.02% of the population has used out of pocket as their main mode of payment for the services utilized. According to an annual report to the people on health by the ministry of health and family welfare, Government of India (December 2011) approximately 71% of the total health care expenditure in the country was borne by households out of their pockets, whereas the payment through the third party was common among western counterparts. Out of pocket expenditure was any direct outlay by households, including gratuities and in-kind payment to health practitioners and suppliers of pharmaceuticals, therapeutic appliance, and other goods and services whose primary intent was to contribute to the restoration or enhancements of the health status of individuals or population groups, as per the definition given by the World bank.^[33,34]

Amount spent for last medical visit

However, 30.33% of the study population has spent around 501–2000 Indian rupees on their last medical visit, whereas it was 1–500 Indian rupees for delivery of services in a study done by Pushpendra Kumar and Abha Gupta (2016)^[35]; while only 26.5% of them spent 501–2000 INR for the institutional delivery.

Experience with treatment received as perceived/told by subjects

Approximately, 69.77% of the study population has reported that they were very much relieved/satisfied after they sought

care from the center, while in a study done by Saritha Vargese *et al.* (2013)^[16] 18.4% reported that the treatment given by the public services were not effective.

Various medical problems of the participants who have not utilized the services

Common cold and cough are not considered as important to visit health care facility owing to the availability of OTC medications and home remedies, which was reported by 17.77%, while knee pains reported by 17.03% of the study population was considered as a common serious problem among aged persons.

Reasons for refraining from seeking medical care

Almost equal proportions of the study participants (17.03%) has reported that they like to try self-care and home remedies, proximity from the place, and OTC were the main reason for refraining from seeking medical care, whereas in a study done by Saritha Vargese *et al.* (2013),^[12] the reasons for not using public health services as 74% said that public services are free, but they take money and 18.5% have reported that the treatment was not effective.

While in other studies, the reasons for refraining from utilization of health are cost, perceived lack of quality, accessibility, transport, and financial constraints that also include lack of insurance.^[36-40]

Generalizability

The sampling process ensured the representativeness of the population and contributes to the internal validity of the study. However, the process of training and standardization that the research went through, and also by the high concordance obtained through kappa test ensured the reproducibility of the data. Non-availability of validated questionnaire necessitated the use self-developed questionnaire. The questionnaire utilized possessed good reliability and also adds to the internal validity of the study. Cross-sectional studies that inquire questions about the past require participant's ability to recall past events. In this study, recall bias was minimal, as it is believed that pain suffered owing to health problem was not likely to be soon forgotten.

Limitations

The results found in this study cannot be inferred to other population owing to differences in socioeconomic status, culture, and access to and use of health services found in India. The cross-sectional design of the study inherits a limitation; it does not allow the determination of the causal relationship between the variables investigated and the outcome. Although a short-time frame was used, recall bias was inevitable.

Conclusion

There is an utmost need to minimize barriers of utilizing the services by motivating people and making them aware about the

health problems so that they develop a positive attitude toward health care utilization. Changing the perception toward health, undertaking an effective program to build science transfer, increasing workforce by strengthening the safety net system, and successfully partnering in all levels of society can bring healthy life. Pliability and acclimatization to change will be essential in the medical education in the future while analyzing the profile and patterns of the disease will help to reduce the economic impact of health and also helps in providing well-balanced care, while it also aids to reduce the variations in performance among physicians to deliver quality health care.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Differences in Oral Health Status Leading to Tooth Mortality Based on Socioeconomic Stratification: A Cross-sectional Study

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Abstract

Background: Oral health is always an inseparable part of general health. The inequalities in socioeconomic status (SES) are the causes of many health disparities including oral health in the world. **Aim:** To document the differences in oral health status leading to tooth mortality based on socioeconomic stratification. **Materials and Methods:** A cross-sectional study was done using stratified random sampling. Centers/facilities providing dental care were stratified into government general hospital (GGH), teaching dental hospital (TDH), and private dental clinics (PDCs). Sample size was taken as 750. Demographic data were taken from each patient; clinical examination of existing teeth was done, while decayed-missing-filled teeth (DMFT) and community periodontal indices were recorded. Data were analyzed by Statistical Package for the Social Sciences software version 20, and Chi-square, ANOVA, and Spearman correlation tests were used for statistical analysis. Significance level was set at $P \leq 0.05$. **Results:** Of the total study subjects, 40% ($n = 300$) were taken from TDH, 33.3% ($n = 250$) were taken GGH, and 26.7% ($n = 200$) were taken from PDCs. A majority (50.5%) of them belongs to the upper-lower class. Majority ($n = 412$) were with community periodontal index (CPI) score of 2, and most of the people ($n = 175$) had loss of attachment (LOA) score below 2–6–8 mm, of which a majority of them belonged to upper-lower and lower SES. Both CPI and LOA were statistically significantly related with SES ($P < 0.001$). As SES decreased, the mean DMFT increased and this relation was highly significant ($P < 0.001$). The mean number of teeth to be extracted was increased with increased SES. **Conclusion:** Inverse correlation was observed between SES and mean DMFT. Majority of the low socioeconomic people were with sub- and supra-gingival calculus. It implies that socio-economically disadvantaged people are in great need of dental treatment. Hence, dental health education programs should be targeted to uneducated and low-income groups to reduce the rate of extractions.

Keywords: Community periodontal index, dental clinics, decayed-missing-filled teeth, loss of attachment, socioeconomic inequalities, tooth loss

INTRODUCTION

One of the fundamental rights of every human being, irrespective of race, religion, economic, and social conditions, is enjoying the highest attainable standard of health.^[1] Health is influenced by many factors such as genetics, lifestyle, environment, and socioeconomic status (SES) and it is multifactorial. In the last few decades, we have witnessed that social and economic factors have as much influence on the health as much as medical interventions. Hence, health cannot be isolated from its social context.^[2] Oral health is always an inseparable part of general health. The inequalities in SES are the causes of many health disparities, including oral health in the world.^[3] Oral diseases place a huge social burden and also an economic burden on the population in terms of many sufferings, expenditure on

treatment, and prevention.^[4] There is a severe influence of oro-dental disease upon the whole community, and it plays an adverse role in general health.^[5] Because of recent shifts into unhealthy diets such as rich in sugar and increase consumption of tobacco, the burden of oral diseases is likely to grow in many developing countries.^[6] Extraction is the terminal event in the life of a tooth and is a frequent episode in the people with uncared and neglected oral cavity.^[7]

Tooth loss and edentulism, one of the major public oral health problems in India, has been reported to be on decline

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in developing countries.^[8] Industrialized countries spend 5%–10% of their national public resources (gross national product) on oral health care of people every year, while no budget allocation for the same in developing countries.^[9] Even though there is an increase in specialization and fields of medical practice in the country, no changes have been seen in the service provision for oral health care and oral hygiene status. We need some modifications which will involve oral healthcare services at the primary, secondary, and tertiary levels of healthcare delivery systems. These bring a noticeable improvement in the knowledge, attitude, and practices in the general people in terms of nutrition, oral hygiene, and health-seeking behavior.^[5] Low priority is given to the dental health in India, and it is not astonishing to find that the oral health problems have received adequate attention.^[10] Differences in the prevalence of oral health problems between individuals of higher and lower SES can be described as the concept of socioeconomic inequalities in oral health. Therefore, the intention was to find the differences in oral health status, leading to tooth mortality based on socioeconomic stratification.^[11]

METHODOLOGY

This study was cross-sectional in design. Ethical clearance was obtained from the institutional review board (No. 138509010). The study was carried out in Guntur city which is one of the medical hubs in India. Guntur offers a wide access to top medical care at reasonable prices and has a variety of charity hospitals that serve as a safety net for the destitute. It possesses major medical facilities (super-specialty hospitals) and related research institutions.

Centers providing dental services in Guntur were stratified into government general hospital (GGH), teaching dental hospital (TDH), and private dental clinics (PDCs). Reason behind this stratification was to include people from different socioeconomic level as they choose different types of healthcare centers based on their priorities and opinions. Necessary permissions were taken from the superintendent of GGH, principal of TDH, and practitioners in selected PDCs in advance.

A pilot survey was conducted and the number of extractions per day at different centers was recorded. The results of the pilot study showed that 35.8% of patients attending GGH, 42.83% of patients attending TDH, and 28.6% of patients attending PDCs underwent extraction over 3 weeks. Therefore, the prevalence of dental extractions on an average was taken as 39%. Absolute level of precision (d) which specifies the width of the confidence interval was kept as 5. The sample size was calculated using below formula.

$$n = \frac{(z_{\alpha} + z_{\beta})^2 pq}{d^2}$$

The estimated sample size was 746. It was rounded to 750 for the convenient division of the sample into three categories.

Stratified random sampling technique was adopted to draw the appropriate and representative sample. Sample drawn from each type of center was in proportionate to the average number of patients undergoing extractions per day as per the records (pilot survey) at different types of centers.

Since there were only one GGH and only one TDH in Guntur that provide services for low cost and conduct free camps throughout the district, they were included in the study without sampling. Fifteen PDCs were randomly selected from a total of 107 dental clinics in Guntur.^[12] All adult patients who were willing to participate and gave consent were included. Patients with restricted mouth opening were excluded.

The pro forma was prepared in English, and the investigator had interviewed the patients in the regional language Telugu. The investigator had prior training on the interview procedure and was supervised by a group of experts in the initial phase of data collection. Demographic details were taken from each patient. SES division was done in accordance with the modified Kuppaswamy scale (2014).^[13] Clinical examination for remaining existing teeth status was done using community periodontal index (CPI), loss of attachment (LOA) index, and dentition status and treatment needs as per the World Health Organization Basic Oral Health Survey 1997. Type III American Dental Association examination^[14] was done using mouth mirror, CPI probe, and adequate illumination. Data were analyzed by IBM SPSS Statistics for Windows, Version 20.0. (Armonk, NY: IBM Corp.), and statistical tests used were Chi-square test, ANOVA, and spearman correlation test. $P \leq 0.05$ was considered statistically significant.

RESULTS

In the study sample, 40% (300) were taken from TDH, 33.3% (250) were from taken GGH, and 26.7% (200) were taken from PDCs. Half of the study subjects belonged to upper lower socioeconomic class, i.e., 50.5%. Most of the upper (52.5%) and upper-middle (59.7%) class people were visiting PDCs and most of the lower-middle (48.7%), upper-lower (47.5%), and lower class (39.6%) people were visiting TDH and GGH [Table 1].

Oral hygiene behavior of the participants was documented, and it was found that there was no significant difference in the oral hygiene behaviors between patients from three different types of oral health facilities consider in the study.

Periodontal status was assessed using CPI by taking CPI scores and LOA scores [Table 2]. The highest CPI and LOA scores among the sextants examined in a person were taken as CPI and LOA scores, respectively, for that person. Half of the subjects ($n = 412$) were with supra- and/or sub-gingival calculus, of which 261 (63.3%) subjects belong either upper-lower or lower class. 57.5% ($n = 431$) of the study subjects were with no LOA and most of the people who had LOA were underscore two (LOA of 6–8 mm), a majority of them belong to upper-lower and lower SES. Both CPI



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Table 1: Based on socioeconomic status, distribution of study population according to a type of service center attended, adverse habits and systemic diseases

Variable	Group	Upper class, n (%)	Upper-middle class, n (%)	Lower-middle class, n (%)	Upper-lower class, n (%)	Lower class, n (%)	Total, n (%)	P
Type of dental care center	Private clinics	21 (52.5)	74 (59.7)	35 (22.7)	58 (15.3)	12 (22.6)	200 (26.7)	<0.001*
	TDH	19 (47.5)	44 (35.5)	75 (48.7)	141 (37.2)	21 (39.6)	300 (39.6)	
	GGH	0 (0.0)	6 (4.8)	44 (28.6)	180 (47.5)	20 (37.7)	250 (33.3)	
	Total	40 (100)	124 (100)	154 (100)	379 (100)	53 (100)	750 (100)	

Chi-square test. *Statistically significant. TDH – Teaching dental hospital, GGH – Government general hospital

Table 2: Distribution of community periodontal index and loss of attachment scores in different socioeconomic groups

Scores	SES					Total (n)	P
	Upper class (n)	Upper-middle class (n)	Lower-middle class (n)	Upper lower class (n)	Lower class (n)		
CPI							
0	9	45	48	65	2	169	<0.001*
1	11	12	17	47	2	89	
2	18	58	75	232	29	412	
3	1	4	9	18	5	37	
4	0	1	2	3	1	7	
9	0	0	0	1	0	1	
X	1	4	3	13	14	35	
Total	40	124	154	379	53	750	
LOA							
0	29	91	105	201	5	431	<0.001*
1	7	11	14	49	4	85	
2	2	14	28	102	29	175	
3	0	2	4	8	1	15	
4	1	2	0	5	0	8	
9	0	0	0	1	0	1	
X	1	4	3	13	14	35	
Total	40	124	154	379	53	750	

Chi-square test. *Statistically significant. SES – Socioeconomic status, CPI – Community periodontal index, LOA – Loss of attachment

Table 3: Difference in mean decayed-missing-filled teeth by socioeconomic status and correlation between decayed-missing-filled teeth and socioeconomic status

SES category	n	Mean±SD	P
Upper class	40	3.83±3.954	<0.001*
Upper-middle class	124	4.17±3.993	
Lower-middle class	154	4.18±4.089	
Upper-lower class	379	4.98±4.198	
Lower class	53	9.47±6.024	
Total	750	4.93±4.472	

One-way ANOVA. *Statistically significant. SD – Standard deviation, SES – Socioeconomic status

and LOA had statistically significant association with SES ($P < 0.001$).

Mean decayed-missing-filled teeth (DMFT) was found to be highest in the lower class. The difference was statistically significant ($P < 0.001$). As SES decreased, the mean DMFT increased [Table 3]. Spearman correlation test showed significant weak positive correlation between SES and DMFT ($r = 0.2$, $P < 0.001$).

As SES decreased, the number of teeth to be extracted increased, but reverse was observed for number of teeth retained after a scheduled extraction [Table 4].

SES showed highly significant positive correlation with the number of teeth to be extracted ($r = 0.234$, $P < 0.001$) and highly significant negative correlation with the number of retained teeth after a scheduled extraction ($r = -0.289$, $P < 0.001$).

DISCUSSION

Tooth loss can be considered as dental equivalent of mortality. It is the end product of many dental diseases and it reflects the SES and attitude of patients toward availability and accessibility of dental care. One of the major handicaps in the elderly population is the loss of tooth affecting mastication, dietary intake, and nutritional status. Many studies have consistently shown the role of specific diseases such as dental caries and periodontal disease as major causes of tooth loss.^[15] Studies have long established a close relationship between SES and health.^[16-18]



Dr. L. KRISHNA PRASAD

DEAN

SIBAR INSTITUTE OF DENTAL SCIENCES
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Table 4: Mean number of teeth to be extracted and mean number of retained teeth after a scheduled extraction in different socioeconomic classes

SES category	Total	Number of teeth to be extracted		Number of teeth after scheduled extraction	
		Mean ± SD	P	Mean ± SD	P
Upper class	40	1.60±1.549	<0.001*	28.73±5.496	<0.001*
Upper-middle class	124	1.69±1.510		27.39±6.202	
Lower-middle class	154	2.23±2.272		26.71±7.080	
Upper-lower class	379	2.50±2.366		25.36±7.597	
Lower class	53	2.94±2.248		14.55±11.54	
Total	750	2.29±2.206		25.39±8.151	

One-way ANOVA, *Statistically significant. SD – Standard deviation, SES – Socioeconomic status

The study results showed that the majority of the people were visiting TDH for oral care. This may be attributed to less treatment cost and/or availability of all specialty treatments in one place. Most of the study participants belonged to lower SES, indicating that people from low SES are getting massive dental problems because of poor oral hygiene practices,^[17] lack of awareness on the etiological factors for oral diseases,^[18] and poor utilization of dental services.^[19-21] Poor utilization may in turn be related to cost, lack of knowledge and motivation toward dental care,^[20-24] lack of awareness on the provision of reimbursement for dental care,^[25] low priority given to dental health, and lack of perception of the fact that the teeth are worth saving.^[26]

The maximum number of upper-class people was visiting PDCs. This may be because they are staying within the city limits, good at affording conservative treatment, lack of time in the morning sections, high quality and quick treatment, the feasibility of dental clinics, convenient appointments, etc.^[27-29]

Most of the subjects were with sub- and/or supra-gingival calculus and LOA 6–8 mm. Higher number of subjects in upper-lower and lower-middle class categories were with CPI scores 1–4. Similar results were seen in a study by Chandra Shekar and Reddy^[26] because of poor oral hygiene practices,^[30,31] nonutilization of dental services, presence of deleterious oral habits such as smoking,^[17] pan chewing,^[30,32] and the lack of awareness on the dental diseases,^[3] which are proven to have a role in determining the periodontal status of an individual. This poor periodontal status for long period ultimately leads to tooth mortality.

In this study, SES and mean DMFT were showing inverse relationship. Mean DMFT was more in the lower SES group, indicating the neglecting oral health behavior. Similar results were seen in the study done by Chandra Shekar BR and Reddy C. Where mean number of teeth to be extracted was increased as the SES decreased.^[26] In lower SES classes, the number of teeth to be extracted was more, and at the same time, the number of teeth retained in the oral cavity was less. This might be attributed to poverty, ignorance, and lack of knowledge about dental care.^[5] They give little or no importance for the preservation of their teeth for the entire lifetime and prefer extraction over restoration^[33] because of less affordability and more time consumption. In contrast,

high income permits access to good oral health services and good environment for health and offer opportunities to adopt to appropriate oral health behavior.^[34] In general, losing teeth in turn shows a great impact on their quality of life.

In our study, the number of teeth to be extracted was positively correlated SES and reverse was true in case of number of teeth retained after a scheduled extraction. It was highly statistically significant.

CONCLUSION

Low SES people are in great need of dental treatments. Dental health education should be targeted to uneducated and low-income groups to reduce the rate of tooth mortality. Every dental professional should take care of their patients and has to encourage toward fulfillment of their dental normative needs and should take care of preventive dental care and create awareness regarding proper utilization of government dental health schemes.

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Conflicts of interest

There are no conflicts of interest.

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Awareness and attitude toward pictographs on tobacco products: A population-based study in field practice area

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ABSTRACT

Background: Tobacco use is a major global public health threat for all countries and no form of tobacco use is safe. Pictorial warnings covering 40% area of tobacco products are made mandatory from May 31, 2009 in India. The present study was aimed to investigate the awareness and attitude toward pictorial warnings on tobacco products among age 15 years and above population in field-practice area.

Materials and Methods: A total of 1064 individuals were selected by systematic random sampling. A model containing the pictorial warnings were shown to the study subjects, and data were collected by semi-structured questionnaire. Data was analyzed using SPSS 20 and statistical tests used were Chi-square test. Significance level set at $P \leq 0.05$.

Results: Of the 1064 individuals participated in the study, 53.9% were male and the mean age of the study population was 31.24 ± 5.8 . Nearly 45.8% reported using tobacco in some form and all of them were males. 51.3% of both males and females reported that they never came across antitobacco messages (pictorial warnings). 71.2% believed that the size and position of pictorial warnings on tobacco products were not sufficient to understand the harmful effects of tobacco. There was statistically significant difference between different age groups in their responses to the aforementioned questions.

Conclusion: The results of present study demonstrate that the need for pictorial warnings on tobacco products to be more clear and explanatory since there is little point in having pictorial warnings when the prime purpose of having them is not being served.

Key words: Attitude, awareness, pictorial warnings, tobacco

INTRODUCTION

Tobacco use in the Indian context imposes a huge burden for public health and being emerged as a major cause for premature deaths.^[1] Considering the public health thrust, there is a need of visible effort to increase awareness regarding the health risks of tobacco use to consumers. Tobacco packages

provide high reach and frequency of exposure and also serve as portable advertisements providing a provision for Health warnings which have emerged as an efficient medium for communicating the health risks, as the frequency of exposure through pack a day, smokers are potentially exposed to the warnings over 7000 times per year.^[2]

In India, the new pictorial warnings notification was issued on October 15, 2014, but mandatory display of

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new health warnings covering 85% of the principal display area on all tobacco products was notified on September 24, 2015, making India 3rd among countries with largest pictorial warnings on tobacco products. This initiation to drive home the point of prevention was implemented by Health Ministry, from April 1 2016, on all forms of tobacco products.^[3,4]

A successful image is about communication as most of the information transmitted to the brain is visual; it must tell the viewer the intended story, through an entirely and solely visual means of communication. The visual portion has much more immediate impact than the text because text requires conscious processing and images do not, which facilitate to empower people to make strong associations between health messages and their perceptions. Majority of the researchers presume that the knowledge and attitude are related to each other where attitude is further linked to the behavior. The assumption believes on "if people become more knowledgeable about the tobacco and its associated diseases, they will, in turn become more aware of the tobacco and its problems and thus, be more motivated to act toward the tobacco and its related diseases in more responsible and vigilant ways."^[5,6]

The present study was done from the Department of Public Health Dentistry, in a renowned dental college that is located in Takkellapadu Village, Guntur, Andhra Pradesh, India. Since Guntur is a major district in production and consumption of tobacco, the public in this area are more prone to get habituated to various forms of tobacco. Hence made an attempt to study the awareness, attitudes and impact of current pictorial warnings in the field practice area, which is with in 15 kilometers radius from the institution.

MATERIALS AND METHODS

Study design

A cross-sectional, semi-structured, self-administered, validated questionnaire-based study was done with ethical clearance obtained from the Institutional Ethics Review Board, where study aim, objectives and confidentiality on their personal information, and responses were well described to the study contestants whilst an informed written consent was obtained from all the participants before the start of the study.

Selection criteria

Inclusion criteria

1. Males and females of age 15 years and above were included in the study
2. Tobacco users, nonusers, and past users were included in the study
3. Persons who are permanent residents of the study area are only included in this study.

Exclusion criteria

1. Mentally challenged and who are unable to answer.

Study procedure

A house-to-house survey was conducted on 1064 participants aged 15 and above in the field practice area located in Pedakakani Mandal (Mandal is a sub-district administrative area), South coastal Andhra Pradesh, India, spreading over 16.60 km² comprising 12 suburban localities with population of 64,693 residing in 14,944 houses.^[7] From each of these, 12 localities a quota of 50 houses selected using systematic random sampling technique.

The data on attitude and awareness on pictorial warnings of tobacco products were obtained using a self-administered, semi-structured questionnaire along with a model-contained a panel of empty packets having pictorial warnings of smoked and smokeless tobacco products stuck on it.

The questionnaire was developed on the basis of the perceptions of authors with Master's degree in the subject of Public Health Dentistry. While framing the questionnaire the context of the study subjects and their usage of tobacco products were taken into consideration. The perceptions were converted to sentences to frame the questionnaire. These questions were then organized in a systematic order and then were translated to Telugu, mother tongue of study participants and then back translated to English by a bilingual expert, the face and content validity of the questionnaire were assessed while the content validity of the questionnaire is 0.80. For illiterates, the questions were read out and the reliability of the questionnaire was assessed in the pilot study, which was conducted on 90 individuals (Cronbach's alpha = 0.88). The final questionnaire contained semi-structured questions after considering minor corrections.

The questionnaire consisted of sociodemographic details such as age, gender, education and occupation; tobacco use practices and questions on awareness along with attitudes in regard to pictorial warnings on tobacco products. Data thus obtained were analyzed using Chi-square test with IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Out of 1064, 53.9% were male with mean age group of 31.24 ± 5.8 and majority of the participants (54%) belonged to the age group of 25–34 years with 26.9% ($n = 287$) of the study participants had primary school education. Regarding occupation, majority of the study participants, i.e., 31.4% are clerical, shop-owner or farmer. Nearly 46% of the study participants are tobacco users and all the tobacco users are only males, thus gender-wise shift to one side could be due to social stratification bias [Table 1].

Table 1: Frequency distribution of the study population

	Tobacco users		Tobacco nonusers	
	Gender (%)			
	Males	Females	Males	Females
	489 (100)	0 (0)	84 (14.6)	491 (85.4)
Age groups (years) (%)				
15-24	58 (12)	0	18 (23)	153 (31)
25-34	291 (60)	0	37 (43)	249 (51)
35-44	106 (22)	0	22 (26)	62 (13)
45-54	22 (5)	0	3 (3)	15 (3)
55 and above	12 (2)	0	4 (5)	12 (2)
Education (%)				
Illiterate	74 (15)	0	5 (6)	104 (21)
Primary school education	128 (26)	0	11 (13)	148 (30)
Secondary school education	114 (23)	0	12 (14)	68 (14)
Intermediate	58 (12)	0	17 (20)	64 (13)
Under graduate	90 (18)	0	32 (38)	94 (19)
Postgraduate	25 (5)	0	7 (8)	13 (3)
Occupation (%)				
Unemployed	7 (1)	0	11 (13)	249 (51)
Unskilled worker	64 (13)	0	4 (5)	75 (15)
Semi-skilled worker	41 (8)	0	9 (10)	48 (10)
Skilled worker	104 (21)	0	9 (10)	20 (4)
Clerical, shop owner, farmer	226 (46)	0	34 (40)	75 (15)
Semi profession	29 (6)	0	9 (10)	24 (5)
Profession	18 (4)	0	8 (9)	0 (0)

In total population, 91% of tobacco users and 14% of nonusers responded that they came across antitobacco pictorial warning signs while this difference was statistically significant ($P = 0.001$). Nearly 56% of tobacco users and 6% of nonusers agreed that textual warning on tobacco products are sufficient and clear to understand the harmful effect and this difference was statistically significant ($P = 0.002$) [Figure 1].

There was also a significant difference ($P = 0.001$) in the opinions of 69% tobacco users and 15% of nonusers about changing the textual warnings on tobacco products to regional language, while 2% of tobacco users and 83% of nonusers were not aware about the colors that should be used on pictorial warnings of tobacco products ($P = 0.008$). Mandated pictorial warnings do not serve the desired purpose since they are not properly understood, people would like to see the warnings mainly in regional language with more clarity in picture [Figure 1]. Further, the respondent's attitudes toward tobacco are measured through an open-ended question and were converted to typical statements and presented.

DISCUSSION

Despite the fact that state has taken enormous measures, none have served in getting the desired result as to properly communicate the ill effects of tobacco consumption to the general public. Hence, the present study states the current level of knowledge on harmful effects of tobacco consumption among the mainstream population.

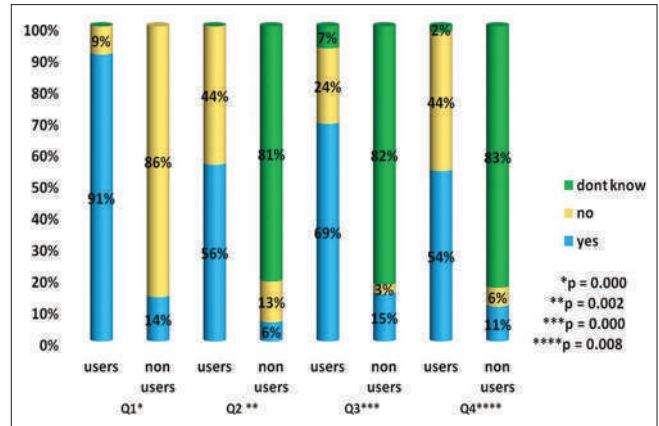


Figure 1: Responses of the participants

Nearly 57.3% of illiterates and 54.2% of the present study population in the age group of 25–34 years were not aware of pictorial warnings on tobacco products as compared to 40.6% illiterates and 32.6% of people aged 25–34 years in a study done by G. N Karibasappa *et al.*^[8] Tobacco use has been found inversely proportion to literacy and directly proportional to age which is similar to a study done by Bhawna, Global Adult Tobacco Survey, India.^[9]

Awareness among the people on warning labels was inadequate. These labels were ineffective in conveying the adverse effects of tobacco use on health which was on par with the studies done by Rani *et al.*,^[10] Oswal *et al.*,^[11] Arora *et al.*^[12]

The perceptions on the effectiveness of pictorial warnings on tobacco products were found to be inadequate among tobacco users (44%) and nonusers (13%), respectively. Dissimilar results were observed in the studies done by Thakur *et al.* and Arora *et al.* where 71.5% of users and 62.75% of nonusers opined that the effectiveness of pictorial warnings to be inadequate.^[12,13]

The following are the statements of the study participants that are organized based on their colloquial, which reflects the study population's attitudes toward the tobacco usage and pictorial warnings.

Attitudes of the study population

“Is it wrong to consume tobacco? If tobacco is really wrong and harmful to our health then why government is allowing to grow the crop and also allowing it for consumption.”

–Male respondent (farmer)

“Consumption of tobacco decreases only when government bans the tobacco completely and government should more concentrate on educating people about risks of tobacco.”

–Male respondent (teacher)

“We don’t have the habit and never saw the pictorial warnings.”

–Female respondent (house wife)

“Though pictorial warnings are printed on tobacco products, it has less impact on behaviours as people will consume tobacco products no matter what and they aren’t going to stop now even though it is bad for health”

–Female respondent (house wife whose husband is a smoker)

“As I am a farmer, I work at midnights in the field so, in order to stay awake I consume tobacco”

–Male respondent (farmer)

“We will discuss about this topic (tobacco consumption) outside the house”

–Male respondent (clerk)

Consumption of tobacco is sin, god punishes them those who consume it.

–Male respondent (pastor)

“I have been a smoker for the past 15 years and have always known the risks of health, as I have to work continuously I smoke”.

–Male respondent (driver)

“My favorite hero smokes on screen so as I”

–Male respondent (student)

The present study, however depicts exact understanding of ill effects caused by tobacco among the residents of our selected field area and the only possible shortcoming might be due to the under response of female participants. Although Indian women right away trying to adapt themselves to the modern habits besides maintaining their ancient culture and traditions, occasionally they don’t like to express their inner feelings, tastes, and habits to the outside world.^[14-16]

It also said that the packaging rules are drastic and impractical as this may lead to increase smuggling of illegal cigarettes, even the Beedi industry are against the new rules saying that they would bring the industry to “Grinding Halt” and “cause grave and irreparable harm and loss” as 8 million people and their families were surviving on this industry. Farmer groups and industrial entrepreneurs are among those taking out large advertisements in newspapers protesting and criticizing the legislation.^[17]

Keeping in mind the ethical standards everyone has right to know the truth about harmful effects of tobacco consumption, as pictorial warnings helps to communicate not only with tobacco users but also with prospective quitters and probable initiators, young people and illiterates who initiate tobacco use without knowledge of its health impact will benefit the most, as a picture is worth a thousand words.

CONCLUSION

Although photographic psychology explores the impact that pictures have on behaviors, the present study depicted mandated pictorial warnings on tobacco products do not serve the desired purpose since they are not in a way that can be easily understood by the target population. Hence, there required an implementation of robust and effective pictorial health warnings along with regional language on all tobacco products with immediate effect. Health professionals can play a decisive role by making use of every opportunity in the fight against the tobacco that can be considered as weapon of mass destruction as today’s teenage beginner is tomorrow’s potential regular user.

Limitations

- For the illiterates, the questions were read out by the investigator, which could have influenced the answers
- All female subjects said that they do not have the habit of tobacco either in smoke or smokeless form, as the possibility of social stratification bias cannot be ruled out as none of the female subjects reported of having the habit
- Traditional tobacco products like “chutta” do not have pictorial warnings
- Cross-sectional studies only reflect the time period when the data were collected.

Implications for policymakers and public

- Toll free numbers of rehabilitation cells should be provided on packaging to aid in quitting the habit
- Needed stronger implementation of COTPA Act (cigarettes and other tobacco products act), 2003
- Regional languages along with English should be printed on tobacco products to make the general population realize about the harmful effects of tobacco
- Loose sale of tobacco products should be avoided, as the purpose of pictographs cannot be served
- Establishment of authoritative committees is required to organize the local sale of indigenous tobacco products such as Chutta with proper pictorial representation
- While making the policies plain packaging of the tobacco products should be encouraged worldwide
- Alternative crops cultivation should be encouraged among the tobacco farmers
- Lower the nicotine content in conventional cigarettes to nonaddictive levels suggested by US Food and Drug Administration

- The close interconnection between tobacco use and psychopathological profiles accentuate the importance of interdisciplinary cooperation among professionals working in treatment programs for nicotine dependence.

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Conflicts of interest

There are no conflicts of interest.

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Physical Activity among Dentists in Neo-capital Area of a South Indian State: A Cross-Sectional Study

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Abstract

Background: Sedentary work, which the dental profession demands, causes repeated strain in muscles, tendons, and other body tissues, which could lead to the development of musculoskeletal disorders (MSDs). The prevalence and severity of MSDs can overcome by performing regular physical exercises. **Aim:** To assess the physical activity among dentists in neo-capital area of Andhra Pradesh, India. **Material and Methods:** A cross-sectional study with the help of a self-administered, closed-ended questionnaire was conducted in dental clinics and dental institutions of capital area of Andhra Pradesh, India. A total of 200 dentists were included in the study. Data analysis was performed using SPSS version 20, and Spearman's correlation coefficient, Chi-square test, and Fisher's exact tests were used to analyze the data, and $P \leq 0.05$ was considered statistically significant. **Results:** The study sample consists of 119 (59.5%) males and 81 (40.5%) females, with 50% of individuals belonging to 31–40 years of age group. Majority of individuals belonged to the category of normal (43.5%) and overweight (43.5%) categories of body mass index (BMI) scores. The prevalence of physical activity among the study population was 57.5%, and 53 (26.5%) individuals had suffered from MSD. There was a statistical significance between age groups and BMI scores ($P = 0.01$). **Conclusion:** The demanding and busy life of a dentist should not be perceived as a deterrent to physical activity. Dentists should be at forefront in doing physical activity because of its positive effect on the physical stress and strain, which, in turn, can have effect on delivering services.

Keywords: Body mass index, musculoskeletal disorders, noncommunicable disorders, physical activity

INTRODUCTION

Physical activity is a crucial method for the advancement and upkeep of individual and population well-being and prosperity.^[1] Physical activity is defined as any bodily movement produced by skeletal muscles that require energy expenditure. Physical inactivity has been identified as the fourth leading risk factor for global mortality, causing an estimated 3.2 million deaths globally.^[2] The World Health Organization appraises that, comprehensively, the prevalence of physical activity among grown-ups was 17%, ranging from 11% to 24% crosswise over various provinces of the world.^[3]

Epidemiological research has confirmed that 15%–20% of comprehensive liability for coronary heart disease, type-II diabetes, colon cancers, breast cancers, musculoskeletal disorders (MSDs), and psychological disorders is inferable from physical activity.^[3] The confirmation shows that

customary physical activity is consistent to avert obesity and to minimize the effort of chronic diseases.^[4] Dentistry, the profession of clinical and practical skills, is confined to an outlook covering just a few centimeters (the mouth) and requires continuous, definite force application while conveying oral health. These circumstances appeal a settled posture that can make occupation hazards for dentists.^[5]

Physical activity is a modifiable hazard calculate for noncommunicable diseases (NCDs).^[6,7]

The Indian Council of Medical Research, noncommunicable disease risk factor surveillance, reported that the job-related moderate and vigorous intensity physical activity in urban,

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slum, and rural population was 35.8%, 55.2%, and 61%, respectively, whereas leisure time, moderate, and vigorous intensity physical activity was 15.6%, 12.1%, and 14%, respectively.^[8]

Dental practice is a high hazard calling, and, consistently, a substantial number of dental practitioners are at danger of employment-related musculoskeletal issue. Distinctive elements, for example, heredity, push, unsatisfactory stance amid work, and absence of normal exercise, can influence the frequency of such issue.^[9] In many investigations, the prevalence of different musculoskeletal pains and that the mechanisms prompting work-related musculoskeletal pain are multifactorial, it was accounted for as over half, yet because of prolonged static postures, suboptimal lighting, poor posture of upper limb, repetitive movements, long-term static contractions, use of high-vibration tools, accumulation, injury in the upper limb, genetic predisposition, mental stress, physical conditioning, and age are higher.^[10] In a study by Sharma and Golchha, 75% of dental professionals were at a hazard for creating work-related musculoskeletal scatters, and the pervasiveness and seriousness of these disarranges diminished by performing general particular activities by 20% and 80%, individually; hence, investment in physical action all through life can help in keeping up musculoskeletal well-being.^[11,12]

Essential working stance is viewed as an imperative word-related medical problem for oral human service clinicians. It is by and large concurred that the physical posture of the operator, while providing care, should be such that all muscles are in a relaxed, well-balanced, and neutral position. Postures outside of this neutral position are probably going to bring about musculoskeletal discomfort position.^[12] In India, 40.8% of dental professionals lead a sedentary lifestyle and are at a greater risk for developing chronic diseases.^[1] Information regarding the physical status among dental professionals in India is scarce. Hence, the aim of the present study is to assess physical activity among dentists in neo-capital area of a south Indian state.

MATERIAL AND METHODS

A cross-sectional questionnaire study on dentists was planned in the neo-capital region of Andhra Pradesh, India, with varied specialization fields. Ethical clearance was obtained from the institutional review board, and informed consent (protocol No. Pr. 06/IEC/SIBAR/2016) was obtained after clarifying the aspiration of the study. The study was done during October 2016.

A total of 200 dentists were included in the study based on snowball sampling methodology. As there is no official data regarding the active workforce in this region, snowball sampling was adopted. The questionnaire was developed in a step-wise manner; in the first step, domains were established, that is, demographics and physical activity and then the formulation of item pool questions which were evaluated for validity and reliability. The questionnaire was

self-administered and consisted of various questions including demographics, habit of doing physical activity, type of physical activity, frequency, and others.

Dentists with a habit of doing some kind of activities such as yoga, brisk walking, jogging, running, and workouts were considered as individuals with physical activity. A pilot study was conducted on a sample of 20 dentists to check the feasibility of the study and validity and reliability of the questionnaire. The Cronbach's alpha value of the questionnaire was 0.78.

The questionnaire was answered in the investigator-blinded method. A total of 200 questionnaires were included in the study after excluding 20 incompletely filled questionnaires.

Data analysis

The data obtained were compiled systematically and transformed from a precoded pro forma to a computer, and a master table was prepared using Microsoft Excel 2013.

The collected data were analyzed using IBM SPSS Statistics for Windows, Version 20.0., IBM Corp., Armonk, NY, USA.^[13] Descriptive and inferential statistics were used to summarize the results. The dependent variables were cross-tabulated with the independent variables and then were examined for significance. Pearson's Chi-square, Fisher's exact test, and Spearman's correlation tests were used, when needed to determine significant differences and correlations. The level of significance was set at $P \leq 0.05$ for all the tests.

RESULTS

A total of 200 dentists completed the questionnaire with a response rate of 90.9% with varied age ranges of 23–70 years with a mean age of 34.91 ± 6.902 . Half of the study population belonged to the third decade of age, and about 82% of the dental health professionals were married. The study sample consists of 59.5% of males and 40.5% of females, with majority of participants having master's degree, MDS (65.5%), and BDS (34.05%). Over half of the study participants are working as clinical practitioners, and 38.5% of the participants were working as both a clinical practitioner and a staff [Table 1].

Majority of the study participants belonged to normal (43.5%) and overweight (43.5%) categories of body mass index (BMI) scores, with least number of participants belonging to underweight category (0.5%). The prevalence of physical activity in the study sample was 57.5% [Table 1].

About three-fourth of the individuals who are not performing any type of physical activity was due to inadequate time (75.4%), followed by too tired with the daily schedule (52.9%) and lack of interest (41.2%) [Table 2].

The relationship between various independent factors and the reasons for doing physical activity was cross-tabulated, and the inferences were drawn. There was no statistically significant relationship between reasons for doing physical activity and age group ($P = 0.440$), gender ($P = 0.384$), and

specialty ($P = 0.680$). There was a statistical relationship between BMI scores and reasons for doing physical activity ($P = 0.002$) [Table 3].

The prevalence of MSDs (29.5%) and hypertension (15.4%) was more in participants who are not performing any types of physical activity than participants who are performing physical activity. The prevalence of diabetes (13.1%) was more in participants who are performing physical activity

Table 1: Distribution of individuals based on the study variables	
Variables	n (%)
Age	
23-30	63 (31.5)
31-40	100 (50)
41-50	32 (16)
51-60	4 (2)
61-70	1 (0.5)
Gender	
Males	119 (59.5)
Females	81 (40.5)
Specialty	
BDS	69 (34.5)
MDS	131 (65.5)
Working as	
Staff	26 (13)
Clinical practitioner	97 (48.5)
Both	77 (38.5)
BMI	
Underweight	1 (0.5)
Normal	87 (43.5)
Overweight	87 (43.5)
Obese Class I	22 (11)
Obese Class II	3 (1.5)
Habit of physical activity	
Yes	115 (57.5)
No	85 (42.5)

BMI - Body mass index

Table 2: Reasons for not doing physical activity among dental practitioners	
Reasons	n (%)
Inadequate time	64 (75.3)
Lack of motivation	19 (22.4)
Lack of interest	35 (41.2)
Lack of confidence	2 (2.4)
Intimidating crowds at Gyms/Grounds	1 (1.2)
Tried in the past and failed	10 (11.8)
Family obligations/personal reasons	4 (4.7)
Too tired with the daily schedule	45 (52.9)
Financial problems	1 (1.2)
Even if we do exercise, disease may occur	1 (1.2)
As I am doing meditation, not interested in exercises	2 (2.4)
Health condition does not permit	0 (0)

Total percentage was >100 as multiple options selected by participants

than participants who are not performing any types of physical activity. There was no statistically significant relationship between habit of doing physical activity and history of any medical conditions ($P = 0.766$) [Figure 1].

There was a moderate positive correlation between them ($r = 0.226$), and there was a statistical relationship seen between age groups and BMI scores ($P = 0.001$) [Table 4].

DISCUSSION

A drastic increase in the maintenance of health was not only due to the health services utilization but also through lifestyle factors (physical activity, diet, habits, and hygiene) of the person. The aspect of physical environment as a key determinant of health has also been accentuated by the WHO.^[14] Dentistry is one of the professions that request delayed static position with constrained versatility. The static forces coming about because of these stances have been appeared to be a great deal more entrusting than dynamic forces.^[15]

Health professionals play an important role in managing patients to embrace sound way of life for aversion of NCDs. McKenna *et al.* concluded that health professionals who are physically dynamic themselves are three times more prone to consistently advance physical activity in their patients.^[16] Recognizing this importance, the present study was conducted to determine the prevalence of physical activity among dental health professionals in neo-capital region of Andhra Pradesh, India.

Majority of the study population belonged to third to fourth decades of life, who are having the highest percentage of individuals with a habit of physical activity. The current study observations are supported by various studies.^[17-19] The reason might be with increasing age, there was a decrease in physical activity with more sedentary behavior.

The present study reveals that males are doing more physical activity than females, which was supported by a study done at Hyderabad.^[17] The reason might be that movement at work and recreational action was carried out mostly by males than females.^[17] The findings of the present study reveal

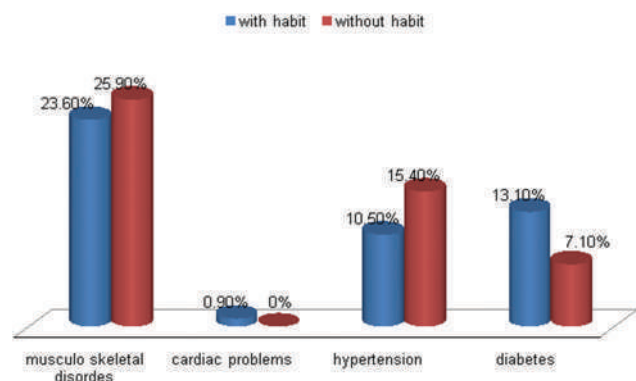


Figure 1: Relation between the habit of physical activity and systemic diseases. $\chi^2 = 5.732$, $P = 0.766$

that MDS graduates are performing more activity than BDS graduates, which are in contrast with the study done on dental practitioners.^[17] The reason for the above comparison might be the MDS graduates practicing dentistry for limited hours, age factor, and had more conscious toward general health.^[17]

Lack of physical activity leads to obesity, which, in turn, is the prime cause for many NCDs.^[20] This evidence was clearly observed in the current study, where the dentists reported the prevalence of many NCDs such as musculoskeletal problems (26.5%), hypertension (9%), diabetes (7%), cardiac problems (0.5%), and other systemic diseases (2%). As the period of time and years of physical activity increases, there is a chance of decreasing the effects of NCDs.

The current study reveals that majority of the professionals belonged to normal and overweight categories of BMI scores, which was similar to the study in which there is a statistical relationship between BMI scores and habit of physical activity.^[21] The reason might be because physical inactivity and excess body mass are not only associated with a number of health-related risk factors but also seem to be an independent risk factor for cardiovascular disease (CVD), type II diabetes, and several types of cancers.^[3] BMI is commonly used to estimate the association of body fattiness and CVD risk in clinical practice and epidemiological studies, but the principal

limitation of BMI is that it does not ascertain fat mass from lean body mass. Individual with a low body mass in relation to height, expressed as the BMI have less difficulty moving their body during weight-bearing activities, such as walking or stair climbing, than individuals with a high BMI.^[21]

In general, physical activity is done to decrease the effects of NCDs, but an interesting point observed in this study was some of the dental professionals started doing physical activity after affecting with NCDs. The reason might be due to lack of free time available for doing physical activity to decrease their work-related stress or disorders.

In the present study, the prevalence of physical activity among health professionals was 57.5%, which was on par with several studies.^[17,22,23] On the other side, the authors reported that physical inactivity was 68% among dental health-care professionals, which was more when compared with the present study findings. This difference in activity levels among dental professionals was because of increased productivity time, which leads to lack of time for physical activity and recreations.^[3]

None of the studies reported about the type of physical activity, and instead they reported the duration of physical activity. The present study reveals that walking (33%) was the most common form of physical activity, followed by yoga (16%). Professionals who perform activities over a long period of time are healthy and are free from NCDs. In the current study as reported by dentists who are doing physical activity more than 10 years are having less chance of NCDs than who are performing activities for a short period (<1 year).

Majority of the dental professionals had opined that inadequate time and too tired with the daily schedule were the two most major reasons for not performing any kind of physical activity.

Table 3: Correlation between body mass index scores and age groups

Variables	Correlation coefficient	P
Age groups	1.000	0.001*
BMI scores	0.226**	

Pearson's correlation, *Statistically significant, **Correlation is significant at the 0.01 level (2-tailed). BMI - Body mass index

Table 4: Relation between independent factors and reasons for doing physical activity

	To be healthy	Cautious to have good physique	On doctors advise	To be healthy and cautious to have good physique	P
Age					
25-30	14	2	2	14	0.440
31-40	28	1	1	28	
41-50	7	0	3	11	
51-60	1	0	1	1	
61-70	1	0	0	0	
Gender					
Male	36	2	4	29	0.384
Female	15	1	3	25	
Specialty					
MDS	34	1	5	36	0.680
BDS	17	2	2	18	
BMI					
Normal	21	1	2	24	0.002*
Overweight	22	2	2	27	
Obese Class I	8	0	1	2	
Obese Class II	0	0	2	1	

Fisher's exact test, *Statistically significant. BMI - Body mass index



Hence, every dentist should allocate some time for physical activity despite their busy schedule, which helps in halting the NCDs.

The limitation of the present study is the sampling technique which is a snowball technique. There is a chance of missing some practitioners because of the misguiding and establishment of a new dental office in those areas which was not known to the participant. Furthermore, there might be a chance of overreporting bias, which is due to social desirability of the professionals when compared to other professionals.

Recommendations

- Social motivation through professional organizations such as the Indian Dental Association
- Knowing the barriers and addressing the issues
- Experts' advise
- Dentists seeking political/administrative involvement in establishing parks and fitness centers through professional organizations
- Tie up with local medical association for the evaluation of systemic diseases.

CONCLUSION

This study reveals that 42.5% of dentists are not performing any kind of physical exercises. This investigation gives knowledge into the level of importance among Indian dental specialist about the significance of customary physical activity. Inside the confinements of the examination, work-related MSDs are observed to be a noteworthy word-related medical issue among the dental practitioners. The part of some physical activity on the quality and amount of work-related MSDs experienced by dental practitioners is critical.

The physical activity among dental practitioners appears to put them at chance for the event of MSDs. MSDs are significant reason driving for loss of work proficiency and in addition early sick well-being retirement among dental practitioners and the predominance and seriousness of these clutters diminish by performing general particular activities as appeared by the present examination. The active recuperation counsel included stance amendment, ergonomic exhort, and extending works out. The part of physical activity among the dental practitioners should be explored advance on bigger gathering to assess particularly the sort of action that diminishes their side effects in the future.

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Conflicts of interest

There are no conflicts of interest.

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Can Inclusion of Humanities be a Silver Bullet Solution to Address Problems in Health-Care Education? A Dental Academician's Perspective

Viswa Chaitanya Chandu*, Srinivas Pachava** and Viswanath V***

The most frequent criticism from patients about the limited time that the doctors are sparing to interact with them highlights the unfortunate shift of the profession from a solemn duty aimed at alleviating the suffering to a financially rewarding line of work. Education at dental colleges focuses on the technical dental practice with limited scope for holistic understanding of the subjects in need. It is less often realized by the dental students that addressing oral health problems of a subject or improving oral health status of a community needs more than technical competency and academic performance. This paper explores the need for inclusion of humanities in dental education in light of the rising public cynicism, creation of incompetent graduates, and the psychologically demanding nature of the dental profession. It is felt that dental graduates are being prepared for the future exclusively technically and therefore incompletely. There is a dire need for inclusion of humanities in the dental curricula. A practical framework for the integration of humanities in dental education is proposed here. The outcomes of humanities in dental education are graduates with humane perspectives, high spirits, substantial clinical acumen, scientific temper, moral reasoning, social responsibility, and political insights who could make the world smile in a real sense.

Keywords: Dental education, health care, humanities, public cynicism

I. INTRODUCTION

In this overachieving world, the unfortunate reality is that the educational systems are not growing into creativity, but out of it. In Sir Ken Robinson's words, "we are actually getting educated out of creativity". The rather mechanistic conception of education predicates on the idea of academic ability which unfortunately narrows down the focus to standard disciplines¹. The essence of education, if education is the body of knowledge received and assimilated by the student in his/hers thoughts and acts, is to develop a basic understanding for things and, thereafter, to explore further

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in quest of knowledge waiting to be discovered and, hence, which have value as ideas sourced in originality and inter-disciplinary lines of thought. This requires moving away from the getting accustomed to routine algorithms of tutored thinking and its *deja vu* of narrow vision to the engaging in constant exploration of multi-disciplinary thought on things, to progress towards building a broader and more comprehensive understanding of the extant body of knowledge.

II. DENTAL EDUCATION AROUND THE WORLD

It is beyond doubt that dental education forms the foundation for a country's progress in dental science. Owing to disparities in the cultural, social, and economic contexts, the prerequisites for gaining admission into dental schools / colleges and the rendition pattern of dental education varies across countries. The degrees awarded after graduation from dental schools / colleges are also different in different countries. It is therefore obvious that the curricula followed in these countries are different with lucid variations in the duration of these courses ². Consequently, an attempt to bring any universal change in global dental education is supremely demanding.

Dental educational institutions are facilities providing clinical care to people, carrying out research in advancement of knowledge, and imparting holistic education to students of dentistry. While it is certain that these three are not mutually exclusive and cannot be, it is also true that there are other facilities like community hospitals which provide clinical care, and there are institutes that exclusively perform research like National Institute for Dental and Craniofacial Research (NIDCR). However, it is the education that is the sole responsibility of educational institutions and therefore must be considered not just as one of the responsibilities, but it's the most important responsibility ³.

III. THE FUNDAMENTAL UNDERSTANDING OF HUMANITIES

Humanities are the lens through which the world around can be better viewed. They are the stories, the propositions, and the expressions which bring people close to making sense of their own lives and the world which they live in. From times immemorial, human beings have been using literature, art, music, history, language, and philosophy to comprehend and document the world ⁴. These aforementioned modes of creative expression are some of the disciplines that are conventionally discussed under the umbrella of humanities. The beauty of humanities is that they introduce us to new people, places, and ideas beyond what unprocessed imagination can do. The highly acknowledged skills of intellect and imagination, creative thinking, independent evaluation, tolerance to different ideologies, critical self – cognition are often regarded as the outcomes of exposure to humanities courses. To put it in a nutshell, the way of science is logical and pragmatic; the way of humanities is one of immersive contemplation based on intuition, creativity, sympathetic understanding, and indwelling.



Educationally, the humanities can develop critical conceptualization and analysis of personal and professional values, and the instinctive, deliberative capabilities of empathy, collegiality, and team work. Individuals with exposure to humanities courses can adapt to work environments rather swiftly and deliver to the extent of their potential. While humanities refer to a plethora of disciplines relating to processing and documenting the human experience, the humanities as applied to health care in particular can be discussed under three broad elements ⁴:

- i. *The Classical Humanities*: These include literature, philosophy, ethics, and culture. They aid in the development of ethical judgment, a sense of social justice, and a respect for persons, all of which are of unsurpassable importance to health care professionals.
- ii. *The Social Sciences*: Anthropology, psychology, economics, history, political science, linguistics, international relations, and communication can be discussed under social sciences. These contribute in development of a comprehensive ideology about health care and its delivery in the context of the individual, the family, the community, the health care team, the nation, and the world.
- iii. *The Arts*: Literature, film, and visual, performance arts comes under the arts and they help in developing and nurturing the skills of self-learning, observation, analysis, empathy, and self-reflection. The arts component of humanity is of special importance to dentistry owing to the nature of work involved.

The fundamental understanding of humanities includes the appreciation of the fact that science and humanities, values and technology are not incompatible. They converge in the clinical decisions; they are both essential, though different, ways of knowing the realities of illness, healing, and health ⁵.

IV. CHALLENGES FOR INTEGRATION OF HUMANITIES IN DENTAL EDUCATION AND COUNTER ARGUMENTS JUSTIFYING THE INCLUSION

While inclusion of humanities in medical education was proposed and fragmentarily executed since 1948 ⁶, the proposition was much less discussed among the dental fraternity affirming the fact that the topics and concepts which start first in medical field will take time to be adapted to dentistry. Despite the afore-stated advantages of inclusion of humanities in health care education, there are different viewpoints in this regard, and there has also been formidable resistance against their inclusion ⁷. Some of the many arguments against inclusion of humanities in dental education are enlisted below, together with the counterarguments justifying their inclusion.

Challenge: What is the rationale for inclusion of humanities in dentistry which is a science?



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Counterargument: The argument questioning the rationale behind inclusion of humanities in dental education is a sheer misunderstanding of the nature of health care practice, in general, and dentistry, in particular. Dentistry, or any health care profession, belongs to humanities as much as it belongs to science. The conceptualization of dentistry exclusively as a science holds the hazard of creation of technically driven entrepreneurs. Humanities offer insights into the human condition, suffering, personhood, community responsibility, and a historical perspective of dental practice, and have a place in dentistry beyond doubt.

Challenge: There is no place for inclusion of humanities in the already overloaded curriculum.

Counterargument: With genuine empathy towards the argument that the curriculum is already overloaded, the assertion is that it is equally important to realise whether the curriculum is serving its very purpose or not. The inclusion of humanities in the curriculum not only helps in betterment of the dental treatment outcomes of patients who are treated by better abled dentists but also assuages the critics who have long advocated this inclusion. There has long been a criticism from supporters of humanities in health care education that the integration of humanities was never complete and there was always a tendency to quarantine humanities from health care subjects, even when included ⁸. Since there is no space for humanities in the overloaded curriculum, it will be wise to resort to problem-based integration of humanities teaching within each of the subjects of the existing dental curriculum rather than teaching it as an alienated peripheral discipline.

Challenge: Funding for this sort of development is a problem.

Counterargument: It is true that the funding necessary for inclusion of humanities courses in health care education is often derived from the income generated by the clinical departments ⁹. Funding is absolutely necessary for the successful implementation of any program, and it is important that the educational experts convince the managements of dental schools / colleges to realize the benefits of humanities in their endeavor of creating responsible dentists. Efforts should be made both at regional and national levels to bring policy changes, financially supporting the integration of humanities in dental education. Advocacy and lobbying become the vital activities in this quest of convincing authorities and policy makers.

V. HUMANITIES IN DENTAL EDUCATION: AN ABSOLUTE NECESSITY

It is important to understand that the dental profession is bowed down with a multitude of challenges in recent years. There is a dire need for the dental education system to undergo progressive metamorphosis in order to negotiate these challenges ¹⁰. An attempt has been made here to group many of the challenges faced by the profession under three headings as follows:



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- i. *Public Cynicism*: There has been rising public cynicism and enormous dissatisfaction with the health care profession as a whole. Doctors are increasingly being recognized by public as over-materialistic, and deprived of the qualities of empathy and compassion, which warns the profession of the precarious chance for slow erosion of public regard for health care professionals¹¹. The most frequent criticism from patients about the unduly limited time that the doctors are sparing to interact with them highlights the unfortunate shift of the profession from a solemn duty aimed at alleviating the suffering to a financially remunerated and technically demanding line of work¹². In today's world, doctors are increasingly being referred to as greedy entrepreneurs and indifferent technocrats with little appreciation for the fact that disease involves the person as much as his organs and tissues. Zhong Nanshan, former president of Chinese Medical Association, opines that the absence of humanities is the prime reason why doctors are being mistrusted by public¹³.

It is true, though unfortunate, that there has been not enough emphasis on nurturing ideals and service attitude among dental students as there has been on testing and grading. This not only results in unjust competitiveness, anxiety, and stress, but also leads to traumatic divestment of ideals, which refers to transformation of students from the ideals with which they got attracted to the profession in the first place to distancing from ideals. In this context, inclusion of humanities, the disciplines of philosophy and literature in particular, in dental education has that immaculate potential to inculcate the humane touch among future dental professionals, indoctrinate empathy for patients, and reinforce their moral ideals. Moreover, the expectations and ideology with which students enter the profession can be sustained and reinforced.

- ii. *Creation of Incompetent Graduates*: Reemphasis on the fact that the expression 'competency' does not just refer to technical and academic competencies is essential at this juncture. The expression also refers to the ability of the dental students to communicate with their patients, to develop scientific temper, to think and act critically, to work in an interdisciplinary environment, to get equipped with the skills required for scientific communication, to understand cultural values, traditions, health related beliefs, and attitudes of people from diverse cultural backgrounds, and to be well aware of the role he / she is going to assume in the future. Studies suggest that there is very limited emphasis on interpersonal communication in the dental curricula worldwide¹⁴. It is less often realized that dentistry, and for that matter of fact, any health care profession, has been situated between science and technology, on one hand, and the needs of the suffering human beings, on the other. The connection between technical



and moral questions in clinical decision making, and the preparation to be both objective and compassionate in a scenario are not being taught.

Also, dental students from countries where English is not the first language are not nourished with effective scientific communication skills, resulting in journals providing language refining services. This forms a strong argument for the integration of humanities in dental education. Principally, the disciplines of history, psychology, ethics, and communication embrace the potential to make students competent from the sense of ability to handle patients but also connect with them as humans in need of doctor's understanding and time. .

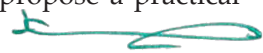
- iii. *Stress, Anxiety, and Suicidal Ideation:* Research suggests that dental students are prone to tremendous amounts of anxiety and stress, and the causal relation that curriculum has in this regard cannot be denied ^{15, 16}. A few reports highlight dentistry as a profession which is highly prone to its students committing suicide compared to other professions, and identified stress as the major cause for this tendency ¹⁷. Recent studies confirm burnout and depression among dental students, and highlight the suicidal ideation ¹⁸.

Also, there is growing insecurity among dental students about their careers, at least in countries like India and Japan, particularly in light of deficient policy actions with regard to dental manpower planning based on the service needs and utilisation patterns. The growing insecurity can be cited as one of the reasons for depression among dental students which clearly is undesired. Another concern is the diminishing ethical standards among students in health care professions with regard to various aspects of academic life ^{19, 20}.

The aforementioned challenges bring up an argument criticising the current framework of medical and dental education. Some experts suggest that integration of humanities in dental education goes a long way in the endeavour of creating humanistic, high-spirited, and competent dental health care professionals. However, it is erroneous to postulate that humanities never existed in the curricula of dental education as every scientific element taught in dental schools / colleges have indisputable contribution from humanistic components. The truth is that humanities are not being stressed to the extent necessary, with adverse consequences to the professionals and the profession.

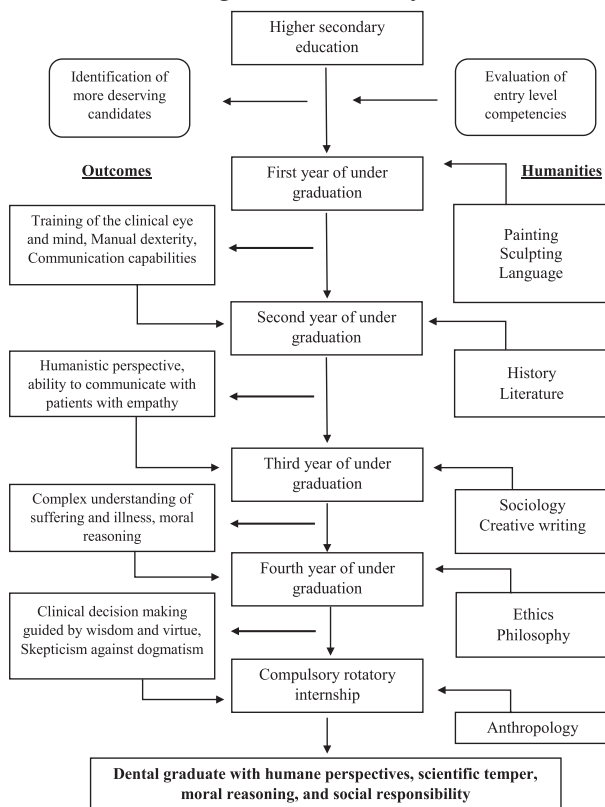
VI. PRACTICAL FRAMEWORK FOR INTEGRATION WITH POTENTIAL OUTCOMES

Though integration of humanities in dental education has increasingly been receiving theoretical support, its practical application was never complete partly owing to the challenges already stated and partly owing to the complacency in formulating an executable framework ²¹. An attempt has been made here to propose a practical



framework to integrate humanities with assertions on possible outcomes. However, as previously mentioned, the curricula in dental education across the world are very diverse from the viewpoint of each place having its own needs and hence a different curriculum; and, a universally acceptable framework cannot be proposed with distinct variations in the duration of these curricula. Therefore, the framework is proposed to suit the dental education in India and other countries which follow similar curriculum.

The integration of humanities in dental education must start from the admission process itself with enough emphasis on competencies like social and interpersonal skills, desire to learn, service orientation, and adaptability at entry level. It is important to highlight at this point that the necessity and relevance of inclusion of humanities in dental education increases only when Indian scenario is exclusively considered, owing to the exceedingly high oral health inequalities requiring just distribution of dental professionals across the country, not to mention uniform disbursement of dental care based on doctors' ability to adapt to patient needs not limited to his socioeconomic condition. Ironically, these inequalities must be understood in light of the fact that there are 313 dental colleges in India at present and the dental educational system in the nation produces around 30,570 graduates each year ²².



Integration of humanities in dental education is certainly essential in India more than anywhere else and could be considered as an upstream approach in reducing the geographical imbalances in distribution of dentists, socioeconomic disparities in utilization of oral health services, and consequently oral health inequalities. In post graduate curriculum, the emphasis must be placed on preparing students on how to think critically, present research effectively, and argue rationally. Application of knowledge through humanities in ethical decision making can be evaluated at the post graduate level. The disciplines in humanities suggested above must be taught liberally, inter-linked with dental science scenarios creating rounded vision and robust approach to patient care, and not as distinct disciplines. The intention in integration of humanities in dental education is not to master the humanities, but to realize the philosophical assumptions that underlie the practice of dentistry and to indoctrinate humane values among future dental professionals besides equipping them with necessary competencies.

VII. CONCLUSION

Health is a social, economic and political issue and, above all, health is a fundamental human right. Educational institutions play an influential role in creation of health care professionals who work for this fundamental right of people. Despite the significant negative influence of the oral health problems on the quality of life, utilisation of oral health services has never been optimal around the world. Moreover, there is growing cynicism among public about the predominantly financial and technically driven attitude of dental professionals. What has happened to the ability of dentists to interact with their patients with humanistic attitude and empathy, to understand the patient as a whole with regard to their social, economic, cultural background with meticulous detail about how exactly the condition affects their personality? The answer to these questions lies in the way the dental graduates are prepared for the future: exclusively technically and, therefore, incompletely. The outcomes of humanities in dental education are inculcating graduates with humane perspectives, instilling high spirits, imbibing of substantial clinical acumen, forging of scientific temper, assimilation of moral reasoning, disbursement of social responsibility, and using of political insights all of which could make the world smile in a real sense.

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Expression of cluster differentiation-44 stem cell marker in grades of oral epithelial dysplasia: A preliminary study

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Abstract

Introduction: Oral cancer is one among the alarming diseases related to oral cavity. Its prevalence and incidence have increased in many folds, in the past decade. This has led the investigators to find the preliminary stages and related early evaluating methods to restrain it. Few clinical lesions such as leukoplakia, erythroplakia, oral submucous fibrosis and lichen planus reflected malignant changes. These premalignant disorders provided scope to assess the underlying cellular and molecular events, which shall be helpful in early detection, aggressiveness and prognosis of the patient.

Materials and Methods: Forty formalin fixed, paraffin embedded blocks were utilized and evenly subdivided into Group I – control tissue, Group II – mild epithelial dysplasia, Group III – moderate epithelial dysplasia and Group IV – severe epithelial dysplasia. The study group was categorized based on the WHO classification of dysplasia 2005. Routine staining was performed to reconfirm the diagnosis of all the samples. Simultaneously, immunohistochemical staining was done with cluster differentiation-44 (CD44) antibody. Positive cells were counted on 10 representative fields with a minimum of 100 cells per field using $\times 20$.

Statistical Analysis: Comparison of four groups with respective to number of positive cells was done using Kruskal–Wallis ANOVA test. Pair-wise comparison of three grades of oral epithelial dysplasia and the controls was done using Mann–Whitney U test.

Results: The mean of Group I is 745.50, Group II is 665.20, Group III is 530.10 and Group IV is 322.90. A statistically significant $P = 0.00001$ was ascertained on comparison of the mean between the groups.

Conclusion: CD44, a cell membrane marker could help in cell adhesion and cell-cell interactions. Loss of CD44 expression enhances the binding of the growth factors with their principle receptors that enhances the cellular proliferation. It can be used as a prognostic marker for identifying the rate of malignant transformation in these disorders.

Keywords: CD44, cell membrane marker, dyaplasia, stem cell

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INTRODUCTION

Oral cancer is a major health problem in many parts of the world. While the incidence is relatively low in western countries, it remains to be one of the most common forms of cancer, in the Indian subcontinent and other parts of Asia.^[1] Its strong correlation with specific risk factors, such as tobacco and alcohol use cause genetic damage. Thus, leading to uncontrolled proliferation of these cells resulting in dysplasia and presents as precancer and cancer.^[2]

Oral leukoplakia represents the most common potentially malignant disorder of the oral cavity. Worldwide prevalence of leukoplakia is 0.2%–4.9% and the overall malignant transformation rates for dysplastic lesions range from 3% to 6%, depending on the type, size of the lesion and length of follow-up. The presence of epithelial dysplasia histopathologically may be even more important in predicting malignant potential than the clinical characteristics.^[3]

Dysplasia is a Greek word meaning abnormal atypical proliferation of tissues. The term “*dysplasia*” was introduced by Reagon in 1958 in relation to the cells exfoliated from lesions of the uterine cervix. Dysplasia is encountered principally in the epithelium. In the past, epithelial dysplasia, epithelial atypia and dyskeratosis were used synonymously.^[4]

Oral epithelial dysplasia (OED) is the diagnostic term used to describe the histopathologic changes seen in a chronic, progressive and premalignant disorder of the oral mucosa. OED is not associated with any specific clinical appearance. However, leukoplakia and erythroplakia are the lesions classically associated with dysplastic changes. It is also consistently seen in the mucosa adjacent to the tumor in patients with invasive squamous cell carcinoma.^[5]

Various markers such as Ki-67, p53, proliferating cell nuclear antigen, argyrophilic nucleolar organizer region and cytokeratins and notch genes have been utilized to identify OED that will progress to malignancy.^[6]

In the past few years, there has been increasing interest in family of surface glycoproteins called cluster differentiation-44 (CD44). These are first described in 1983 as lymphocyte homing receptor. It is encoded by a single gene containing 20 exons located on chromosome 11p13. CD44 was originally implicated as a hyaluronic acid and a homing receptor directing the migration of circulating lymphocytes across the high endothelial venular membranes of the lymph nodes and inflamed synovia.^[7]

CD44 is not only expressed in lymphocytes but also a wide variety of epithelial tissues. Functionally speaking, CD44 is involved in organ integrity through its ability to contact extracellular matrix. It serves as a co-receptor for numerous transmembrane proteins such as matrix metalloproteases, members of the ERB family of receptor tyrosine kinases, and the long known tumor-associated antigens EpCAM (CD326, ESA1).^[8]

CD44 was in the focus of molecular oncology in the early 1990s when it was recognized that variants of it, chiefly CD44 v6, regulate tumor progression, invasion and metastasis formation.^[9] The nature of this transmembrane adhesion molecule and the role it plays in the tumor development and progression are matters which have interested not only the basic researchers but also cancer clinicians and pathologists. The availability of different exon-specific monoclonal antibodies against CD44 variants has enhanced the ease and accuracy of immunohistochemical analysis. However, there have been comparatively few studies involving CD44 expression and premalignant oral lesions.^[10]

The current study aims to evaluate the immunohistochemical expression of CD44 in OED and whether it can serve as a prognostic marker.

MATERIALS AND METHODS

The present study was carried after the ethical clearance from the Institutional Review Board (Protocol no.22/IEC-SIBAR/17) on 24th December 2017. A total of 40 samples were utilized. Formalin-fixed, paraffin embedded blocks, categorized according to 2005 WHO classification of dysplasia were included in the study. Apparently normal healthy oral mucosa without any of the clinically obvious lesions was included in the control group. Those patients who are having a habit of smoking, drinking alcohol and chewing are excluded. The study sample was divided into Group I – control tissue (10), Group II – mild epithelial dysplasia (10), Group III – moderate epithelial dysplasia (10) and Group IV – severe epithelial dysplasia (10).

Serial sections of 4 μ were obtained from the archival material. The sections of all the samples were first subjected for routine hematoxylin and eosin examination to reconfirm the diagnosis. Later, other sections of all the four groups were subjected for immunohistochemical analysis using CD44 antibody. The positive CD44 expression was seen as a light brown stain in the cells of the epithelium [Figures 1-4]. These cells were counted on 10 representative fields with a minimum of 100 cells per field using $\times 20$.

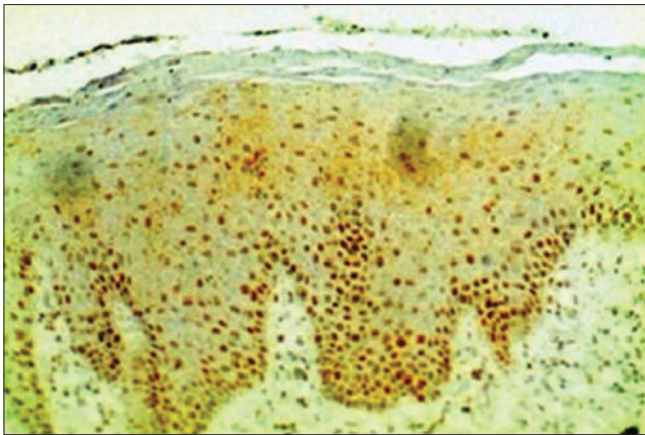


Figure 1: Cluster differentiation-44 immunopositivity of normal mucosa, $\times 10$

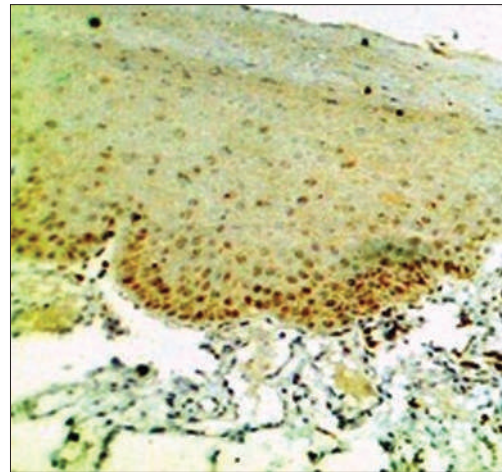


Figure 2: Cluster differentiation-44 immunopositivity of mild epithelial dysplasia, $\times 10$

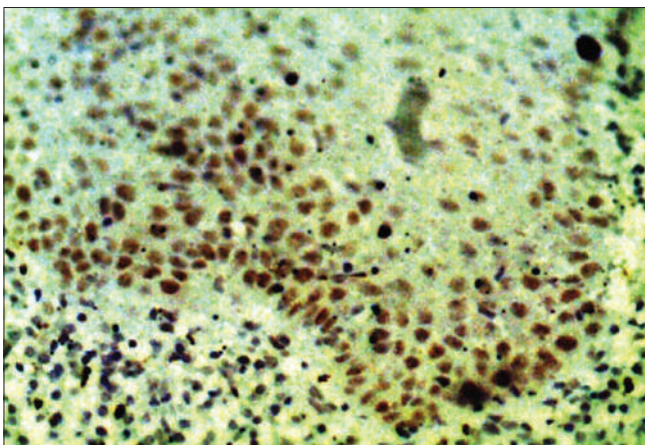


Figure 3: Cluster differentiation-44 immunopositivity of moderate epithelial dysplasia, $\times 20$

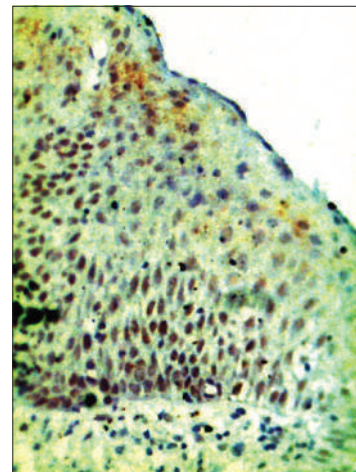


Figure 4: Cluster differentiation-44 immunopositivity of severe epithelial dysplasia, $\times 20$

Research microscope BX51 manufactured by Olympus, a jenoptik CCD camera and image analyzer software (Pro Express) were utilized.

Statistical analysis

The collected data were entered into the Excel sheet, and statistical analysis was done using software, Statistical Package for Social Sciences version 20.0 (IBM Business Corporation, Chicago, USA). The comparison of four groups with respect to number of positive cells was done using Kruskal–Wallis ANOVA test. Pair-wise comparison of three grades of OED and the controls was done using Mann–Whitney U test.

RESULTS

The mean and standard deviation of each group was determined Kruskal–Wallis ANOVA. The mean of Group I is 745.50, Group II is 665.20, Group III is 530.10 and Group IV is 322.90. The standard deviation of four groups is 68.17, 68.94, 56.69 and 42.77, respectively. A statistically

significant $P = 0.00001$ was ascertained on comparison of the mean between the groups, as shown in Table 1.

Pair-wise comparison of four groups with respect to number of cells was done using Mann–Whitney U-test. A significant P value was noticed on the comparison between the groups [Table 2].

DISCUSSION

Oral precancerous lesions are altered epithelial lesions which have an increased likelihood to progress toward oral squamous cell carcinoma. Recently, the term premalignant lesions and conditions have been replaced with a common favorable terminology “potentially malignant disorder.”^[11]

Most common potentially malignant disorders of the oral cavity are leukoplakia and erythroplakia. Around 50% of the oral squamous cell carcinoma arises from these lesions.

Table 1: Comparison of four groups with respect to number of cells by Kruskal-Wallis ANOVA test

Groups	Mean	SD	P
Group I	745.50	68.17	0.00001*
Group II	665.20	68.94	
Group III	530.10	56.69	
Group IV	322.90	42.77	

*P<0.005. SD: Standard deviation

Table 2: Pair-wise comparison of four groups with respect to number of cells by Mann-Whitney U-test

Groups	Mean	SD	Z	P
Group I	745.50	68.17	-2.2678	0.0233*
Group II	665.20	68.94		
Group I	745.50	68.17	-3.7041	0.0002*
Group III	530.10	56.69		
Group I	745.50	68.17	-3.7796	0.0002*
Group IV	322.90	42.77		
Group II	665.20	68.94	-3.3639	0.0008*
Group III	530.10	56.69		
Group II	665.20	68.94	-3.7796	0.0002*
Group IV	322.90	42.77		
Group III	530.10	56.69		
Group IV	322.90	42.77	-3.7796	0.0002*

*P<0.005. SD: Standard deviation

The potentially malignant lesions can be assessed using hematoxylin and eosin for the architectural and cytological changes which is generally referred as epithelial dysplasia.^[12]

The specific alterations of the epithelial cells are important to determine dysplastic cells. The nuclei take a more primitive appearance, similar to those of basal cells, often show nuclear enlargement, dark-staining nuclei and an increased nuclear-to-cytoplasmic ratio as well as variation in the shape of the cells and nuclei. These are unusual outside cancers and precancers.^[13]

The 14th International Cancer Congress in Hungary suggested the following microscopic changes for the diagnosis of OED: drop-shaped rete processes, disturbed nuclear polarity, basal cell hyperplasia, disturbed epithelial maturation, pleomorphic cells, anisocytosis, hyperchromatic nuclei, prominent nucleoli, increased nuclear-cytoplasmic ratio, cell crowding, increased number of mitoses, abnormal mitoses and reduced cellular cohesion.^[14]

The abnormalities in the nuclear morphology are frequently seen in the dysplasia such as the nuclear diameter, shape, nuclear area, number of nucleoli and membrane outline. This can be correlated with the rate of malignant transformation and prognosis of the disease.^[15] The oral epithelial dysplasias are more likely to progress into cancer. The actual mechanism of progression is poorly understood, and there is no evidence that a dysplastic lesion will surely progress into cancer.^[16]

Grading of OED's will help us to lower the inter- and intra-observer variation, and it give us a clear separation between the patients who need treatment to prevent malignancy.^[17] There are several grading systems followed to grade OEDs such as Shafer's (1993), Neville (1995), Ljubljana (2003) and WHO (2005). Among these most commonly followed is the WHO system, in which it is divided into three grades such as mild, moderate and severe epithelial dysplasia. The WHO grading system was considered in the present study which is universally accepted. These grading processes are basically structured on the potential risk for malignant transformation showing a range from 3% to 6%.^[18]

There are several methods for identification, but the gold standard of identification is tissue biopsy. Molecular markers with immunohistochemical procedures can determine the prognostic features and rate of malignant transformation.^[19]

Stem cells constitute a distinct subset of cells characterized by their capacity to self-renewal and differentiation into multilineage cellular constituents of a specific tissue or organ.^[20]

The stem cells are of different types such as embryonic stem cells, adult stem cells and cancer stem cells. The embryonic stem cells are isolated from the inner cell mass of the blastocyst are pluripotent and can differentiate into three germ layers (ectoderm, endoderm and mesoderm). Adult stem cells are found in various adult tissues, which are typically more limited to with respect to differentiation, they are considered as multioligopotent.^[21]

Cancer stem cells are a small subpopulation of cancer cells that have a unique ability of self-renewal and are potent to differentiate into progenitor cells. The fundamental characteristics which segregate cancer stem cells from other stem cells are ability to initiate and regenerate the tumor, representing a phenocopy of the original tumor. These cells exhibit *in vivo* self-renewal capability and demonstrate a unique capacity to differentiate into various lineages, allowing them to give rise to a heterogeneous progeny.^[22]

In the recent decade, stem cell markers such as aldehyde dehydrogenase 1, CD271, CD24 and CD44 have been used in the identification of OED and oral squamous cell carcinoma.^[23]

In the present study, there was a decline in the expression of CD44 in three grades of epithelial dysplasia when

compared to normal mucosa. The correlation between the degree of dysplasia and CD44 v6 down-regulation might reflect the fact of early cellular changes from normal cell-cell and cell-matrix interactions toward the bizarre, pathophysiological heterotypic cell surface adhesion property, which may be contributory for the cells to achieve invasion and early development of malignant tumors in the oral cavity.^[24-26]

The mean of CD44 immunopositive cells was more in normal mucosa when compared to mild, moderate and severe epithelial dysplasia. Severe epithelial dysplasia cases showed down-regulated expression of CD44. The correlation between the degree of dysplasia and CD44 down-regulation has been related to proliferation as well as the grade of cellular differentiation implicated in motility and invasion of the lesion.^[23,27,28]

CONCLUSION

CD44 mediates the adhesive properties and signals for the orientation of epithelial cells to migrate upward. It regulates the interaction of growth factors and their corresponding receptors. Increased cellular proliferation is a result of enhanced binding of the growth factors with their principle receptors, which is correlated to loss of CD44 and its reduced expression. Thus, CD44, a cell membrane marker could help in cell adhesion and cell-cell interactions. It can be used as a prognostic marker for identifying the rate of malignant transformation and by detecting the severity of the disease.

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Conflicts of interest

There are no conflicts of interest.

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A preliminary study on plasma concentration achieved following intrapterygomandibular space injection of dexamethasone as a route of drug delivery with lignocaine inferior alveolar nerve block—correlation of clinical effects

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Abstract

Purpose To determine the quantity of dexamethasone plasma concentration achieved following intrapterygomandibular space injection of dexamethasone when co-administered with inferior alveolar nerve block correlating with the clinical effects in the postoperative phase.

Objective A preliminary prospective study to evaluate the dexamethasone plasma concentration achieved following intrapterygomandibular space injection of dexamethasone with 2% lignocaine inferior alveolar nerve block to achieve hemimandibular anesthesia for minor oral surgical procedures and derive clinical correlations.

Background Dexamethasone is a glucocorticoid, chiefly used for the management of postsurgical sequelae like trismus and swelling in maxillofacial surgical practice. Conventionally, parenteral dexamethasone is administered via intravenous or intramuscular route. Intrapterygomandibular space injection is a novel route of steroid delivery described in literature. For minor oral surgical procedures in maxillofacial surgical practice requiring inferior alveolar nerve block, dexamethasone can be administered along with local anesthetic through a single injection as a mixture (twin mix).

Methods Prospective double-blind randomized clinical trial was designed to evaluate plasma concentration of dexamethasone achieved following injection of a freshly prepared mixture of 1.8 ml of 2% lignocaine with adrenaline (1:200000) and 1 ml (4 mg) dexamethasone [2.8 ml solution of twin mix] in the pterygomandibular space. The 30 candidates included for the trial were randomly split into three study groups (ten each)—(1) control group (C); (2) intramuscular group (IM); (3) intraspaces group (IS).

Results The mean plasma dexamethasone concentration at 30 min postinjection in group IM was 226.41 ± 48.67 ng/ml and for IS group it was 209.67 ± 88.13 ng/ml. Post hoc (Bonferroni-Holm test) intergroup comparison for plasma dexamethasone concentration (IM and IS) was found statistically insignificant ($P = 0.605$).

Conclusion Intraspace route of drug administration can be utilized to deliver dexamethasonized local anesthetics safely with predictable clinical effects in the patients requiring mandibular minor oral surgery under local anesthesia.

Keywords Local anesthesia · Inferior alveolar nerve block · Dexamethasone · Intraoral nerve block

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Introduction

The chosen route of drug delivery should offer lesser patient discomfort and ease of drug administration by the clinician. Corticosteroids are time tested adjuncts to surgery for suppressing tissue mediators of inflammation, thereby reducing transudation and lessening edema in the postoperative phase [1]. Evidence from systematic review and meta-analysis evaluating the use of corticosteroids in reducing postoperative morbidity after third molar surgery has indicated that the administration of steroids clinically produce reduction in edema and improvement in range of motion after third molar surgical



removal [2]. Corticosteroids exert their anti-inflammatory action through its multimodal effect by inhibiting vascular dilatation, reducing liquid transudation and edema formation, decreasing cell exudates, and reducing fibrin deposit around the inflamed area through its mechanisms responsible for these effects like inhibition of leukocyte chemotaxis to the inflammatory focus, inhibition of fibroblast function and endothelial cells, and suppression of the production or inhibitory effects of numerous chemical inflammation mediators including leukotriene pathway suppression [3]. Usual practice is to deposit/inject steroids via intramuscular or intravenous route to reduce the postoperative sequelae following mandibular transalveolar extractions [2, 4, 5]. For minor oral surgical procedures requiring inferior alveolar nerve block, dexamethasone can be administered along with local anesthetic solution through a single injection as a mixture [2]. A randomized prospective double-blind study was planned to evaluate the efficacy of dexamethasone following inrapterygomandibular space injection administered along with local anesthetic solution. Presented study also correlates the dexamethasone plasma concentrations achieved with the clinical effects of the drug in the postoperative period. The preliminary report of the research outcome is presented.

Material and methods

A prospective double-blind randomized clinical trial was designed to evaluative plasma concentration of dexamethasone achieved following injection of a freshly prepared mixture of 1.8 ml of 2% lignocaine with adrenaline (1:200000) and 1 ml (4 mg) dexamethasone [2.8 ml solution of twin mix] in the pterygomandibular space and correlate the same with the clinical effects of the steroid administered (Table 1). The conventional inferior alveolar nerve block technique as described by Ashley Lindsay was followed for all the procedures in this study. In cases receiving the “study mixture,” 2.3 ml of the twin mix solution was deposited in the pterygomandibular space and 0.5 ml solution was deposited distal and buccal to erupted last molar for anesthetizing the long buccal nerve, where as in cases receiving standard local anesthetic solution,

1.3 ml was deposited in the pterygomandibular space and 0.5 ml solution was deposited distal and buccal to last molar for anesthetizing the long buccal nerve [6]. The clinical trial was approved by the institutional ethical committee via reference no. P***/ACAD/8/2016/24. A total of 30 patients with impacted mandibular third molar were selected for minor oral surgical procedure, planned to be performed under inferior alveolar nerve block local anesthesia. Inclusion criteria for the selection of study patients included, Class II-Position B mandibular wisdom tooth impaction as described by Pell and Gregory [7, 8], ASA Class I, and the candidates willing to sign the written informed consent for inclusion in the trial. The 30 candidates included for the trial were randomly split into three study groups (ten each)—(1) control group (C); (2) intramuscular group (IM); (3) intraspace group (IS). In the control group, patients’ dexamethasone was not used. For “IM group,” patients’ 4 mg dexamethasone was administered via gluteal intramuscular injection just before the surgical procedure whereas the “IS group” received dexamethasone as a mixture [1.8 ml of 2% lignocaine with adrenaline (1:200000) and 1 ml (4 mg) dexamethasone] through inrapterygomandibular space injection. Control group (C) and intramuscular group (IM) group patients received standard 1.8 ml 2% lignocaine with adrenaline (1:200000) pterygomandibular nerve block before the surgical procedure. All the transalveolar mandibular surgeries for the study were performed by a single operator who was blinded. The evaluation parameters for the study included—(1) plasma concentration of dexamethasone in IM and IS group patients; (2) reduction in mouth opening to assess trismus (interincisal distance, measurement from central incisor of maxillary first quadrant to mandibular central incisor in fourth quadrant as per FDI notation); (3) increase in tragus-oral commissure distance postoperatively to assess swelling. VAS pain scores were recorded but not included for this study to eliminate subjective (multifactorial patient related) data input bias. For estimation of plasma concentration of dexamethasone, 5 ml venous blood sample was withdrawn via the antecubital fossa 30 min after injection with 4 mg dexamethasone either by intraspace route or by intramuscular route, and samples were subjected to high performance liquid chromatography (HPLC) using standard protocols described elsewhere [9]. The observer of the study parameters to be evaluated was also blinded. Statistically, $P < 0.001$ was considered significant for the study.

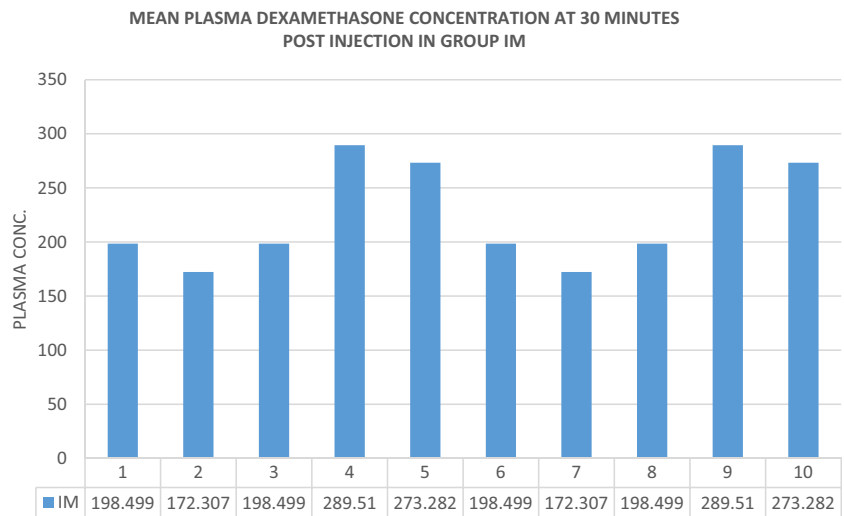
Table 1 Composition of individual drugs used to formulate twin mix

Corticosteroid (1 ml)	Local anesthetic (1.8 ml)
Dexamethasone sodium phosphate IP 4 mg/ml	Lignocaine hydrochloride IP 21.3 mg/ml
Sodium methylparaben IP 0.15% w/v	Adrenaline (as bitartrate) IP 0.005 mg/ml
Sodium propylparaben IP 0.02% w/v	Sodium chloride IP 6.0 mg/ml
Water for injection IP q.s	Sodium metabisulfite IP 0.5 mg/ml
	Methyleparaben IP 1.0 mg/ml
	Water for injection IP to make 1 ml

Results

The mean age (in years) and gender distribution of the study patients in group C was 27.9 ± 5.02 (M 7, F 3); group IM 28.7 ± 4.97 (M 6, F 4), and group IS 29 ± 5.94 (M 8, F 2). The mean plasma dexamethasone concentration at 30 min

Fig. 1 The mean plasma dexamethasone concentration at 30 min postinjection in group intramuscular (IM) in ng/ml for each individual sample (1–10)



postinjection in group IM was 226.41 ± 48.67 ng/ml (Fig. 1) and for IS group it was 209.67 ± 88.13 ng/ml (Fig. 2, Table 2). Post hoc (Bonferroni-Holm test) intergroup comparison for plasma dexamethasone concentration (IM and IS) was found statistically insignificant ($P = 0.605$). The mean reduction in mouth opening (in mm) 24 h postsurgery recorded in group C was 4.80 ± 1.93 , group IM was 3.60 ± 1.71 , and in group IS was 3.00 ± 1.05 with probability of intergroup comparison using ANOVA = 0.055. The mean increase in tragus-oral commissure distance postoperatively (to assess swelling in mm) 24 h postsurgery recorded in group C was 5.00 ± 1.41 , group IM was 3.00 ± 1.25 , and in group IS was 3.20 ± 1.23 with probability of intergroup comparison using ANOVA = 0.003 (Statistics for comparative analysis of the obtained data using College of Saint Benedict and Saint John’s University Statistical Calculators).

Discussion

Surgical trauma in the oral cavity causes tissue injury characterized by hyperemia, vasodilatation, and increased capillary permeability with fluid accumulation in the interstitial space which in turn causes edema and postoperative discomfort to the patients undergoing such surgery [10, 11]. As concluded by Sortino and Ciccù, “postoperative swelling is a common event after surgery of impacted third molar and may affect, only for a few days, the social and working life of the patient.” Clinicians should manage the postoperative discomfort after the surgery as well as they can emphasizes the importance of managing the postsurgical sequelae following such surgical procedures [12]. Steroids are potent anti-inflammatory agents that have been widely used to reduce the sequelae following transalveolar surgery of third molars. The benefits of

Fig. 2 The mean plasma dexamethasone concentration at 30 min postinjection in group intraspace (IS) in ng/ml for each individual sample (1–10)

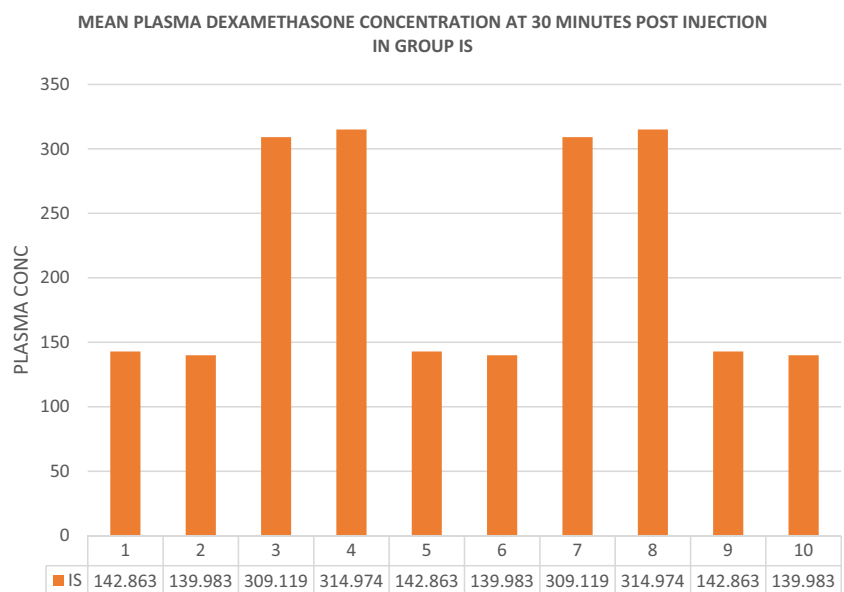


Table 2 Quantitative analysis of mean plasma dexamethasone concentration at 30 min postinjection using HPLC (in ng/ml)

Mean plasma dexamethasone concentration at 30 min postinjection	Quantitative analysis using HPLC (in ng/ml)	Standard deviation	<i>P</i> based on post hoc Bonferroni-Holm test for intergroup comparison
For patients in intramuscular (IM) injection group	226.41	48.67	<i>P</i> = 0.605 (NS)
For patients in intraspace (IS) injection group	209.67	88.13	

NS not significant

administering corticosteroids for improving the postoperative quality of life of patients following surgical extraction of third molars are well documented [13].

Administration of dexamethasonized local anesthetic via intrapterygomandibular space for mandibular minor oral surgeries have a clear advantage of injecting local anesthesia and dexamethasone by a single-needle prick [14, 15]. Other advantages offered by this route of drug delivery apart from single prick for dual drug administration include ease of administration of the drug for oral and dental clinicians, lesser stinging of local anesthetic injection due to altered pH of the combination, short latency for local anesthetic, prolonged duration of the soft tissue anesthesia, and improved quality of life in the postoperative period after the surgical procedure [4, 14]. pH of the local anesthetic solution containing 2% lignocaine with 1:200,000 epinephrine is in the range of 3.5–4.5. Addition of dexamethasone solution (pH = 8.5) to 2% lignocaine with 1:200,000 Epinephrine alters the pH of the mixture to 6.0 [4]. The pain of the local anesthetic injection has its contribution from the acidic nature of the anesthetic solution. By alkalinizing local anesthetic solution, acidity can be dealt with by altering the pH of the solution which clinically produces less “sting” on injection [16, 17].

In a previously described method for plasma dexamethasone identification by the authors through their study on high performance liquid chromatography determination of dexamethasone in plasma to evaluate its systemic absorption following intraspace pterygomandibular injection of twin mix, it was concluded that intraspace pterygomandibular route of drug administration can achieve statistically similar plasma concentration of a drug as when the same dose is administered intramuscularly [8]. In the presented study, the achieved dexamethasone plasma concentrations via intraspace pterygomandibular route and intramuscular route and its clinical effect has been compared with randomly selected control group.

In the presented study, both swelling and trismus was found to be less in the groups that received dexamethasone either by intraspace route (IS) or by intramuscular route (IM) when compared to the control group (C) which did not receive dexamethasone. Intraspace route of dexamethasone administration was found to have achieved statistically comparable and indifferently ($P = 0.605$) plasma dexamethasone concentrations as seen in patients where it was administered intramuscularly. The clinical effect of the drug (dexamethasone) was also found to be similar in both intramuscular group (IM) patients

and in intraspace group (IS) patients. Intraspace group (IS) patients had the advantage of single-prick delivery of both local anesthesia and the steroid.

Apart from the above advantages offered by intraspace administration of dexamethasone, administration of steroid by pterygomandibular route for mandibular surgery like transalveolar extraction of wisdom tooth, will offer local availability and anti-inflammatory action of the steroid at the surgical site apart from the conventional action via systemic absorption of the drug. This will augment the control of inflammation at the surgical site by lipoxygenase pathway inhibition and prevention of leukotriene production [18–20].

Further study has to be conducted to establish its efficacy with large sample size and its use in bilateral third molar extractions. Intraspace route of drug administration can be utilized to deliver dexamethasonized local anesthetics safely with predictable clinical effects in the patients requiring mandibular minor oral surgery under local anesthesia.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval Ethical approval was obtained. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Proposed Mechanism of Action for Twin Mix Anaesthesia When Used as Intra-space Pterygomandibular Injection for Inferior Alveolar Nerve Block with Emphasis on Effects of Perineural Injection of Dexamethasone

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Abstract

There has been recent research on the use of dexamethasone as an adjunct to local anaesthetics to enhance the block characteristics and improve post-operative pain outcomes. Numerous studies have shown that perineural dexamethasone improves post-operative analgesia, along with other clinical benefits. Intra-space pterygomandibular twin mix anaesthesia is a novel technique for inferior alveolar nerve block used for mandibular anaesthesia. Twin mix anaesthesia has its advantages in shortening the latency and prolonging the duration of the soft tissue anaesthesia, along with improving the quality of life in the post-operative period after mandibular oral surgical procedures. The concern regarding the use of perineural dexamethasone has been discussed.

Keywords: Anaesthesia, dental anaesthesia, dexamethasone, inferior alveolar nerve block, lignocaine, local anaesthesia, mandible, twin mix

INTRODUCTION

Co-administration of steroid and local anaesthetics (LAs) has shown some clinical benefits in the recent research. Perineural dexamethasone injection improves post-operative pain outcomes when given as an adjunct to LA blocks with no clinical evidence of persistent neural damage or functional alteration after perineural administration of the drug.^[1] Authors had proposed the co-administration of 2% lignocaine with 1:200,000 epinephrine and 4 mg dexamethasone (twin mix) as intra-space pterygomandibular injection for inferior alveolar nerve blocks for surgical removal of impacted mandibular third molars.^[2] Twin mix intra-space pterygomandibular anaesthesia has its advantages in shortening the latency and prolonging the duration of the soft tissue anaesthesia, along with improving the quality of life in the post-operative period after surgical extraction of mandibular third molars. Patients who receive a mixture of 2% lignocaine with 1:200,000 epinephrine and

4 mg dexamethasone show lesser swelling and better mouth opening in the post-operative period when compared to patients who receive lignocaine with epinephrine blocks. This review discusses the possible mechanisms involved in the action of this mixture when used for inferior alveolar nerve block.

CURRENT CLINICAL EVIDENCE

Evidence-based practice backs the use of twin mix for inferior alveolar nerve blocks specifically in cases where

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surgical procedure in the mandible is expected to produce post-operative swelling and trismus.

A prospective randomised double-blind study to assess the latency and efficacy of twin mix and 2% lignocaine with 1:200,000 epinephrine in surgical removal of impacted mandibular third molars in 20 patients (40 interventions) ascertained that addition of dexamethasone to lignocaine and its administration as an intra-space injection significantly shortens the latency and prolongs the duration of the soft tissue anaesthesia, with improved quality of life in the post-operative period after surgical extraction of the mandibular third molars.^[2]

Through a clinical comparative study on effects of intra-space injection of twin mix versus intraoral-submucosal, intramuscular, intravenous and per-oral administration of dexamethasone on the post-operative sequelae after mandibular impacted third molar surgery, it was concluded that steroid groups had a better clinical outcome with improved quality of life post-operatively when compared to the non-steroid study group. Intra-space injection of dexamethasone in the pterygomandibular space as twin mix was found to have similar clinical effects as conventional methods of administering steroids via intraoral-submucosal, intramuscular, intravenous and per-oral routes.^[3]

In a clinical trial for comparative evaluation of efficacy of twin mix versus 2% lignocaine with 1:200000 epinephrine, it was concluded that there was better post-operative outcome with administration of dexamethasone and lignocaine as an intra-space injection in decreasing the post-operative patient discomfort. The anaesthetic efficacy of the twin mix admixture was found to be statistically similar to the control solution of 2% lignocaine with 1:200,000 epinephrine. The ultraviolet spectrometry study for chemical stability of the mixture suggested that there was no change in the active pharmacological compounds (lignocaine or dexamethasone).^[4,5]

A report by Knezevic *et al.* demonstrated that the addition of dexamethasone to LAs delayed the block onset. This report remains in contradiction to the author's findings in context to the use of twin mix, as the use of the mixture of 2% lignocaine with 1:200,000 epinephrine and 4 mg dexamethasone, as intra-space pterygomandibular injection has shown to reduce the latency period in the clinical trials.^[6] The possible reasons for the contradictory finding may be due to the use of different LA drugs in different anatomical space and the use of epinephrine by the authors in the twin mix solution.

PROPOSED MECHANISMS INVOLVED FOR THE CLINICAL EFFECTS OF TWIN MIX ANAESTHESIA AS PTERYGOMANDIBULAR NERVE BLOCK FOR SURGICAL REMOVAL OF IMPACTED MANDIBULAR THIRD MOLARS

The active pharmacological agents that produce the clinical effects of the mixture are 2% lignocaine, 1:200,000

adrenaline and 4 mg dexamethasone. Lignocaine is an amide LA, chemically 2-(diethylamino)-N-(2,6-dimethyl phenyl)-acetamide. The basic mechanism by which lignocaine produces LA is by blocking the voltage-gated sodium channels in the cell membrane of post-synaptic neurons, thereby preventing depolarisation of the neural tissue and inhibiting the generation or propagation of nerve impulses.^[7]

Adrenaline in the concentration of 1:200,000 used in the mixture has several beneficial effects that include decrease in the peak plasma concentration of the LA agent, thereby reducing chances of toxicity, increasing duration and quality of anaesthesia, reducing minimum concentration of anaesthetic needed for nerve block and decreasing blood loss during surgical procedures.^[8]

Dexamethasone, a corticosteroid, is a potent anti-inflammatory agent and an immunosuppressant. Its anti-inflammatory potency is 20–30 times when compared to cortisol. Dexamethasone exerts potent anti-inflammatory action by inducing the synthesis of endogenous proteins, which block the enzymatic activation of phospholipase A2. This in turn inhibits arachidonic acid release by the cell membrane, with inhibition of the synthesis of prostaglandins, leucotrienes or substances related to thromboxane.^[3,9] Dexamethasone is known to block superoxide production and lysosomal enzyme release in human polymorphonuclear neutrophils inhibiting the functional responses of degranulation. The probable action of dexamethasone on human polymorphonuclear leucocytes is by membrane-bound calcium release.^[10]

Usually, addition of vasoconstrictor renders the LA solution more acidic with its pH in the range of 3.5–4.5. This acidic pH of the solution clinically produces a 'sting'-like sensation when injected into the tissue. More basic LA solutions (without vasoconstrictors) do not produce the sting on injection and minimise the tissue injury, otherwise caused by acidic solutions of LA.^[11] Addition of dexamethasone to 2% lignocaine with 1:200,000 epinephrine renders the final pH of the mixture more basic (pH=6). LAs in solution exist in equilibrium between the basic uncharged (non-ionised) form, which is lipid soluble, and the charged (ionised) cationic form, which is water soluble. Lipid-soluble, non-ionised form of the LA penetrates the neural sheath and membrane (tissue penetration). The ionised form of the LA binds with the sodium channel and prevents propagating of impulses (clinical action). Altering the pH to a more basic solution, as in case of twin mix, will increase the amount of non-ionised form compared to ionised form which will speed onset. Increasing the pH of lidocaine decreases the pain associated with its infiltration.^[2,12] Dexamethasone solution when mixed with LA solution increases the pH of the LA solution from 4.5 to 6 of the mixture and clinically demonstrated faster onset of anaesthesia and longer duration with reduction of sting-like sensation on injection.^[2] Proposition, yet not proved, for steroid-induced shorter onset and prolonged duration, apart from change in pH, may also be due to the vasoconstriction

property of dexamethasone or by increase in the activity of the inhibitory potassium channels on nociceptive C-fibres (via glucocorticoid receptors), thus decreasing their activity.^[13,14] On investigation, dexamethasone individually is found to have analgesic effects by increasing the activity of inhibitory potassium channels on nociceptive C-fibres.^[15,16] Yet, another school of thought that holds importance is that glucocorticoids produce vasoconstriction, which might reduce LA absorption, hence prolonging LA nerve contact time.^[17,18]

SAFETY ISSUES WITH THE USE OF PERINEURAL DEXAMETHASONE INJECTIONS

In a systematic review by Knezevic *et al.* on perineural dexamethasone added to LA for brachial plexus block, it was concluded that perineural dexamethasone in addition to LA solutions significantly improved post-operative pain without increasing complications, and also, smaller doses of dexamethasone (4–5 mg) were as effective as higher doses (8–10 mg).^[6] These results are in concurrence of author's proposition of using 4 mg dexamethasone in the twin mix anaesthetic solution.

Concerns remain about the vasoconstrictor effects of dexamethasone, which may cause ischaemic changes in the nerve tissue if injected in nerve proximity. Wang *et al.* evaluated the effects of topical corticosteroids on the sciatic nerve in adult Wistar rats and suggested that caution is required when using large doses of corticosteroid in nerve proximity as topical dexamethasone adversely affected neural conduction in a dose-dependent manner.^[19] Shishido *et al.* studied the effects of topically applied 0.4% dexamethasone on acute changes in the nerve blood flow and subsequent histologic changes in rat sciatic nerve fibers and concluded that dexamethasone causes statistically significant reductions in normal nerve blood flow at 30 min and 4 h after topical application; however, the reduction is on average below the threshold for causing ischemic changes in the structure of peripheral nerve fibres.^[18] Williams *et al.* opine to exercise caution on use of dexamethasone perineurally in doses of 8 mg or greater as this dose does not show any additional clinical benefits over lower doses of dexamethasone.^[20]

Choi *et al.*, through their systematic review and meta-analysis of randomised trials on effects of dexamethasone as an LA adjuvant for nerve blocks in the British Journal of Anaesthesia, concluded that to date, dexamethasone appears to be the best method for prolonging analgesia as an adjuvant over clonidine, epinephrine or midazolam. They also highlighted that the clinician should evaluate the risk–benefit ratio with ‘off-label’ use of perineural dexamethasone.^[21] Noss *et al.*, in a systematic review on the use of dexamethasone with LA, have commented that ‘while dexamethasone is not approved for perineural use, this application is well described in textbooks and peer-reviewed literature. There has been reported neither an incidence of neurotoxicity nor an increased incidence of complications or

side effects associated with perineural use of dexamethasone in humans in the literature.’^[22]

CONCLUSION

Twin mix, a mixture of 2% lignocaine with 1:200,000 epinephrine and 4 mg dexamethasone, as intra-space pterygomandibular injection for inferior alveolar nerve block when used for mandibular surgical procedures, holds promising clinical benefits based on the current clinical evidence. Currently available systematic reviews and meta-analysis of randomised trials have demonstrated the use of perineural dexamethasone, in the doses used, as clinically safe.

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Conflicts of interest

There are no conflicts of interest.

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Case Report

Orthomorphic and Orthognathic treatment for Aesthetics and Function in Facial Asymmetry Post TMJ Ankylosis - A Case Report

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ABSTRACT:

Introduction: Orthomorphic surgery is performed when alone orthognathic is unable to correct the condition. It refers to correction offacial asymmetry or jaw deviation without changing the occlusion. It is the best treatment option available so far for such deformities.

Case Report: A 23-year-old male patient presented to us with the complaint of flattening of the right side of face. Patient gave a history of childhood trauma which lead to temporomandibular joint ankylosis and was operated for the same at the age of 10 years. He reported for unaesthetic appearance and was diagnosed as facial asymmetry with occlusal cant. Treatment was planned in two stages by giving priority to correctfunction first and then proceeding for the aesthetics 6 months later. **Conclusion:** An optimal function and aesthetic was achieved post-operatively. The described technique has the quality to alter the dentofacial deformity in any magnitude and directionin accurate dimension.

Key words: Orthognathic surgery, orthomorphic surgery, facial asymmetry, two stage procedure.

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INTRODUCTION:

All normal human faces have some degree of asymmetry. The ancient Greeks were probably the first to notice these variations between the two sides of the face, as discovered much later by analysis of their statuary, which included mild to moderate facial asymmetries. Normal asymmetries such as these often go unnoticed by the general public. Esthetically pleasing and apparently symmetrical faces do indeed exhibit skeletal asymmetries and one side of the face can be rather different from the other and still be considered completely normal. The level at which asymmetry becomes unacceptable to a patient is variable and depends on many factors, most of which are psychological.[1] Facial

asymmetry, when obvious, has enormous sociopsychological impact on the affected individuals. It can occur as a consequence of developmental anomalies or disease or after trauma or surgery. Surgical reconstruction is usually indicated in most instances involving a noticeable facial asymmetry. This is usually accomplished by reconstructing the deformed portion with its normal counterpart working as a reference.[2] Patients who present with significant facial asymmetry are not only concerned with restoring functional occlusion but also with improving esthetics and beauty. This has often been a source of social scorn for many of these patients. Beauty and symmetry have often been thought of synonymously; hence, the belief that

unattractiveness is the result of asymmetry.[3] Unilateral temporomandibular joint ankylosis occurring during the active growth period if left without treatment, or when improperly treated, is often complicated by the development of secondary changes in the structure, shape, and size of the mandible together with the surrounding tissues.[4] Mandibular asymmetry may be caused by infection and trauma during the growing period. Primary trauma may lead to asymmetry and in some cases ankylosis. Asymmetry may also follow a surgical procedure or a malunited fracture.[5] Orthognathic surgery for the correction of facial deformity arising from discrepancy in spatial relationship or dimensional differences is well established. However, when the cause of the deformity includes an alteration of the shape of the jaws, orthognathic surgery is unable to correct the resulting contour deformity.[6] For this reason in the management of facial asymmetry, orthomorphic principles of management are an adjunct to orthognathic surgery or Osseo distraction.[7] The surgical correction consisting of an osteotomy aimed at restoring the morphology is denoted the term “orthomorphic” to distinguish it from conventional orthognathic surgery. The orthomorphic correction aims to correct deformities related to shape and contour of the jaws without affecting the status of occlusion.[6]

CASE REPORT:

A 23-year-old male patient presented to us with the complaint of flattening of the right side of face. Patient gave a history of childhood trauma which lead to temporomandibular joint ankylosis and was operated for the same at the age of 10 years. He reported for unaesthetic appearance and was diagnosed as facial asymmetry with occlusal cant. Treatment was planned in two stages by giving priority to correct function first and then proceeding for the aesthetics 6 months later. On occlusal examination an occlusal cant was present.(Figure 1) Clinically a clear-cut asymmetry was seen in the lower border of

mandible.(Figure 2) Lateral Cephalogram and Posteroanterior Cephalogram Confirmed the diagnosis of facial asymmetry by deviation of jaw on the left side due to underdevelopment of growth from childhood trauma leading to TMJ ankylosis. (Figure 3)Grumman’s analysis and Three-dimensional computed tomography elicited mandibular deviation on left side. (Figure 4). After confirmation of diagnosis first occlusal correction was planned by performing a differential Le Fort I osteotomy as the vertical height was more on the left side compared to right side. So, the occlusal cant was corrected. After 6 months of correcting occlusion, facial asymmetry correction was planned. An extended lateralsliding genioplasty was done for correction of mandibular underdevelopment. We achieved patients and our expectation by combining orthognathic correction primarily and orthomorphic correction secondarily after 6 months.

Figure 1: Clinical Picture & Occlusion Cant Pre and Post-operative.

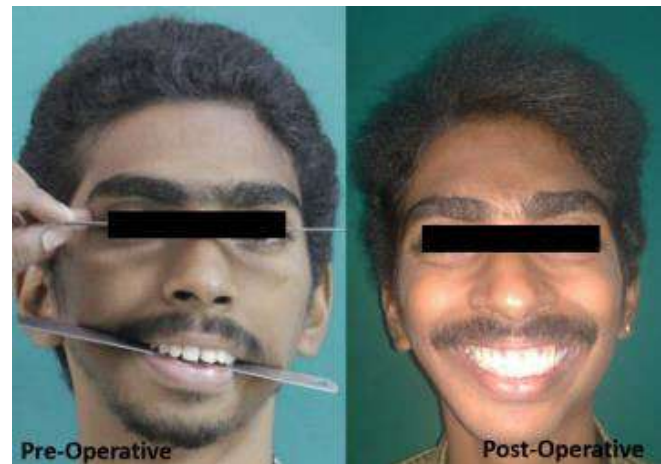


Figure 2: Deviation & Occlusion Pre and Post-operative.



Figure 3: Lateral and PA Cephalograms Pre and Post-operative

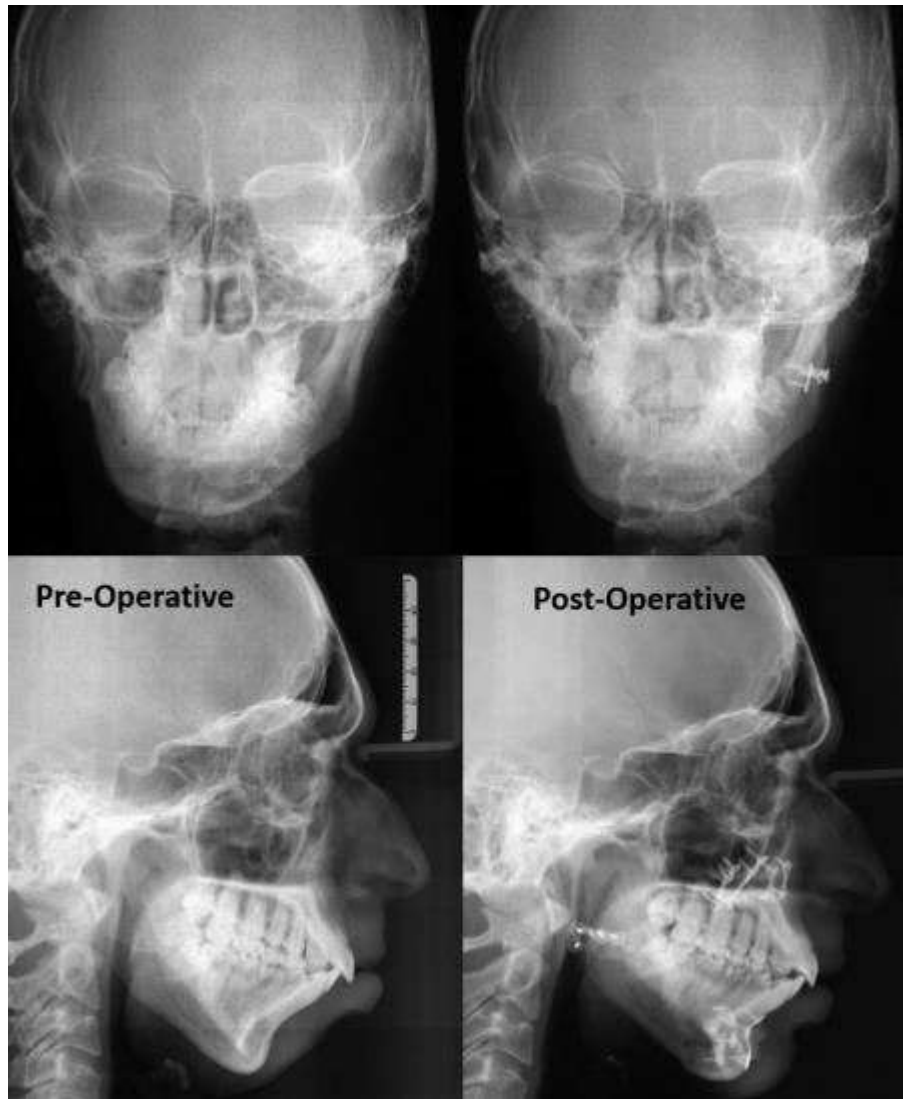


Figure 4: Grumman's Analysis & 3DCT Pre-operative.




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DISCUSSION:

Surgical correction of facial asymmetry is more challenging than the correction of symmetrical deformities. Often, unilateral mandibular deficiencies are associated with slight downward growth of the maxilla on the affected side, altering the position of midline structures of the middle-third of the face and creating an occlusal cant. We resorted to a line drawn perpendicular to the inter-inner canthal line as the facial midline. [8] Inferior border osteotomy of the mandible performed intraorally can be employed independently or in conjunction with other procedures to correct facial asymmetry of the mandible. Genioplasty is a well-documented procedure to move the chin to a more desirable position. The anterior chin can be moved in all 3 dimensions of space. Extended lateral sliding genioplasty is the technique followed by the first author since 1992 for the correction of facial asymmetry, especially for residual deformities of unilateral TMJ ankylosis. In TMJ ankylosis, because of the destruction of the growth center and the missing functional stimulus, the mandible on the affected side is short due to deficient growth. The opposite side of the mandible is dragged towards the affected side. This leads to facial asymmetry, with false fullness of the affected side and deficiency of the unaffected side, and resultant deviation of the mandible. The conventional technique of aesthetic correction of the above-described deformity is by ramus osteotomies and bone grafting. This technique often jeopardizes the existing occlusion. In most patients, because of the pre-existing trismus, the teeth are unhealthy, hence orthodontic treatment may not be feasible. Benefits of the 'long' osteotomy cut extending to the gonial region bilaterally have been put forward by Tessier. [9] They include maintenance of a smooth natural inferior mandibular border, noninterference with the occlusion, and simultaneous increase in the width of the jaw line. Extended lateral sliding genioplasty is an intraoral procedure where the inferior mandibular border is cut from one gonial region to the opposite gonial region below the inferior alveolar canal. Wolfe estimated the position of the inferior alveolar canal to be 6 mm below the mental foramen. [10] We made the osteotomy cut on the deficient side approximately 5 mm below the mandibular canal. However, the cut was slanted downwards by approximately 45° towards the lingual side so that the nerve canal was spared. Multiple holes were made on the lingual cortex and the bone was split using osteotomes and/ or a split spreader. None of the patients experienced transection of the nerve inside the canal. The divided bone segment was slid forward and laterally, so that the chin and the sunken lateral aspect of the mandible were augmented. The deficient side of the mandible was lengthened and the deviation of the chin was corrected. The same technique can be employed in conjunction with other osteotomies such as sagittal ramus split, maxillary osteotomies, etc., for correction of complex asymmetric deformities. Stepping on the lateral mandibular contour is often created after lateral sliding of the extended

genioplasty segment and, in such cases, autogenous bone grafting can be used to interpose or obliterate the step deformity. When aesthetic correction is performed simultaneously with functional correction of ankylosis, the resected bone may be used as the graft material thus eliminating the need for donor site surgery. Management of the mental nerve is an important issue. Hinds and Kent favor dissection and protection of the mental nerve during osteotomy. [11] Posnick et al also advocated dissection and retraction during osteotomy and reported persistent sensory deficit in 10% of their patients after 1 year. [12] Spear and Kassar [13] and Lindquist and Obeid [14] advised identification and protection of the mental nerve. These authors reported permanent mental nerve deficit in 6% [13] and 10% [13] of patients, respectively, and are against the idea of dissecting the nerve. Converse and Wood-Smith were of the opinion that the mental nerve may be divided if it obstructs the osteotomy. [15] To obtain adequate space to perform inferior border osteotomy of the mandible, extensive dissection or traction is required on the mental nerve. Moreover, inadvertent avulsion of the nerve is a possibility and is a more serious problem than intentional division of the nerve because it does not lend itself to correction by microsurgical repair. The advantages of extended lateral sliding genioplasty may be summarized as follows: a simple procedure accomplished through an intraoral degloving incision in the buccal vestibule, less time-consuming than the conventional ramus osteotomies for the correction of unilateral mandibular deficiencies, existing occlusion is not disturbed, deficient side of the mandible is lengthened, the chin is brought close to the midline, the apparent deficiency on the unaffected side is corrected by the lateral shift of the inferior border segment and thus fullness is achieved, the procedure can be done concomitantly with the correction of TMJ ankylosis, the procedure can be combined with other orthognathic procedures such as ramus sagittal split and maxillary osteotomy, harmony and balance of the face (vertical and horizontal proportions) are improved, psychological rehabilitation of the patient is rapid.

CONCLUSION:

The surgical correction of facial asymmetry is extremely challenging because the asymmetry may be centered at the hard and/or soft tissue; any of a combination of dimensions; and it may involve the maxilla, mandible, and symphysis or any combination of the three. It is the effective treatment of the hard tissues that brings about the most dramatic change, as soft tissue defects are usually corrected after skeletal correction.[16] Combination of orthognathic and orthomorphic surgery is useful in correcting cases of mandibular deformity which increases quality of life of patients.



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Review Article

Endodontic Surgery – A Review

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ABSTRACT:

Endodontic surgery has now evolved into endodontic microsurgery. Nowadays each tooth may be managed with endodontic surgery and concept of extraction has been abolished. Carious lesion with periapical pathology is managed with the endodontic therapy. The access opening, bio- mechanical preparation followed by obturation and restoration are steps of endodontic therapy or root canal treatment. There is no need of raising the surgical flap. Whenever, access is achieved via surgical flap, the procedure is known as surgical endodontics. The present review article focuses on various indication and category of endodontic surgery.

Key words- Bio- mechanical, Carious, Endodontic surgery

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INTRODUCTION

Surgical endodontics is the branch of dentistry that is concerned with the diagnosis and treatment of lesions of endodontic origin that do not respond to conventional endodontic therapy or that cannot be treated by conventional endodontic therapy. Carious lesion with periapical pathology is managed with the endodontic therapy. The access opening, bio- mechanical preparation followed by obturation and restoration are steps of endodontic therapy or root canal treatment. There is no need of raising the surgical flap. Whenever, access is achieved via surgical flap, the procedure is known as surgical endodontics.¹

Success rates for contemporary endodontic therapy are in excess of 90%, depending on the skill of the clinician and the teeth involved. Surgical endodontic procedures are usually undertaken when conventional (orthograde) endodontics has failed. However, the chances of successful

re-treatment of a tooth with a failed root filling are higher when non-surgical endodontics is repeated rather than by undertaking a surgical approach. Surgical endodontics may therefore not be the first option when conventional root canal treatment fails.²

Options for the management of these failures can be non-surgical root canal retreatment or surgical endodontics. Non-surgical retreatment may provide a better opportunity to clean the pulp space than a surgical approach. However there are clinical situations when non-surgical root canal retreatment is inappropriate.³

A wide range of success rates for surgical endodontics has been reported (44–95%). Systematic reviews comparing the outcome of non-surgical root canal retreatment and surgical endodontics reveal that, to date, there have been only two randomised controlled trials. The data from this limited evidence suggests that although surgery may offer a more favourable outcome in

the short term, non-surgical retreatment offers a more favourable long-term outcome.⁴

Indications for surgical endodontics

Following are the indications of surgical endodontics-

1. Peri-radicular disease associated with a tooth where iatrogenic or developmental anomalies prevent non-surgical root canal treatment being undertaken.
2. Peri-radicular disease in a root-filled tooth where non-surgical root canal retreatment cannot be undertaken or has failed, or when it may be detrimental to the retention of the tooth.
3. Where a biopsy of peri-radicular tissue is required.
4. Where visualisation of the peri-radicular tissues and tooth root is required when perforation or root fracture is suspected.
5. Where it may not be expedient to undertake prolonged nonsurgical root canal retreatment because of patient considerations.⁵

Contraindications

Most contraindications are relative, and they are usually limited to three areas: the patient's medical status, anatomic considerations, and the dentist's skills and experience. Contraindications for endodontic surgery include the following-

1. The tooth has no function (no antagonist, no strategic importance serving as a pillar for a fixed prosthesis).
2. The tooth cannot be restored.
3. The tooth has inadequate periodontal support, or the tooth has a vertical root fracture.⁶ Additional general contraindications may be an uncooperative patient or a patient with a compromised medical history for an oral surgical intervention.

Advances in medicine have dramatically increased life expectancy and the survival rate from most of today's diseases. Dentists are, with increasing frequency, being asked to treat medically compromised patients. When considering performing any surgical procedure on a patient who reports a major systems disorder (cardiovascular, respiratory, digestive, hepatic, renal, immune, or skeletomuscular), a thorough medical history is mandatory. Following the identification of all potential medical complications and a review of the patient's current drug regimen, a consultation with the primary care physician or specialist may be in order.⁷

Categories of endodontic surgery

Following are the endodontic surgeries

- 1- Periapical surgery.
- 2- Hemisection/root amputation.
- 3- Intentional replantation.
- 4- Corrective surgery.

Periapical surgery

The success of apical surgery varies considerably, depending on the reason for and nature of the procedure. With failed root canal treatment, often retreatment is not possible or a better result cannot be achieved by a coronal approach.⁸

Indication for periapical surgery

1. When an unusual entity in the periapical region requires surgical removal and biopsy for identification
2. When the cause of root canal failure cannot be identified so surgical exploration may be necessary.⁹
3. Anatomic problems preventing complete debridement /obturation.
4. Restorative consideration that compromise treatment.
5. Horizontal root fractures with apical necrosis.
6. Irretrievable material preventing canal treatment or retreatment.
7. Procedural error during treatment.
8. Large periapical lesion that do not resolve with root canal treatment.¹⁰

Hemisection/root amputation

Root end resection is often, but not always, indicated. It is useful in two situations:

1. To gain access to the canal for examination and placement of a root end preparation and restoration.
2. To remove an undebrided or unobtured (or both) portion of a root.

This may be necessary in cases with dilacerated roots, ledged or blocked canals, or apical canal space that is inaccessible because of restorations, as well as in accessing of lingual structures.¹¹ Amputation may be vertical root amputation and horizontal root amputation. Remaining root prone to fracture due to occlusal forces of restoration not in line with long axis of the root, consider minor tooth movement to align root so occlusal forces are along long axis, do not restore as a cantilever.¹²

Intentional replantation

Sometimes, tooth which get exfoliated accidentally such as in case of trauma, endodontic surgery is performed and the tooth is intentionally replanted in the socket.¹³

Corrective surgery

Corrective surgery is managing defects that have occurred by a biologic response (i.e., resorption) or iatrogenic (i.e., procedural) error. These may be anywhere on the root, from cervical margin to apex. Many are accessible; others are difficult to reach or are in virtually inaccessible areas. A corrective procedure is necessary. Generally, the procedure involves exposing, preparing, then sealing the defect.¹⁴

CONCLUSION

Surgery is a very important aspect of endodontics. Endodontic surgery has now evolved into endodontic

microsurgery. With a high percentage of successful treatment outcomes of conventional endodontics together with high success of surgical endodontics almost all teeth with endodontic lesions can be successfully treated.

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Apertognathia- A review

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Abstract

An anterior open bite is considered to be one of the most dentofacial deformities to treat in orthodontics. The complexity of this malocclusion is attributed to a combination of skeletal, dentoalveolar, functional and habit related factors. There is common agreement amongst orthodontists that patients with anterior open bites are difficult to treat and relapse is common after treatment with orthodontics alone or with orthognathic surgery.

Keywords: Open bite, Bimax, Protrusion.

Introduction

Caravelli (1842) was the first to introduce the term "open bite" as a separate class of dentofacial deformity.¹ Kim (1974) introduced the overbite depth indicator (ODI) evaluated the nature and skeletal pattern of open bite with the help of ODI analysis and concluded that the incidence of open bite is greatest when the ODI value is below the mean 74.5.² The clinical definition of open bite, diagnostic criteria, appraisal of etiologic factors and treatment of open bite was given by Subtelny and Sakuda (1964).³ An anterior open bite has been defined by various authors;

According to Graber It is defined as a condition where a space exists between the occlusal or incisal surfaces of maxillary and mandibular teeth in the buccal or anterior segments when the mandible is brought into habitual or centric occlusion.⁴ According to Moyers, It is defined as the absence of incisal overlap, and/or the absence of an occlusal stop or contact. True open-bite can occur with Class I, Class II division 1, and Class III malocclusions.⁵ Subtelny and Sakuda states that an open bite exists when there is an open vertical dimension between the incisal edges of the maxillary and mandibular anterior teeth although loss of vertical dental contact can occur between the anterior or the buccal segment.⁶ According to Chase Defined open bite as a condition characterized by space discrepancies between the occlusal and incisal surfaces of the maxillary and mandibular teeth when the mandible is brought into habitual or centric occlusion.⁷

In the Glossary of Orthodontic Terms, an open bite is a developmental or acquired malocclusion whereby no vertical overlap exists between maxillary and mandibular anterior or posterior teeth.⁸

Incidence: The incidence of anterior open bite varies among races and with dental age. In African Americans incidence is reported to be 6.6%, than in Caucasians it is reported 2.9% and Hispanics 2.1% incidence was

reported. Chronologically, as children develop dentally, anterior open bite incidence decreases, because it tends to self-correct through the mixed dentition phase.⁹

Classification: Moyers classified open bite as:

1. Simple or Dentoalveolar and
2. Complex or Skeletal.

Simple Open Bite: When the basal skeleton is normal and the open bite is confined to the teeth and an alveolar process, the condition is called a simple open bite.

Simple open bite can be subdivided into anterior and posterior according to the location.

Anterior simple open bite (Fig. 1): It usually results from digital sucking or abnormal tongue behavior. It is more common in children than adolescents, because by adolescence many simple tongue thrusts are lost. By adolescence, too, some vertical effects on the facial skeleton must have occurred and what was an earlier simple anterior open bite becomes more complex.

Posterior simple open bite (Fig. 2): It is rarer than simple anterior open bite and frequently is the result of a lateral spreading of the tongue at rest. The abnormal tongue posture usually begins when it is necessary to secure a posterior seal during the swallow because ankylosed primary molars are present or their early loss creates a vertical open space. The persistence of the spreading tongue posture impedes eruption and full vertical development of succedaneous permanent teeth. This condition is sometimes confused with "idiopathic failure to erupt." Maxillary apical base insufficiency also may necessitate an abnormal posterior spreading of the tongue.



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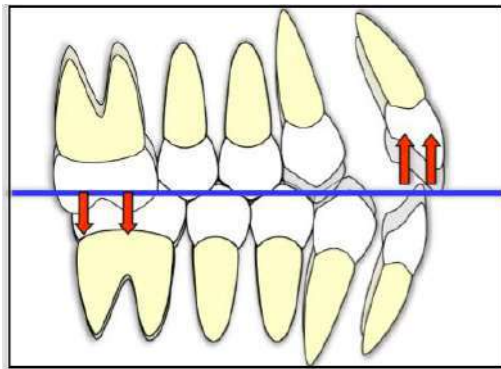


Fig. 1: Anterior simple open bite (Dentoalveolar)

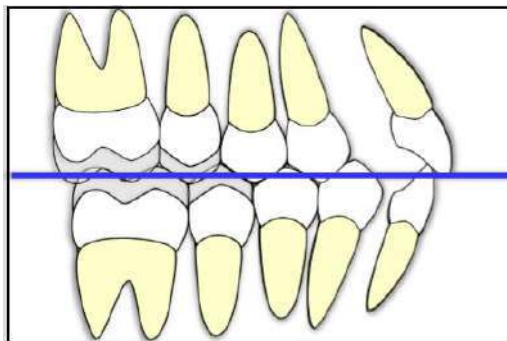


Fig. 2: Posterior simple open bite

Complex or Skeletal Open Bite: Complex or Skeletal open bite is the result of a vertical dysplasia so severe

that compensatory alveolar growth cannot cope. The abnormal lip and tongue functioning observed with a complex open bite is usually adaptive in coping with the skeletal dysplasia, though the condition is held by many to be associated with “mouthbreathing” and chronic nasorespiratory dysfunction.⁶

Subtelny and Sakuda (1964)

Classified open bite based on etiologic origin.

Generally, three etiologic factors have been considered to be associated with open-bite:

1. Vertical growth deficiencies in both the anterior and posterior region of the maxilla;
2. Disproportionate muscle growth or aberrant muscle function caused by enlarged, excessively fronted, or protrusive function of the tongue which is thought to prevent the full eruption of anterior dental units or to exert a disfiguring influence on the molding of the anterior dentoalveolar processes;
3. Thumb and finger sucking habits.¹¹

Sassouni and Nanda (1964) classified skeletal open bite into:

- Class I,
- Class II and
- Class III. (Fig. 3)¹¹

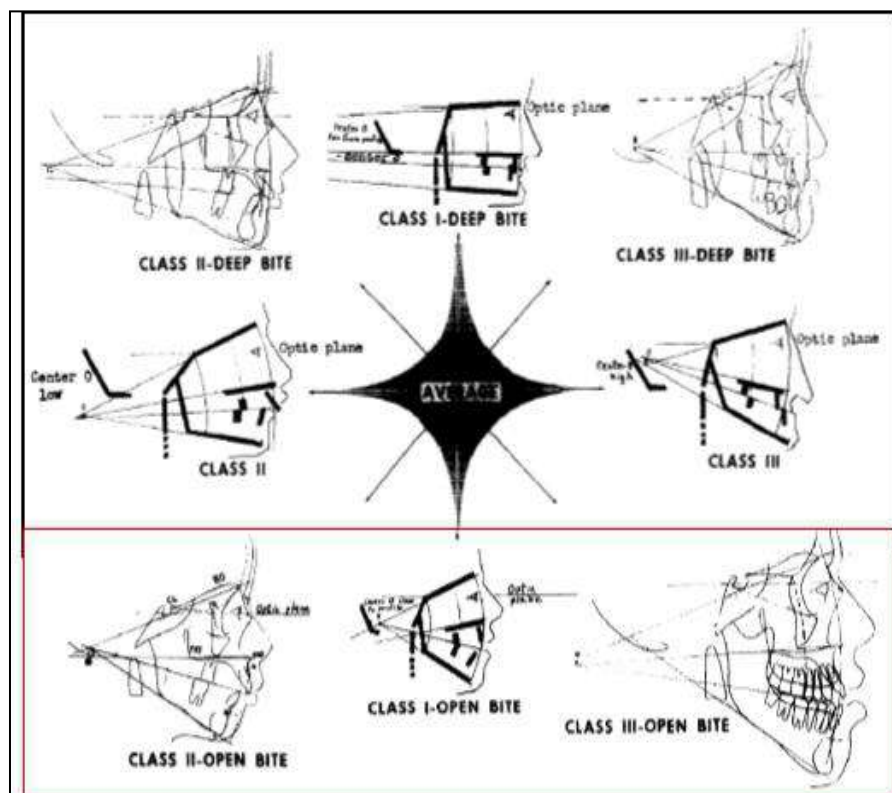


Fig. 3: Classification of skeletal malocclusion (Sassouni V, Nanda S. Analysis of dentofacial vertical proportions. Am J Orthod 1964;50(11):817.

Y. H. Kim (1974) classified open bite as mild, moderate and severe.¹⁰

Kamiyama and Takiguchi (1958) and Horowitz and Hixon (1966) in a similar manner, categorized open bite into the dentoalveolar type and the skeletal type.

Etiology

Table 1:

	Environmental Factors
1	Habits: Finger sucking Tongue thrusting Mouth breathing due to upper airway obstruction
2	Retained infantile swallow
3	Altered tongue posture
4	Macroglossia
5	Skeletofacial or dentoalveolar trauma
6	Degenerative disorders of the condyle like idiopathic condylar resorption and juvenile rheumatoid arthritis
7	Neuromuscular deficiencies causing masticatory muscle atrophy
8	Craniofacial anomalies like cleft lip cleft palate, pierre robin syndrome

Genetic Factors: Inherited growth potential most commonly inherited increased anterior facial height

Diagnosis

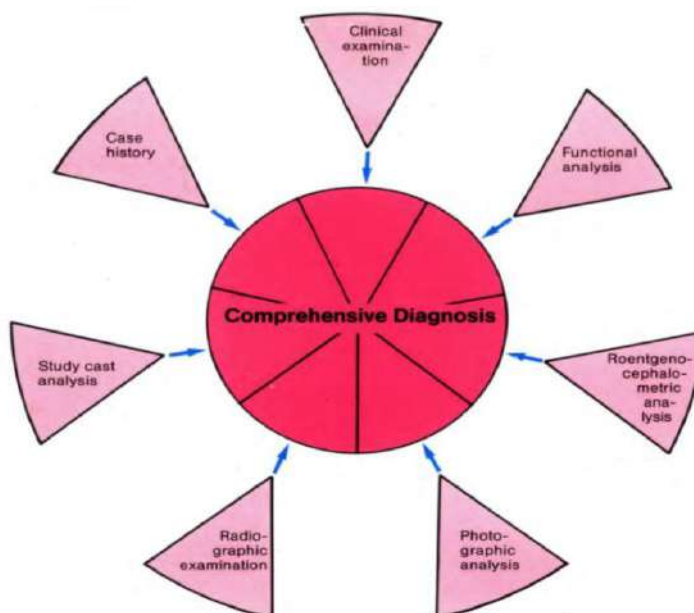


Fig. 4: Etiological factors of open bite

Clinical Findings:

According to Moyers,

In Anterior Open Bite: In patients with thumb or finger sucking habit, the maxillary arch is often narrow and there could be excessive height to the alveolar process, producing undesirable gingival display. Mandibular postural retraction may develop if the weight of the hand or arm continually forces the mandible to assume a retruded position in order to practice the habit.

Concomitantly, the mandibular incisors may be tipped lingually. When the maxillary incisors are tipped labially and an open bite has developed, it becomes obligatory for the tongue to thrust forward during swallowing in order to affect an anterior seal, therefore a simple tongue-thrust is essentially related to digital sucking habit.

This disturbance within the force system in and around the maxillary complex, it often is impracticable for the nasal floor to drop vertically to its expected position during growth. Therefore, thumb-suckers are found to own a narrower nasal floor and a high palatal

vault. The maxillary lip tends to be hypotonic and the mandibular lip becomes hyperactive, since it should be elevated by contractions of the orbicularis muscle to a position between the malposed incisors during swallowing. During sucking and swallowing these abnormal muscle contractions stabilize the deformation.

In Posterior Open Bite: Open bites in the posterior region are rare in young children and usually result from a lack of vertical alveolar development, either ankylosed primary molars or "idiopathic failure to erupt." Lateral "tongue-thrusts" are largely tongue postures adaptive to an open bite resulting from another cause. Ankylosed primary molars result in a localized cessation of alveolar development, creating a posterior open bite. The tongue must spread laterally to seal the open bite space during reflex swallowing. When the primary teeth are removed, the tongue continues its lateral swallowing movements that may impede the eruption of the bicuspid.⁵

According to Bjork: Most of the skeletal and dental characteristics commonly seen in open bite patients were initially described by Bjork. His paper discussed the morphologic characteristics associated with downward and backward mandibular rotation during growth. These skeletal and dental characteristics include: distal condylar inclination, short ramus, antegonial notching, obtuse gonial angle, excessive maxillary height, straight mandibular canal, thin and long symphysis, long anterior facial height, short posterior facial height, steep mandibular plane, divergent occlusal planes, acute intermolar and interincisal angulation, anteriorly tipped-up palatal plane, and extruded molars. Of all these characteristics, the steepness of the mandibular plane has been considered the key skeletal finding associated with a skeletal anterior open bite.¹²

Cephalometric Findings and Radiographic Examination: Nanda stated that the difference between skeletal and dental open bite is evident as excessive vertical growth of the dentoalveolar complex, especially in the posterior molar region in skeletal open bite.

And reduced incisor dentoalveolar vertical height is seen in dental openbite.⁹

Moyers stated that vertical analysis is a necessary part of cephalometric diagnosis. The features seen in open bite patients were: palatal plane tipped upward; mandibular plane steeper than normal; anterior face height excessive relative to posterior face height; the skeletal dysplasia may be confined to anterior lower face height; gonial angle may be obtuse and the ramus angled posteriorly; mandibular alveolar process height may be excessive anteriorly and the occlusal and mandibular lines divergent. The use of Vertical Analysis and the vertical growth measures provides

localization of the problem in the craniofacial skeleton and makes possible monitoring of treatment during growth.⁵

Treatment Planning: After thorough evaluation of the etiology, clinical findings and radiographic assessment of the malocclusion, a definitive treatment plan is arrived at by following the following steps:

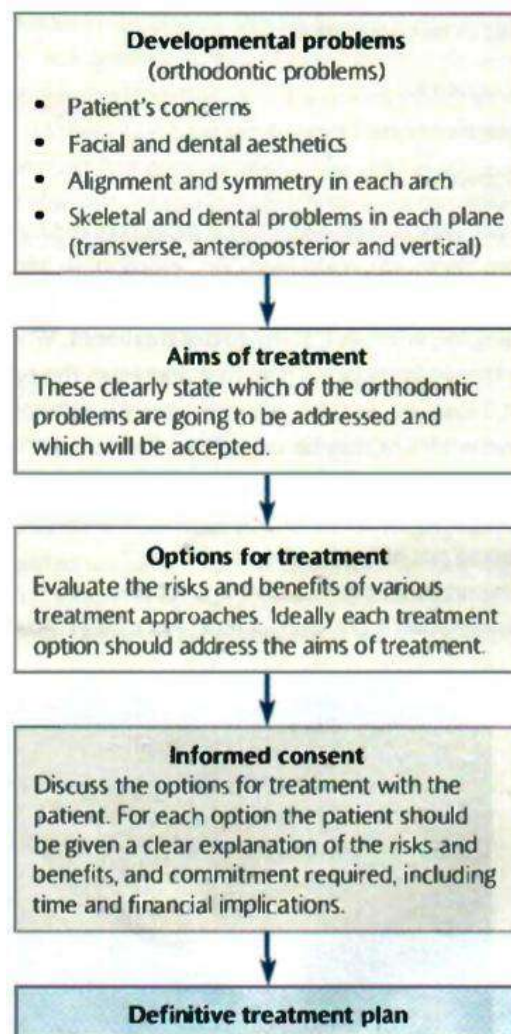



Fig. 5: Treatment planning in apertognathia

According to the Index of Treatment Needs (IOTN):
 Grade 4 (severe/need treatment) – extreme lateral or anterior open bites greater than 4mm.
 Grade 3 (Moderate/Borderline need) – lateral or anterior open bite greater than 2mm but less than or equal to 4mm
 Grade 2 (Mild/Little need) – anterior or posterior open bite greater than 1mm but less than or equal to 2mm.
 Grade 1 (No treatment needed) – no open bite⁷


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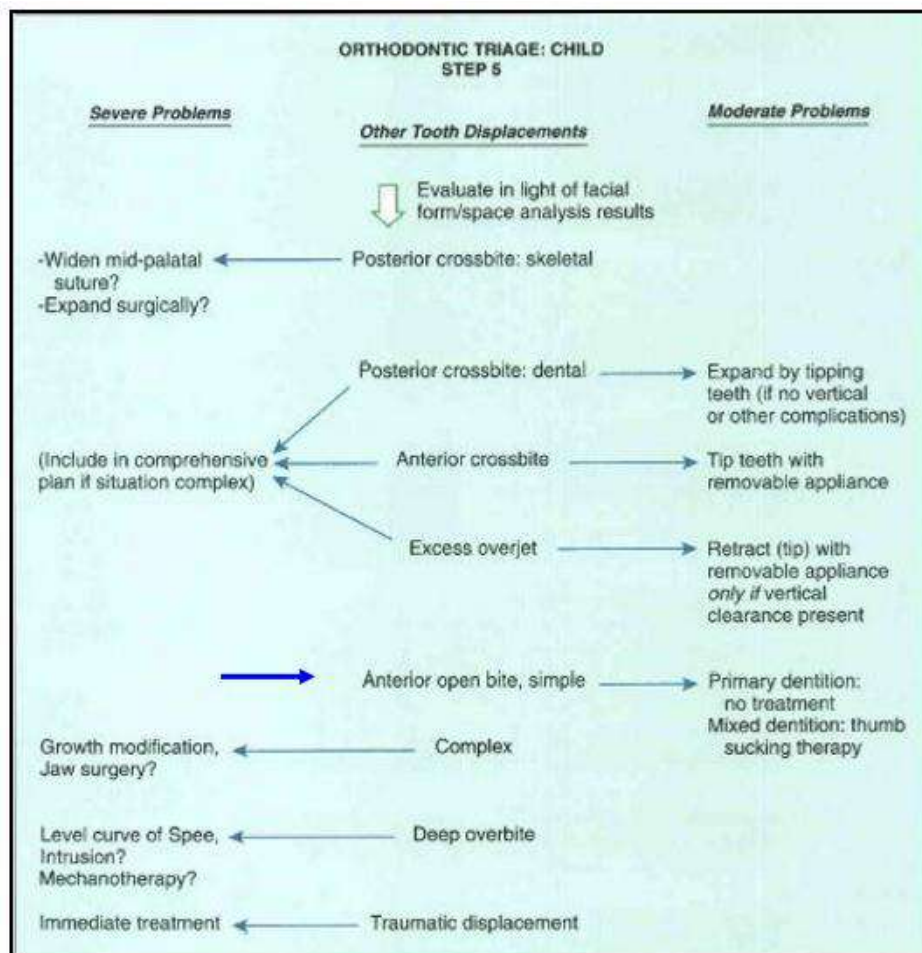


Fig. 6: Orthodontic triage

According to Moyers, it is necessary to note the relationship of the osseous bases to the dentition while planning the treatment. Always suspect a habit of some sort when an open bite is first seen in a child, since the majority of simple anterior open bites are caused by digital sucking habits or abnormal tongue posture. If, during eruption, the teeth repeatedly encounter a finger, thumb, or tongue, eruption is impeded and an open bite results.

The complex or skeletal open bite is a symptom of a variety of serious skeletal dysplasias including varied morphologies such as Class II, vertical type 1 (steep mandibular plane, or excessive anterior total face height), mandibular prognathism, and several of the craniofacial syndromes (e.g. Downs' syndrome). The most frequently encountered skeletal open bite is that seen in vertical type 3 (upward tipped palatal line and inadequate development of anterior upper face height). Skeletal contributions to open bite are often overlooked in young children and then they are more difficult to treat later. A few guidelines and principles for early treatment of complex or skeletal open bite are:

1. The earliest possible diagnosis is essential because the condition is not self-correcting and usually worsens with time;

2. Removal of all possible etiologic factors as soon as diagnosed is important. Consultation with the pediatrician or otolaryngologist may be important. When referring the patient to the physician, the cephalogram and a summary of cephalometric findings should be sent along;
3. Since these cases may be very difficult and require prolonged and varied appliance therapy, they challenge the experience and skills of the best orthodontists.

Early mistakes in treatment can compound the problem.⁵ According to Proffit, anterior open bite in a young child with good facial proportions usually needs no treatment, because there is a good chance of spontaneous correction, especially if the open bite is related to an oral habit like finger sucking. A complex open bite (one with skeletal involvement or posterior manifestations), or any open bite in an older patient, is a severe problem. Excessive growth of the maxilla in children with Class II malocclusion often shows more of a vertical than an anteroposterior component (i.e., there is excessive growth downward than forward). Both components can contribute to skeletal Class II malocclusion, because if the maxilla moves downward, the mandible rotates downward and backward. The effect is to prevent mandibular growth from being

expressed anteriorly. The goal of treatment is to restrict growth of the maxilla while the mandible grows into a more prominent and normal relationship with it. The application of extraoral force is the obvious approach but functional appliance treatment also can be helpful. Children with the long face growth pattern typically have a maxilla that is rotated down posteriorly and/or a short mandibular ramus, which accounts for the steep mandibular plane and the huge discrepancy between posterior and anterior face height. The ideal treatment for these patients would be to control all subsequent posterior vertical growth so that the mandible would rotate in an upward and forward direction. This could be accomplished by controlling all tooth eruption if there was adequate mandibular vertical ramus growth. Unfortunately, vertical facial growth continues through adolescence and into the post-adolescent years, which means that even if growth can be modified successfully in the mixed dentition, active retention is likely to be necessary for a number of years. Although dramatic improvement can be demonstrated in selected patients, probably the most sensible use of any of the appliances to control vertical skeletal and dental development is to use them for the minor to moderate problems and intervene in adolescence toward the end of the growth period. That way, the problem is more manageable and treatment and retention are more circumscribed. Type of the appliance and duration of the treatment when started, retention would be critically necessary till growth is completed in the late teens or early 20's.¹⁴ According to Graber (1959), in patients with thumb- and finger- sucking habit, a thorough oral examination is made on the first visit, but the case history is taken with the child out of the room. An attempt is made to get information on the etiology, intensity, frequency, duration, chronology, mental attributes, family environment, school and play environment, siblings, response under stress, associated abnormalities, parental attitude, previous habit- breaking attempts, psychic superstructure, etc. Children with behavior problems, in whom finger-sucking is only one of many symptoms, are referred for psychiatric guidance. Children from broken homes or environments of great tension or conflict are considered poor risks for interceptive appliances. They are carried on a "visit-and-talk" routine, in the hope of establishing a rapport and, thus, therapy through suggestion. Some of these children had become appliance cases later, but only after a better understanding of the child's actions and motivations had been gained.

Adolescents with Questionable Growth Potential:

When evaluating orthodontic patients with primarily vertical problems, it is easy to dwell on antero-posterior problems because most long face patients will have a receding chin and Class II malocclusion. Frequently the chief complaint is that the upper incisors are very prominent. An astute patient may be perceptive enough to describe the gummy smile as a problem that should

be corrected but most know only that they do not like the prominence of their upper incisors, without differentiating vertical and horizontal components. A camouflage treatment set up supported retraction of the upper incisors is also recommended if the orthodontist views the problem primarily as Class II malocclusion, without recognizing the involvement of the skeletal discrepancy. Correcting the overjet by retraction of incisors for long-face adolescents is extremely unattractive. Extraction of premolars to retract the upper incisors can cause them to elongate even further and increase the nasolabial angle. Because this correction is often accomplished through the use of Class II elastics, the mandible is likely to further rotate down and back, accentuating an already long- face pattern. Before the option of vertical maxillary impaction became available, the negative facial aesthetics that resulted were considered an inevitable consequence of improving the dental occlusion. The continuation of growth well into the late teens tends to worsen the deformity; however, the fact that there is remaining growth provides an opportunity for implementation of growth modification techniques. Growth modification after the adolescent growth spurt is theoretically possible but actually improbable because very few adolescents will wear a functional appliance with bite blocks or a headgear. Anterior open bite in adolescents (or adults) typically will be corrected with orthodontic treatment by intruding the posterior teeth; however that's virtually not possible without surgery. A multiloop edgewise appliance, in conjunction with anterior vertical elastics, claims to produce posterior intrusion and improvement of the skeletal problem. Recent reports show that the open bite correction happens virtually altogether by elongating the incisor teeth. Successful camouflage of a long-face problem is a function of both the patient's perception of the success of treatment and the soft-tissue adequacy (the fuller the lips, the better they can cover the teeth). If the chief complaint is excessive display of the teeth and a gummy smile, elongating the incisors will not correct it. However, if the patient's major concern is the open bite, an increased display of the anterior teeth may be tolerable. The orthodontist must be sure the patient understands the aesthetic implication of the latter decision. Patients who are treated with other extrusive, non-extraction orthodontic approaches may end up with their occlusion reasonably well corrected, but both facial aesthetics and long-term stability are questionable. In this particular situation, the lower incisors will remain too protrusive relative to the chin for good stability, the chin will still be deficient, and the lip incompetence will still be present. If the nonextraction treatment is considered unsuccessful and the patient is retreated with extractions, esthetics will be compromised even more. For borderline cases such as these, a lower border osteotomy of the mandible to bring the chin upward and forward can greatly improve

both dental and facial aesthetics because the lower lip relaxes and moves up as the chin is elevated. The lower border osteotomy is not a complicated procedure and may be accomplished in an ambulatory care setting.

Adults with Little or no Growth Potential: Long-face patients with no prospect for successful growth modification have no real alternative to surgery for a successful and stable outcome. Orthodontic camouflage is not a real option in long-face problems. It may be better not to treat a patient with a true vertical problem who refuses to consider surgical correction because aesthetics is likely to be severely compromised.¹³

Surgical Management: An accurate diagnosis and the variety of surgical techniques available should reduce complications and the degree of relapse following treatment. However, there are still some cases which are so severe that some compromise in treatment may be unavoidable, in the past, the majority of patients were treated by mandibular procedures. The most favorable surgical results are currently achieved through maxillary procedures, particularly the LeFort I osteotomy with adjunctive procedures. Various bi jaw procedures are explained in literature which are shown in the table below:



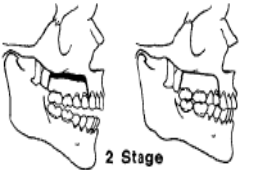
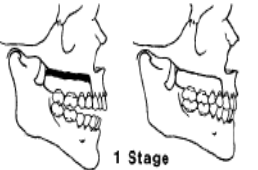


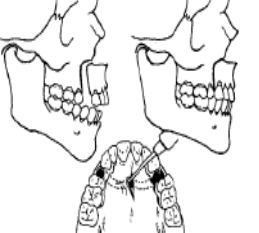
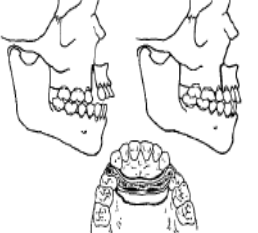





Mandibular Procedures		Maxillary Procedures	
<p>V OSTEOTOMY (HULLIHEN)</p> 	<p>KÖLE MODIFICATION</p> 	<p>SCHUCHARDT TECHNIQUE</p>  <p>2 Stage</p>	<p>KUFNER TECHNIQUE</p>  <p>1 Stage</p>
<p>Y OSTEOTOMY (THOMA)</p> 	<p>TRAPEZOID OSTEOTOMY (THOMA)</p> 	<p>WASSMUND TECHNIQUE</p> 	<p>WUNDERER TECHNIQUE</p> 
<p>C OSTEOTOMY</p> 	<p>SAGITTAL SPLIT OSTEOTOMY</p> 	<p>Le FORT 1 OSTEOTOMY</p> 	
<p>VERTICAL OSTEOTOMY</p> 	<p>PICHLER & TRAUNER OSTEOTOMY</p> 		

Fig. 7: Maxillary and mandibular procedures for treating apertognathia

Summary: An anterior open bite is one of the most difficult dentofacial deformities to treat in orthodontics. Etiology being multifactorial, a thorough clinical and cephalometric examination should be done while treating a patient with open bite. The difficulty of this malocclusion is accredited to a combination of skeletal, dentoalveolar, functional and habit related factors. It is characterized by a large anterior dentoalveolar height in both the jaws, increased total and lower anterior face height, a disproportionate ratio of upper to lower anterior face height, decreased posterior face height, an increased gonial angle, a high mandibular plane angle, a low posterior to anterior face height ratio and a short ramus. Till recently, the orthodontic treatment modalities included habit correction, extrusion of anterior teeth using intermaxillary elastics, uprighting

of the molars and inhibition of molar eruption during growth. These methods were unsatisfactory due to the skeletal and esthetic compromises that ensued. However recently with the advent of clearaligners, patients with high esthetic demands and having a open bite malocclusion can also be treated in an effective manner. Another advancement is the use of skeletal anchorage, with absolute intrusion of the teeth using temporary anchorage devices, it is possible to autorotate the mandible in a closing counter clockwise direction and correcting the open bite without surgery. Miniimplants are used not only as a treatment option but also as an aid in retention. With numerous treatment modalities to correct open bite, the problem still exists with retention and stability of this malocclusion. However many retention protocols have been

documented to be effective, stable results are obtained only if a comprehensive assessment is done with regards to the age of the patient and the etiology of open bite. The treatment of open bite depends on the etiology, age and severity of the problem. Hence, treatment options may include habit correction, extraoral appliances with or without bite blocks in growing patients. Treatment options in non growing patients include intrusion of posteriors using temporary anchorage devices. In severe cases, orthognathic surgery (maxillary or mandibular or both) is indicated. Early and timely treatment of open bite could avoid use of fixed appliances and surgery and but however this requires patient compliance and co operation in a growing patient. However, when the deformity is severe in a non growing patient, orthognathic surgery is the treatment of choice. This may include maxillary expansion, impaction with or without mandibular advancement or setback along with advancement and reduction genioplasties as required. Different approaches have been advocated for retention after treatment of open bite. Day time wear of removable retainers and night time wear of either high pull headgear, or functional appliance with bite blocks (an open bite bionator). Others suggested retainers with occlusal coverage to control molar eruption. Prolonged retention is required in most cases. To summarize, openbite continues to be a challenging malocclusion to be treated by an orthodontist but comprehensive approach towards planning treatment and retention protocol can give successful results in most cases.

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Acanthomatous ameloblastoma – A case report

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Abstract

Ameloblastoma is a common pathological disease of maxillofacial region. It is frequently seen in Asian continents, but huge variants are seen in African countries. Hereby we are presenting a case report of acanthomatous ameloblastoma operated for excision, curettage and chemical cauterization under general anesthesia.

Keywords: Ameloblastoma, Oral Pathology.

Introduction

Ameloblastoma is a true epithelial odontogenic tumor usually unicentric, nonfunctional, intermittent in growth, anatomically benign and clinically persistent.¹ It usually consists of proliferating odontogenic epithelium usually with a follicular or plexiform pattern lying in a fibrous stroma.² Cussack in 1827 was the first to recognize lesion of odontogenic origin.³ Malassez in 1855 first defined as adamantinoma.⁴ Ameloblastoma has predominancy to affect young people.⁵ Clinical presentation and characteristics regarding size, age, and sex distributions differs in the published literature.⁶⁻⁸ Sex predilection also differs as some author suggests it is higher in men and some suggest both these sexes are equally affected.⁹⁻¹¹ There are different radiologic and histologic patterns of ameloblastoma. Radiologically it can be unilocular or multilocular or multilobular.¹² Histologically it is of four types' unicystic, solid multicystic, extrasosseous peripheral and desmoplastic.² Treatment varies according to histological variant.

Case Report

A 36 year old male reported with a swelling on right side of lower jaw since 2 months and pain while chewing food since 3 weeks. Swelling was insidious in size and gradually increased to the present size. There was no history of trauma or tooth ache or decrease in size of swelling or any discharge from the swelling however patient was experiencing pain while chewing hot food. On clinical examination there was a solitary ill-defined diffused mild swelling over the right mandible on the lower 3rd of the face. (Fig. 1) measuring about 6 x3 cm extending Medio laterally 2 cm lateral to the midline on the right side till the angle of the mandible and superiorinferiorly from the corner of the mouth to lower border of the mandible. The surface was smooth and the skin overlying the swelling was not much stretched or distended and was of normal colour and texture of skin with no secondary changes to be found. It was tender and hard to the palpating fingers. An intraoral examination

revealed an ill-defined, solitary swelling in the right lower posterior buccal vestibule extending anteroposteriorly from canine to the third molar region and mediolaterally 1.5 cm from the buccal surface of molars towards the interdental gingiva of the involved teeth (Fig. 2). The mucosa overlying was stretched but was similar to the adjacent mucosal colour. It was tender and hard in consistency with buccal cortical plate expansion. Considering the clinical findings, we came to a tentative diagnosis of benign tumor of right side lower jaw. Ameloblastoma was the primary differential diagnosis. Incisional biopsy was performed subjected to histopathological examination. Orthopantomogram elicited a well-defined radiolucency with scalloped border involving the periapical region from the right lower central incisor to the first molar. Root resorption was present with the premolars and first molars. Lower border of the mandible was intact with 3mm solid bone against the radiolucency. (Fig. 3) Histopathological examination showed multiple strands and islands of odontogenic epithelium with peripheral hyper chromatic palisaded cuboidal cells and central stellate cells. Focal areas of squamous metaplasia and associated stroma has dense collagen fibers with multiple island of odontogenic epithelium suggestive of ameloblastoma of acanthomatous type. Treatment implicated for the management of lesion included excision, curettage and chemical cauterization with Carnoy's Solution leaving the lower border intact. Routine hematological investigations, chest radiograph, Electrocardiogram and pre anesthetic checkup was performed before taking the patient under general anesthesia. Extraction of the involved tooth was done, a trapezoidal flap with bilateral relieving limb was given, and lesion was exposed. Complete excision of the lesion was done with thorough curettage and smoothing of borders with round bur with copious betadine irrigation. (Fig. 4) Carnoy's solution was used for chemical cauterization. Lingual perforation was present on the cortex of 2nd premolar region. A 12 hole reconstruction plate fixed 1cm above

the lower border of mandible leaving 5mm from the apices of roots as a safe side to prevent fracture of mandible from occlusal forces. (Fig. 5) Support for fixation of reconstruction plate was taken bilaterally from buccal cortex. Medicated gauze pack was kept in the defect and water tight closure was done. Medicated gauze pack was removed after 48 hours. Patient was kept under I.V drugs and discharged after 3 days. (Fig. 6)



Fig. 1: Extra Oral Photograph of the Patient



Fig. 2: Intra Oral Photograph of the Patient



Fig. 3: Orthopantomogram



Fig. 4: Excised Lesion



Fig. 5: Reconstruction plate Fixation



Fig. 6: Post-Operative Medicated Gauze

Discussion

The recurrence rate of unicystic and solid multicystic ameloblastomas ranges widely and depends on histological subtype¹³ usually small unicystic lesions are managed by enucleation as they are less likely to recur. But, in background the lesions other than unicystic or giant unicystic lesions had higher chance of recurrence. So the preferable option comes out as jaw resection. This approach has become more common due to wider options of microvascular grafts which are reliable to repair the defect. It is still a dilemmatic question for the operating surgeons about the merits and demerits of ameloblastomas to hand for aggressive approach. no doubt the published literature elicits that in the decades of 1940's to 1970's as per the advances in maxillofacial surgical field, enucleation was the preferable option compared to resection and reconstruction in ameloblastoma cases.¹⁴⁻²¹ Huge blow was given by Stoelting to the literature when he proved the reduction in recurrence of keratocyst from 60 percent to less than 10 percent by using carnoy's solution which


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was very much beneficial to treat ameloblastoma's by chemical cauterization.²²⁻²³ Some authors have reported that the multilocular appearance is the most common radiological finding.²⁴ But, some suggest follicular pattern is the dominant histological type.²⁵⁻²⁶

Conclusion

Epidemiological variations are a pointer to variations in the experience of different groups, which may be better explained in terms of differences, awareness of condition and availability of facilities and resource personal for early diagnosis and treatment. Every case of ameloblastoma must be planned properly to increase the quality of life of patients.

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Review Article

A Review on one rare complication “Avascular Necrosis of Maxilla” after Le Fort I Orthognathic Surgery

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ABSTRACT:

The sequelae of insufficient vascularity following maxillary orthognathic surgery can vary from loss of tooth vitality, to periodontal defects, to tooth loss to loss of major maxillary dentoalveolar segments. The literature is replete with reports of the successful surgical treatment of maxillary and mandibular skeletal deformities. It is unusual, however, to see a report of the case that failed. Understanding the blood supply of the maxilla and how possible patient related, anesthetic and operative factors affect it, is important in understanding how the vascularity of the maxilla can become compromised in a surgical procedure. Avascular necrosis of the maxilla is a rare complication of orthognathic surgery with few cases reported in the literature. There are identifiable risk factors that can influence the blood supply of the maxilla. Careful preoperative assessment is required to exclude patient factors that have the potential to affect tissue vascularity. This in conjunction with sound anesthetic and surgical technique should all minimize the risk of avascular necrosis. Even so it is still possible for this rare complication to occur.\

Key words: Avascular Necrosis, Orthognathic Surgery, osteonecrosis.

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BACKGROUND:

Orthognathic maxillary surgery is a safe, predictable and stable procedure. The incidence of perioperative complications is low and the number of life-threatening complications with this surgery is even lower. However, avascular necrosis of the maxilla after Le Fort I osteotomy is a rare complication that has been reported to occur in <1% of cases.¹ During down fracture of the maxilla, the blood supply is by the ascending pharyngeal artery, the ascending palatine branch of the facial artery and the rich mucosal alveolar network overlying the maxilla.² Table 1 outlines the factors that may result in impaired blood supply to the maxillary segments and increase the risk of ischemia.

When the descending palatine artery is sacrificed, the main blood supply of the maxilla will now be the soft tissue pedicle, which incorporates the anterior faucial pillar and the palatal mucosa (Fig. 1). Maxillofacial orthopedic surgery is a safe, predictable, and stable procedure.^{3,4} The number of life-threatening complications associated with this surgery appears to be very small.^{3,5,6} Other minor intraoperative and perioperative complications have been reported, but their incidence is considered low.³⁻⁶ Among these complications, avascular necrosis of the maxilla after Le Fort I osteotomy has been reported by a few studies.^{5,7,8} Usually, these complications are related to the degree of vascular compromise⁹ and occur in fewer than 1% of

cases.¹⁰ Rupture of the descending palatine artery (DPA) during surgery, postoperative vascular thrombosis, perforation of palatal mucosa when splitting the maxilla into segments, or partial stripping of palatal soft tissues to increase maxillary expansion may impair blood supply to the maxillary segments.⁸ Sequelae of compromised vasculature include loss of tooth vitality, development of periodontal defects, tooth loss, or loss of major segments of alveolar bone or the entire maxilla.^{5,8,11} The risk and the extent of complications seem to be enhanced in patients with anatomical irregularities, such as craniofacial dysplasia's, orofacial clefts, or vascular anomalies.⁵ Accordingly, the risk of ischemic complications is enhanced in patients who present anatomical irregularities that require extensive dislocations or transversal segmentation of the maxilla.⁵ The treatment of avascular necrosis of the maxilla is not easily attained.¹² Although no treatment protocol has been established, aseptic necrosis of the maxilla should be treated by maintenance of optimal hygiene,¹³ antibiotic therapy to prevent secondary infection,^{8,13} heparinization,^{8,14} and hyperbaric oxygenation.^{8,13,14} A recent report¹⁵ described treatment of avascular necrosis of the maxilla related to a previously performed orthognathic surgery by hyperbaric oxygenation, bone grafting, and oral rehabilitation by an implant-supported fixed prosthesis, with a successful outcome. The aim of this report is to present a clinical case of avascular necrosis of the maxilla during the first postoperative days after a bimaxillary orthognathic surgery performed in a middle-aged woman, emphasizing treatment of this condition and correlating it with the current literature.

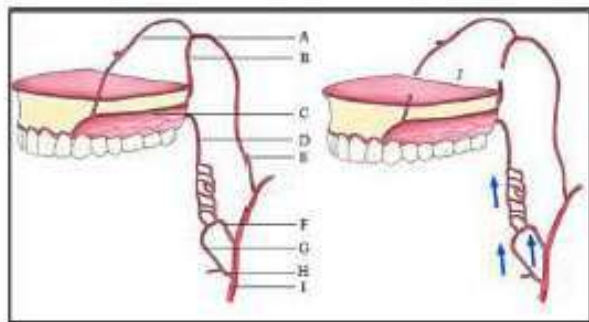


Figure 1: Blood supply of the maxilla. (A) Nasopalatine artery, (B) Descending palatine artery, (C) Greater palatine artery, (D) Lesser palatine artery, (E) Maxillary artery, (F) Ascending pharyngeal artery, (G) Ascending palatine artery, (H) Facial artery, (I) External carotid artery, (J) Le Fort I downfracture.

RARE COMPLICATION:

Blood flow to the maxilla is reduced by 50% in the first postoperative day after sacrifice of the descending palatine arteries.¹⁶ However, there is excellent collateral blood supply particularly if only one artery is sacrificed, as in this case. Experimental studies have shown that loss of the descending palatine arteries results in a transient ischemic period that is compensated for by a vascular proliferation that allows tissue healing. The collateral microvasculature

from other vessels including the ascending pharyngeal and facial arteries (Fig. 1), maintains the viability of the palatine pedicle. The pedicle can withstand stretching greater than 10mm of anterior repositioning of the maxilla. The patient had sickle cell trait. The haemoglobinopathy screen confirmed Hb A (Adult Hemoglobin) and Hb S (Sickle Hemoglobin). The Hb F (Fetal Hemoglobin) level was <0.5% and the Hb S level was 39.4%. These parameters are typical for sickle cell trait. However, for patients in Sickle Cell crisis or those expected to be exposed to severe hypoxia, Hb S level <30% is advised.¹⁷ Craniofacial dysplasia's may represent areas of anatomical variation where the blood supply may be susceptible to disruption. Although the patient had a biopsy showing normal bone, the maxillary tuberosity was expanded and hyperplastic clinically. This in itself would not however have accounted for the degree of avascularity seen in this case. Literature suggests that segmental osteotomy is at a higher risk of ischemic complications.^{18,19} Necrosis of the maxilla can be minimized in the following ways: – Divide into as few segments as possible and avoid small segments anteriorly. – Maintain the integrity of the palatal mucosa. – Perform sagittal segmentation in paramedian sites as the mucosa is thicker and the bone thinner than the midline. Although hypotensive anesthesia was not purposely utilized for the procedure, it is a technique commonly used to help maintain a bloodless surgical field.²⁰ A mean arterial blood pressure (MAP) 30% below a patient's usual MAP, with a minimum MAP of 50mmHg in American society of anesthesiologists Class I patients and a MAP not <80mmHg in the elderly, is suggested to be clinically acceptable.²¹ It is recommended that with respect to hypotensive anesthesia: – It should be adjusted in relation to the patient's preoperative blood pressure rather than to a specific target pressure. – It should be limited to that level necessary to reduce bleeding in the surgical field. – It should be confined to that part of the surgical procedure deemed to benefit by it. – There is little need for blood transfusion perioperatively.²² The anesthetic concerns include the management of perioperative hypoxia, acidosis, hypovolemia and hypothermia. Points to consider with this case are: (1) Hypoxia never occurred and is usually avoided as anaesthetized patients are always maintained with an FiO₂ >0.35. (2) Acidosis may trigger a vaso-occlusive crisis and would affect all micro-circulation; it is easily prevented by proper perfusion and maintaining circulating blood volume. (3) Hypothermia was prevented by patient warming. (4) Hypovolemia could be contentious, especially with hypotension. With this patient, the mechanism of avascular necrosis could not clearly be explained. In the analysis of patient, surgical and anesthetic factors, there seemed to be issues that may have implied a greater risk.

DISCUSSION:

Aseptic necrosis of the maxilla is one of the possible consequences of ischemic problems occurring during Le

Fort I osteotomies, along with gingival recession.^{5,8,10} Lanigan et al⁸ reported 36 cases of aseptic necrosis of the maxilla after Le Fort I osteotomies, usually related to multiple segmentation of the maxilla in conjunction with superior positioning and transverse expansion or palatal perforations. However, other studies^{23,24,25} suggested that segmentation of the vascular pedicle by extensive anterior dislocation of the maxilla and transection of the descending vessels exhibit no relevant effect on revascularization or bone healing. Kramer et al⁵ showed that the rate of intra- and perioperative complications of Le Fort I are infrequent and mostly associated with anatomical complications, including deviation of the nasal septum and nonunion of the osteotomy gap. Another possible cause of maxillary would be related to ischemic complications, reported to occur in approximately 1% of cases, including aseptic necrosis of the alveolar process and retraction of the gingival margin. Ischemic complications have occurred in a small number of patients without recognized damage to palatal vessels during surgery. All patients showing ischemic complications had anatomical irregularities, as also suggested by Drommer,²⁶ transversal segmentation, or extensive anterior dislocation of the maxilla of 9 mm or more. Also, Bays et al²⁷ reported a 0.7% incidence of aseptic necrosis of the maxilla after Le Fort I osteotomy with routine bilateral ligation of the DPA in 149 patients. However, Acebal-Bianco et al⁶ and Panula et al⁴ found no loss of maxillary bone segments resulting from vascularization problems in large-sample studies. The main cause of avascular necrosis of the maxilla is DPA ligation during surgery.^{8,10,24,28,29} The main advantages of ligating DPAs during surgery are decreased risk of postoperative bleeding, easier maxillary mobilization, and shortening of surgical time.^{9,30} Hemorrhage has been considered as a major complication in the first 48 postoperative hours, requiring blood transfusion in 1% to 1.1% of the cases.^{3,5} However, blood transfusion should be avoided because of associated morbidity.^{3,31} Excessive blood loss occurring during surgery or several hours later is mainly related to maxillary osteotomies. Controlled hypotensive general anesthesia and post positioning the patient in a slight anti-Trendelenburg position, along with use of a local anesthetic containing a vasoconstrictor, may help to reduce blood loss.³ However, when using hypotensive anesthesia, laceration of the vessels may be masked, and even intraoperative inspection of vessel integrity does not guarantee that no bleeding will develop afterward.³⁰ Preserving DPA vessels during surgery is justified by the hypothetical benefit of maintaining blood flow and decreasing the risk of ischemic complications.³⁰ The known risk is postoperative bleeding caused by unrecognized laceration of the DPA.^{11,13,30} A reasonable approach, therefore, is to routinely preserve the integrity of these vessels when feasible, and to ligate them when enhanced accessibility or visualization is required, such as superior or posterior repositioning of the posterior maxilla, allowing

visualization and access to the tuberosity-pterygoid plate junction, and facilitating repositioning of the maxilla.¹⁰ In clinical practice, bone contiguous to vessels is routinely removed by careful and meticulous use of a sharp osteotome and Kerrison forceps.¹¹ Vertical movements, especially setbacks, could perhaps lead to injuries of the DPA with subsequent thrombosis, even if no rupture is observed. DPAs can be damaged intraoperatively during initial osteotomy cuts, maxillary down fracture, when achieving transverse modifications or during the intrusion or advancement procedure.⁸ Advantages of identifying and protecting the DPA include protecting maxillary blood supply, particularly in multiple segmented osteotomies, removing mechanical bony obstruction especially in impaction and setback movements, and preserving sensorial functions of the palatine nerves.³¹ The pyramidal osseous release technique around DPAs with a rotary drill described by Johnson and Arnett³² prevents leaving bony contacts posterior to the artery, which could lead to immediate post fixation anterior open bite, as the condyle sets back in the fossa. Preservation of the DPA is also imperative when maxillary segmentation is necessary,³² and does not significantly lengthen surgical time.⁹ A modification of this technique was proposed by O'Regan and Bharadwaj²⁸ by the use of a spatula, which provides better visualization and lower risk of inadvertent injury of the vessel. In the present clinical case, although the patient had developed unexpected intense bleeding during surgery, no ligation or electrocauterization of the DPA was performed, as hemostasis was promptly achieved by compression with gauze, and minor anterior displacement of the maxilla was anticipated by facial analysis software. The rationale for preserving DPAs during Le Fort I osteotomy is to optimize maxillary integrity by maintaining blood nutrition to the anatomical area, decreasing the risk of ischemic necrosis.⁸ Another possible reason for aseptic necrosis of the maxilla is palatal perforation, which compromises the already tenuous blood supply to the anterior maxilla, and leads to avascular necrosis.⁸ When the maxilla is to be expanded more than 3 to 5 mm, there is a risk of avulsing portions of the attached palatal pedicle during forceful manipulations,¹³ which was not observed in the present clinical case. In addition, avascular necrosis of the maxilla can be related to impaired blood supply to marginal gingiva. After Le Fort I down fracture, blood supply of the maxillary and palatal regions is established primarily through major and secondary palatine branches of DPA, soft tissue branches of posterior superior alveolar artery, palatal branch of the ascending palatine artery and palatal branch of the facial artery.⁶ An 84% to 95% reduction in blood flow to the osteotomized segment in a group of animal subjects that had the vessels bilaterally severed suggests that this pedicle should be maintained during surgery.²⁹ A significant decrease in anterior maxilla gingival blood flow (GBF) occurs during the intraoperative course of Le Fort I osteotomy,³³ which could explain

buccal and lingual gingival recession observed in this case. According to Dodson et al,⁹ ligation of the DPA was not associated with a change in anterior maxillary GBF during Le Fort I osteotomy. The trans osseous and soft-tissue collateral blood circulation and the freely anastomosed plexus of the gingiva, vestibule, palate, nose, maxillary sinus, and periodontium provided the necessary blood supply after sectioning of the descending palatine vessels.^{28,29} Although bone segmentation, stretching of the vascular pedicle, flap design, and bilateral sectioning of the DPA have been implicated in impairing the hemodynamics of the maxillary pedicle,⁶ Bell et al¹¹ showed that vascular alterations are only transient and are compatible with clinical success without resultant ischemic complications. Stretching the vascular pedicle by a 7- to 10-mm anterior displacement of the maxillary segment did not interfere with eventual osseous healing and revascularization.³⁴ It is not known to what degree, and for how long, blood flow can be impaired so as not to disturb continuous blood supply to the tissues, as well as the range of individual variability. Aseptic necrosis, with loss of tooth vitality, may occur much more commonly than is clinically apparent after maxillary surgery because obvious clinical signs and symptoms frequently do not accompany this situation.⁸ Tooth vitality tests commonly used in dentistry, involving either electrical or thermal stimulation, are unreliable after orthognathic surgery because sensory fibers from the trigeminal nerve are severed at surgery. Gingival bleeding is reduced in patients who smoke, even in the presence of moderate to severe periodontal disease, accompanied by reduction of other clinical signs of inflammation.³⁵ These findings can be related to the diminished existence of large blood vessels in the buccal gingiva of patients who smoke compared with those who do not smoke, along with proliferation of small blood vessels, without significant alterations in vascular density.³⁶ Patients who smoke can show increased periodontal bone loss even in the absence of dental plaque,³⁷ suggesting that smoking is an important risk factor for periodontal disease.^{38,39} Cessation of smoking habits results in stabilization of the periodontal condition after long-term follow-up,^{40,41} although the periodontal conditions of former smokers have been found to be worse than those of nonsmokers.⁴² Recent studies⁴³ have suggested that risk factors for aseptic osteonecrosis can be induced by intravascular thrombosis, for which risk factors are smoking and excessive consumption of alcoholic beverages, with no relation to blood dyscrasias. These findings were also reported by Wolfe and Taylor-Butler,⁴⁴ who described avascular necrosis of the shoulders in a patient reporting long-term use of corticosteroids, smoking, and alcohol intake. Although the use of bisphosphonates has been extensively implicated in osteonecrosis of the jaws,⁴⁵ its incidence appears to be greater in obese patients and patients who smoke.⁴⁶ Smoking is significantly associated with osteonecrosis of the jaws and has been linked to effects in all organs of the human body.

Specifically, in the oral cavity, carcinogens present in cigarettes, cigars, and pipe tobacco delay wound healing and are associated with a worsening of periodontal conditions of smokers.⁴⁷⁻⁴⁹ Nicotine may cause vasoconstriction in bone, leading to ischemic states that underlie the pathological mechanisms of osteonecrosis.^{50,51,52} Both periodontal disease and oral osteonecrosis seem to result from pathogenic mechanisms influenced by the interaction between environmental genotoxic risk factors and genetics, conferring individual susceptibility. According to Baldi et al,⁵³ osteonecrosis can occur in patients exposed to high doses of DNA-damaging agents, such as chemotherapy and radiotherapy for cancer treatment, and bisphosphonates for the treatment of osteoporosis. Oxidative damage caused by smoking plays a pathogenic role in periodontal disease, as established by the detection of mitochondrial DNA damage in the gingival tissue of patients with periodontitis. Endogenous risk factors in dental diseases include polymorphisms for many metabolic enzymes, metalloproteases, cytokines, prothrombin, and DNA repair activities. Considering that, both osteonecrosis of the jaws and periodontal disease could be related to risk factors associated with environmental mutagenesis. Given that, in the present case, the patient was not obese and did not report use of bisphosphonates, corticosteroids, or excessive alcohol consumption, the only risk factor associated with both periodontal disease and avascular necrosis of maxilla was smoking. Correction of avascular necrosis of the maxilla is not easy to attain.¹² The risk and the extent of complications seem to be enhanced in patients with anatomical irregularities, such as craniofacial dysplasia, orofacial clefts, or vascular anomalies. In addition, the risk of ischemic complications is enhanced in patients requiring extensive dislocations or transversal segmentation of the maxilla,^{5,29} which was not performed or diagnosed in the present case. Treatment of aseptic necrosis initially involves the establishment of optimal hygiene measures of the area, accomplished by frequent irrigation with saline solution.⁶ Ideally, the patient should be treated with hyperbaric oxygen, and antibiotics should be considered to prevent secondary infection.^{8,13} Surgical debridement is required to remove necrotic bone fragments, allowing earlier wound healing.⁸ Hyperbaric oxygen may hasten the delineation of the necrotic segments and allow a definitive debridement to be done at an earlier time.^{8,13} However, hyperbaric oxygenation does not reverse the development of aseptic necrosis once it has started, although it may limit the extent of such necrosis.⁸ No protocol for hyperbaric oxygenation has been proposed in literature. Recently, Singh et al¹⁵ reported the treatment of a patient who had experienced avascular necrosis of the maxilla after an orthognathic surgery performed 8 years previously. The patient complained of maxillary pain, pronounced facial asymmetry, malocclusion, and difficulty in eating and was diagnosed with sinusitis, mobile maxillary teeth, and transverse alveolar collapse of maxilla,

mandibular anterior posterior excess, and facial asymmetry. Treatment was performed by 30 sessions of hyperbaric oxygenation, followed by extraction of condemned teeth, debridement of necrotic bone and maxillary right sinus, and reconstruction of the alveolar ridge with an iliac crest graft. Three months later, Osseo integrated implants were inserted, and the patient was rehabilitated with an implant-supported fixed prosthesis with adequate esthetic outcomes. Hyperbaric oxygenation is capable of normalizing the vascular bed within 10 days of treatment, probably because of synthesis of new vascular elements, and the same size of the vascular bed could be seen after 30 days. Blood flow, on the other side, was reduced after 10 days, probably because of new synthesis of blood vessels.¹⁴ Heparinization is also reported as a treatment option.^{8,14} Perfusion to ischemic regions may be improved by reducing blood viscosity, which is a primary factor in blood flow. Heparin administered subcutaneously reduced morphological tissue damage to the teeth and bone, but this treatment was complicated by significant hemorrhage from the surgical site.⁶ However, this treatment was not performed in the present case, thereby avoiding excessive bleeding from the wound at the postoperative period. Careful assessment of the circumstances involved when small or large bone segments are lost usually indicates that basic biological principles have been violated, such as inadequate soft tissue flap design or impairment of blood supply to maxillary segments. Excessively long and traumatic surgery, inappropriate selection of interdental sites for osteotomy, strangulation of the circulation by imprudent use of palatal splints, and excessive stretching of the palatal mucosal pedicle are other causes of compromised wound healing.¹¹ Basic biological principles were strictly followed during and after orthognathic surgery in the present case, with no ligation of descending palatine vessels to warrant proper bone supply to the osteotomized maxilla, and no hemorrhage from the surgical site at immediate postoperative follow-up. These findings suggest that aseptic necrosis of the maxilla was probably related to smoking rather than to anatomical irregularities or iatrogenic. This condition was completely resolved with hyperbaric oxygenation therapy along with antibiotic therapy and optimal hygiene care of the wound area, requiring no removal of necrotic bone. In an attempt to minimize the possibility of aseptic necrosis we suggest the following, even though we are cognizant of the fact that this complication can still occur even with the best surgical techniques due to inherent biological variability:

1. Preserve the descending palatine arteries whenever possible. In cases where a significant intrusion of the maxilla is performed, especially if the maxilla is also returned, an attempt should be made to limit the kinking of the vessels by gently teasing them out of the surrounding bone superiorly. This procedure is only useful provided the vessel is not encased in a thick bony buttress, because otherwise the trauma of the dissection could lead to

increased damage to the vessel, resulting in hemorrhage or thrombosis.

2. Divide the maxilla into as few segments as possible and try to avoid small segments anteriorly. Good preoperative orthodontics should allow the number of sections to be minimized. In some cases, it may be better to compromise and accept a lack of good contact in the second molar region rather than perform a four-piece maxillary osteotomy to get these teeth into occlusion. In three-piece maxillary osteotomies it may be possible to avoid extending the palatal bone cut across the midline. If, however, the maxillary anterior teeth have to be rotated into the splint to avoid torquing them, this could place increased tension on the palatal mucosa and compromise blood supply more than making the osteotomy cut. If premolar teeth are extracted at the time of a Le Fort I osteotomy, and the anterior maxillary segment is repositioned posteriorly, this must be done very carefully to avoid damaging the palatal vascular pedicle because the palatal mucosa will already be buckled to some extent. Consideration could be given to preoperatively repositioning the anterior teeth orthodontically, or to performing a Wanderer anterior maxillary osteotomy, thereby predicting this segment to the labial mucosa, combined with bilateral posterior maxillary osteotomies, instead of a conventional multisegmental Le Fort I osteotomy.

3. Avoid compression of the palatal mucosa or gingivae by a palatal splint, or compression of the labial gingivae and alveolar bone by skeletal infraorbital rim areas. The necessity for palatal splints and skeletal fixation has fortunately decreased with the use of rigid internal fixation.

4. If significant transverse expansion is necessary, consideration should be given to initial surgically assisted orthodontic palatal expansion. A Le Fort I osteotomy might be necessary at some time in the future for final correction in the vertical, anterior/posterior, or transverse dimensions. In cases where marked transverse problems exist, and two-jaw surgery is to be performed, consideration could also be given to narrowing the mandibular arch via a symphyseal osteotomy rather than doing all the transverse changes in the maxilla.

5. Consider a horseshoe-shaped palatal cut rather than a midpalatal split if a large amount of transverse expansion is required.

6. Have good preoperative orthodontic separation of teeth in areas of osteotomy cuts to avoid damage to the interdental bone and roots.

7. Disimpaction forceps are probably best avoided whenever possible because their use may slightly injure palatal mucosa and could compromise blood supply. The maxilla should be able to be downfractured in most instances with only hand pressure.

8. If unexpected occurrences happen at the time of surgery, it may be prudent to be conservative and either stop the procedure, or plan to accomplish less than was originally intended. If, for example, after completion of a Le Fort I

down fracture the maxilla is noted to be pale white, suggestive of arterial ischemia, or dark purple from venous congestion, the procedure should be temporarily stopped, the maxilla repositioned in its normal location, and the hypotensive anesthesia reversed. After a time, if the color does not improve it may not be prudent to proceed with significant anteroposterior or vertical changes, or transverse modifications. If a maxilla in a cleft palate patient is noted to develop poor color after an intended major anterior advancement, it may be advisable to rely on an intermediate splint where the maxilla has been advanced to a lesser extent. There is undoubtedly a time limit wherein one can afford to wait to see if the situation improves, beyond which the situation is likely to deteriorate to irreversible aseptic necrosis. It would probably be imprudent to delay more than 8 to 12 hours before returning a patient to surgery to move the maxilla back to its original, or an intermediate position, to see if its color improves. Improvements in vascularity are more likely to occur when the surgical changes have primarily been anteroposterior, rather than vertical or transverse, since the latter modifications are less easily reversed. These situations demand judgment calls when a decision has to be made in the operating room or in the early postoperative period, and when a conservative approach may result in a less-than-optimal final result or necessitate additional surgery at a later time. It may, however, in certain instances avert a disastrous result. Consideration should be given to having duplicate models available, especially for cleft palate patients, in case an intermediate splint has to be fabricated in the early postoperative period to aid in repositioning the maxilla to a more suitable location.

9. Other suggestions made by surgeons responding to the questionnaire included performing the surgery under minimal hypotensive anesthesia, avoiding electrocautery for maxillary buccal vestibular incisions, and having patients avoid smoking postoperatively. Treatment of aseptic necrosis initially involves good hygiene in the area, with frequent irrigation with saline. Ideally the patient should be treated with hyperbaric oxygen, and antibiotics should be considered to prevent secondary infection. A surgical debridement will be required to try to speed up the resolution of the necrotic process.

Hyperbaric oxygen may hasten the delineation of the necrotic segments and allow a definitive debridement to be done at an earlier time. It will not, however, prevent or reverse the development of aseptic necrosis once it has started, but may limit its ultimate extent. Later reconstruction may require closure of the defects with soft tissue flaps, bone grafts from the iliac crest, or hydroxyapatite. The resulting defects can then be corrected by fixed or removable prosthetic appliances. Osseo integrated implants may be a useful adjunct.¹³Till date, little experimental work has been done to investigate the prevention of avascular necrosis in the jaws. Nilsson et al⁵⁴ examined the effects of heparin, dextran 40, dextran 70,

and hyperbaric oxygen to reduce tissue damage to teeth and bone after mandibular osteotomies in rats. Tissue damage was recorded morphologically, whereas blood flow was determined by isotope techniques. Hyperbaric oxygen had a beneficial effect on the amount of tissue damage that occurred as compared with nontreated animals. It was hoped that perfusion to ischemic mandibular regions via collateral circulation could be improved by reducing blood viscosity, which is a primary factor in blood flow. Although in other experimental studies intravenous dextran had been reported to improve the microcirculation.⁵⁵ T"dextran 40 or dextran 70 had no preventive effect on tissue damage in this investigation. Heparin administered subcutaneously reduced morphologically determined tissue damage to teeth and bone, but this treatment was complicated by significant hemorrhage from the operative field. Jaques⁵⁶ has found in recent experiments that when heparin is given orally the endothelial cell concentration is from 100 to 1,000 times the plasma concentration. It is therefore possible that if heparin is given orally postoperatively it could improve the microcirculation without leading to significant bleeding complications. The possible usefulness in preventing or limiting the extent of aseptic necrosis with the use of agents such as heparin or dextran sulphate requires further investigation.

CONCLUSION:

It is hoped that the publication of this review will accentuate the importance of a sound background in anatomy, physiology, and basic surgical principles which one must have before attempting any operation on a patient. At least, one should be expected to have a thorough understanding of a procedure before attempting it. It is incumbent upon our profession to prepare our trainees to be aware of the recent advances in oral surgery and to be capable of performing these procedures or to recognize what they are not competent to handle.

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Mortality in Maxillofacial Trauma – A Review

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Abstract: Trauma remains one of the principal causes of mortality in the world, especially among young adults. The most serious immediate life-threatening complication following maxillofacial trauma is airway obstruction. The onset can be sudden, as with foreign body aspiration, or following soft-tissue damage that can lead to a later stage to airway-compromising edema. The medical literature regarding facial trauma appears to support the hypothesis that maxillofacial trauma alone is rarely life threatening or will not lead to life-threatening conditions unless associated with airway compromise. There are some causes of life threatening complications following trauma to the maxillofacial region such as massive bleeding or undiagnosed cervical spine injury. However, there are some situations that may cause irreversible damage unless immediate operation is undertaken. The almost complete lack of reports dealing with death or irreversible damage in trauma involving the maxillofacial region prompted us to review the mortality following trauma to the maxillofacial region.

Keywords: Trauma, Mortality, Death, Face.

INTRODUCTION

Patients with maxillofacial fractures have varying degrees of concomitant injuries. Etiologic factors, socioeconomic factors, geographic location, and type of facility can influence both the type and severity of these injuries[1,2]. Trauma continues to be a major threat to public health. Maxillofacial trauma occurs in a significant number of severely injured patients and may be a clue to concomitant serious or life-threatening injuries.

Knowledge of associated injuries can lead to the rapid assessment and initial treatment of these patients. In addition, our understanding of causes, severity, and specifics such as facial fracture site, hospital complications, and discharge status will improve our abilities of prevention. Motor vehicle accident (MVA) and assault remain the primary causes of maxillofacial fractures[3,4]. Alcohol intoxication at the time of trauma continues to be an important factor related to injury. Serious concomitant injuries with facial fractures have been documented, but further study is important for both patient care and directed research[4-6]. Facial injuries have been the focus of attention in many parts of the world because of its high incidence and diversity[7]. Worldwide indices show road traffic accident to be the major cause of maxillofacial injury. An estimated 1.2 million people round the globe are killed as a result of road traffic injuries each year and the situation in a developing country like India is no different[8]. Many factors affect mortality rate and outcome after trauma. Age of the

patient, concomitant head injuries and increased bleeding are some of the variables of increasing death rates after maxillofacial trauma[9,10]. Another factor affecting mortality is the standard of surgical care in maxillofacial patient. Studies in industrialized countries have shown a rate of death from inpatient surgery of 0.4 to 0.8% and a rate of major complications of 3% to 17%. These rates are likely to be much higher in developing countries[11,12]. Knowledge of injuries associated with maxillofacial fractures provides useful strategies for patient care and prevention of further complications. Cerebral and pulmonary injury are often associated with maxillofacial fractures in severely injured trauma patients. Coordination of trauma teams, emergency room physicians, and surgical teams such as neurosurgeons is vital for the early stabilization and treatment of patients with facial fractures. Because of high hospital complication rates, ongoing care of these patients is an important factor in their morbidity and mortality rates. Ultimately, prevention of injury from

violence and MVA will prove most valuable to society in terms of both public health and cost.

COMPLEXITY OF SITUATION

The maxillofacial trauma patient often presents a problem of difficult mask ventilation and difficult intubation. The trauma usually disrupts the normal anatomy and causes edema and bleeding in the oral cavity. The mask cannot be properly close-fitted to the face, to enable effective mask ventilation. Furthermore, an injured airway may prevent efficient air transferring from the mask to the lungs. The challenge in performing the intubation arises mainly from a difficulty in visualizing the vocal cords with conventional direct laryngoscopy. The oral cavity, pharynx and larynx may be filled with blood, secretions, debris, soft tissue and bone fractures, all of which preclude good visualization of the vocal cords. The indications for tracheostomy have been revised as follows: (1) Acute airway obstruction and failed endotracheal intubation (2). Expected prolonged intubation due to difficulties weaning from the ventilator (3). Multiple facial fractures combined with basal skull fractures (4). Complete destruction of nasal anatomy combined with multiple facial injuries. Severe pain, independent of medical therapy, may cause sudden, unexpected death. Cardiac arrest is the cause, and practitioners need to know how to spot a high-risk patient. Sudden, unexpected death may occur in a severe, chronic pain patient, and the terminal event may be unrelated to medical therapeutics. Fortunately, sudden death is not as commonly observed in pain patients as in past years most likely due to better access to at least some treatment. Sudden death still occurs, however, and practitioners need to know how to spot an "at-risk" patient. Unexpected, sudden death due to severe pain is poorly appreciated, since many observers still view severe pain as a harmless nuisance rather than a potential physiologic calamity. In many cases, just prior to death, the patient informs their family that they feel more ill than usual and seek relief in their bed or on their couch. Unfortunately, some of these patients don't awaken. Other patients die, without warning, in their sleep or are found collapsed on the floor. Modern medicine's aggressive toxicology and forensic procedures after death have contributed to the poor understanding of pain's death threat. In some cases, a pain patient that was being treated appropriately with an opioid or other agent with overdose or abuse potential has suddenly and unexpectedly died. Drugs were found in body fluids after death, and in my opinion a coroner wrongly declared the death to be an "accidental overdose" or "toxic reaction" to drugs rather than implicate the real culprit, which may have been an "out-of-control" pain flare.

Mechanisms of death

Severe pain is a horrific stress[13,14]. Severe pain flares, acute or chronic, cause the hypothalamic-pituitary adrenal axis to produce glucocorticoids

(cortisol, pregnenolone) and catecholamines (adrenalin and noradrenalin) in an effort to biologically contain the stress[15,16] Catecholamines have a direct, potent stimulation effect on the cardiovascular system and severe tachycardia and hypertension result[17]. Pulse rates may commonly rise to more than 100 beats per minute and even rise to more than 130 beats per minute. Blood pressure may reach more than 200 mmHg systolic and more than 120 mmHg diastolic. In addition to adrenal catecholamine release, pain flares cause overactivity of the autonomic, sympathetic nervous system, which add additional stimulation to catecholamine-induced tachycardia and hypertension. Physical signs of autonomic, sympathetic overactivity, in addition to tachycardia and hypertension, may include mydriasis (dilated pupil), sweating, vasoconstriction with cold extremities, hyperreflexia, hyperthermia, nausea, diarrhea, and vomiting. The combined physiologic effects of excessive catecholamine release and autonomic, sympathetic discharge may put such strain on the heart to cause coronary spasm, cardiac arrhythmia, and sudden death[18]. Pain patients who have underlying arteriosclerosis or other cardiac disease are at higher risk of sudden death. For example, a patient with angina or generalized arteriosclerosis is at high risk and should be aggressively treated. Anecdotal reports have been made in which a patient whose pain was well controlled on opioids died unexpectedly with an underlying cardiac disease. In one report, a 40-year-old pain patient on opioids was found dead and the autopsy revealed previously unrecognized coronary artery disease, which was determined to be the cause of death. Some patient deaths may be due to other comorbid conditions, whether known or not known, and may not be related to the pain problem.

DISCUSSION

Trauma accounts for thousands of deaths and financial burden on any country. It has been labeled as "neglected disease of modern society". It involves universal young productive lives and male predominance. For every death, two people suffer permanent disability. Maxillofacial injuries need special attention due to many reasons. These injuries are with or without head injury and cervical spine fractures or polytrauma. Early airway control requires sound judgment and considerable experience. Skillful experienced personnel are mandatory. In order to have a good outcome with minimal risks and maximal success in airway management, should be in collaboration with the anesthesiologist or trauma team leader is must[19]. ATLS protocol must be followed in all cases of maxillofacial trauma with immediate attention to life-threatening injuries[20]. Gruen *et al.* found that, failure to intubate, secure or protect the airway was the most common factor related to patient mortality, responsible for 16% of inpatient deaths[21]. Emergency trauma care was not part of this study since primary care was given separately. The time lag between the injury and surgery

is variable depending on primary care institutional protocols and may range from few hours to few days according to associated injury, facial edema and preoperative optimization of general condition. Resolution of facial edema during this time allows for more accurate clinical evaluation of airway and ease of intubation. Capasi *et al.* suggested that the delay in final reconstruction of facial fractures in critically ill patient has an acceptably low complications rate and may be advantageous in decreasing operative risk[22]. Hutchinson *et al.* addressed six specific situations associated with maxillofacial trauma, which may adversely affect the airway: 1. Postero-inferior displacement of a fractured maxilla parallel to the inclined plane of the skull base, 2. bilateral fracture of the anterior mandible, 3. hemorrhage 4. soft tissue swelling and edema, 5. trauma to the larynx and trachea, 6. foreign bodies – dentures, debris, shrapnel, exfoliated teeth, bone fragments[23]. Planned reconstruction schedule is required to achieve maximum, satisfactory function and appearance as unnecessary delay in surgery may predispose to complications like malunion and infections. Approach to the maxillofacial trauma patient's airway evaluation and preparation is the key to a successful anesthetic management. Extent of injury, the composition and the anatomy of the injury along with Mallampatti classification, atlantoaxial mobility and thyromental distance provides good airway assessment[19]. But these all may not be accurate in the presence of tissue edema, disrupted anatomy and muscle spasm. The risk of airway-related complications during the peri-operative period was studied by Peterson *et al.* [24]. They analyzed the American Society of Anesthesiologists Closed Claims database to identify the patterns of liability associated with the management of the difficult airway. They found that complications arose throughout the peri-operative period: 67% upon induction, 15% during surgery, 12% at extubation and 5% during recovery. As with every difficult airway situation, the equipment for difficult intubation should be prepared and ready to use. The approach should be chosen according to the patient's injuries, airway status and the care provider's experience with such equipment and procedures. Management of the airway is a major concern in patients with maxillofacial trauma (gunshot wounds, facial fractures, cervical spine injuries, laryngotracheal injuries) because a compromised airway can lead to death. The method of intubation to use in these patients remains a controversial topic. Although there are many options available, each one has specific indications, and the choice will ultimately depend on the patient's situation and the expertise of the anesthesiologist[25]. Several studies have explored the association among craniofacial trauma, intracranial injury, and death[26-29]. Although many of these studies were primarily descriptive, they suggested important differences in the outcomes according to the regional facial involvement. Death and intracranial injury have been observed to be increasingly common

with the involvement of more superior facial regions[27,30]. Lee *et al.* [28] had previously found that head trauma after midface fracture is most often minor and postulated that the fragile bones of the midface might act as a cushion for the neurocranium. Mithani *et al.* [30] expanded on these findings in one of the largest comparative facial trauma series to date and noted that serious head injury was relatively less common in midface fractures overall. However, with bilateral midface trauma, a significant association was found with basilar skull fractures and mortality[30,31] Immediate management of maxillofacial injuries is required mainly when impending or existing upper airway compromise and/or profuse hemorrhage occurs. Hutchinson *et al.* [23] addressed six specific situations associated with maxillofacial trauma, which may adversely affect the airway: 1. Postero-inferior displacement of a fractured maxilla parallel to the inclined plane of the skull base may block the nasopharyngeal airway. 2. A bilateral fracture of the anterior mandible may cause the fractured symphysis to slide posteriorly along with the tongue attached to it via its anterior insertion. In the supine patient, the base of the tongue may drop back, thus blocking the oropharynx. 3. Fractured or exfoliated teeth, bone fragments, vomitus and blood as well as foreign bodies – dentures, debris, shrapnel etc. – may block the airway anywhere along the upper aerodigestive tract. 4. Hemorrhage, either from distinct vessels in open wounds or severe nasal bleeding from complex blood supply of the nose, may also contribute to airway obstruction. These situations should be addressed immediately using various manual and/or instrumental techniques, in accordance with the "A" step in the ABC treatment protocol suggested by the ATLS[19]. Endotracheal intubation should be considered if it was not performed earlier. 5. Soft tissue swelling and edema resulting from trauma to the head and neck may cause delayed airway compromise. 6. Trauma to the larynx and trachea may cause swelling and displacement of structures, such as the epiglottis, arytenoid cartilages and vocal cords, thereby increasing the risk of cervical airway obstruction. A high index of suspicion, meticulous physical examination and close observation of the patient may assist in the early detection of such situations and facilitate proper and timely management in order to avoid future complications. Once airway management has been completed and all hemorrhage sites controlled, definitive management of bone and soft tissue injuries resulting from maxillofacial trauma may be deferred until life- and/or organ-threatening injuries have been properly managed. Early intervention is always needed to improve the prognosis. Airway, breathing and circulation compromises may be affected. It is important to understand the uniqueness of the facial region, both in terms of its anatomy and the neighboring structures. Early diagnosis and treatment of life-threatening situations and compromised vital structures, will improve the prognosis. The team approach to maxillofacial trauma should involve general, oral-

maxillofacial and ophthalmology surgeons for optimal care.

CONCLUSION

It can be concluded that Oral and Maxillofacial Surgery is specialty with an extremely low mortality rate. Respiratory complications are the most common cause of death in patients who survive from maxillofacial trauma. General anesthesia poses a negligible mortality risk to ASA class I patients. The only anesthesia related death was that of an ASA class III patient. We recommend that All patients of maxillofacial trauma or otherwise should be thoroughly examined for any respiratory and cardiac abnormalities and referred promptly to other specialties when required. The position of a full time emergency medicine specialist should be mandatory in all maxillofacial units that exist outside of a medical hospital. Another study of the same kind should be repeated after every 5 years as a form of a clinical audit to see any improvements or otherwise in the management of patients at this unit. An annual mortality and morbidity conference should be held in the department involving maxillofacial surgeons and allied specialists from the medical field to conduct a critical appraisal of the multidisciplinary treatment approach of patients and suggest ways to decrease the existing morbidity and mortality rate. Residents of Oral and Maxillofacial Surgery should have a minimum of 2 months rotations in a medical or surgical ICU at par with other mandatory rotations fulfilling the requirements for MDS.

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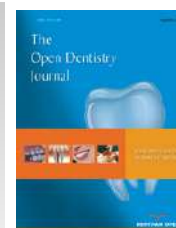
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RESEARCH ARTICLE

A Perioral Soft Tissue evaluation after Orthognathic Surgery Using Three-Dimensional Computed Tomography Scan

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Abstract:

Background:

Facial appearance is an important factor, affects social and psychological well-being. The ideal positioning of jaws and soft tissues is crucial during orthognathic surgery for a better outcome, but the response of facial soft tissues does not always reflect the exact movements of the underlying jaws in 1:1 ratio. So, soft tissue changes following orthognathic surgery require utmost attention during surgical correction to make successful treatment.

Aims and Objectives:

Evaluation of perioral soft tissue changes after orthognathic surgical procedures. The objectives of the study were to assess and compare pre and post-operative perioral soft tissue changes of lip width, nasolabial and mentolabial angle using Three Dimensional Computed Tomography scan (3DCT).

Patient and Methods:

The study involved ten patients for evaluation requiring orthognathic surgical procedures (maxillary or mandibular anteroposterior excess or deficiency, transverse deformities, vertical maxillary excess and facial asymmetry) presented to the department of oral and maxillofacial surgery during 2014-2016. Pre and post-operative 3DCT scan were taken after 12 months using iCT 256 slice whole body CT scanner and evaluated for changes using Dicom PMS D view.

Results:

Significant changes were observed in nasolabial angle after maxillary advancement (1.81°) and maxillary setback procedure (2.73°). The mentolabial angle was significantly increased with mandibular setback procedures (3.27°). Mandibular advancement procedures showed both increase (3.6°) and decrease (7.6°) in mentolabial angle.

Conclusion:

3DCT showed a significant difference in perioral soft tissue changes in nasolabial and mentolabial angle but no significant change was observed in lip width. 3DCT is a reliable tool for 3D assessment. The conventional thought of changes in Nasolabial angle after surgery is changing due to the underlying factors which should be considered for prediction.

Keywords: Assessment, Esthetic surgery, Perioral tissues, Prediction, 3D Analysis, Three Dimensional Computed Tomography (3DCT).

1. INTRODUCTION

Orthognathic surgery is the hallmark procedure for the correction of jaw function and esthetics of the face. The

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response of soft tissues always does not reflect the exact movements of the underlying jaws in 1:1 ratio [1]. Several soft tissue changes will occur following skeletal repositioning of the face which requires attention. The need of each patient will be fulfilled through some form of corrective surgery (single or bi-maxillary surgeries associated with augmentation, reduction and soft tissue surgery like rhinoplasty). The goal of orthognathic surgery is to achieve balanced occlusion and good facial aesthetics [1]. Facial esthetics has become a very important objective of Orthognathic surgery. It is of utmost importance to properly analyze and correctly diagnose the case for best treatment planning to achieve a better prognosis. As we perform the movements of jaws during surgery in 3 dimensional then why to perform the pre-operative work in 2 dimensions. In this context, 3-D imaging is the best way available for treatment planning which gives accurate measurements in anteroposterior, superoinferior and mesiodistal planes. Prediction tracing, mock surgery and post-operative analysis performed in 3 Dimensional software give us a better understanding of the case as it gives the whole positive replica of the jaws. The bony movements performed during these various orthognathic procedures by maxillofacial surgeons contemplate in the facial soft tissue [2]. Analyzing the hard and soft tissues of the face in three dimensions is needed to achieve good post-operative results [3, 4]. Two-dimensional analysis by radiographs and cephalometry have its own limitations for 3D assessment. When the two-dimensional evaluation is performed, it gives the data in only 2 axis but the 3-dimensional study of anything provides us the data in all the 3 planes. Available published literature regarding 3-dimensional analysis and post-operative changes after Orthognathic surgery is available only from the west and very few from Asian countries and same data is scarce in India too [5]. So, this study was planned to assess perioral soft tissue changes after orthognathic surgery using 3DCT scan.

1.1. Aim

The aim of the study was to evaluate and compare changes in lip width, nasolabial and mentolabial angle after the orthognathic surgical procedure using Three-Dimensional Computed Tomography scan (3DCT).

1.2. Patients and Methods

The patients visited for assessment of sleep apnea study with 3DCT volumetric airway assessment requiring orthognathic surgical procedures were randomly involved for assessment of perioral soft tissue changes. Total of 10 (4 males and 6 females) patients with age range of 18 to 26 years willing to participate and consent for use of their data for assessment in the study protocol have been enrolled in the department of oral and maxillofacial surgery during the year 2014-2016. Patients involved in the study are allotted with a lottery method because of the duration of course of study and longer duration of follow up which has restricted us to involve more number of sample size. Even though the sample size is small, the study is giving relevant and important information regarding three-dimensional changes in the series of 10 patients. The study protocol was approved by the Institutional ethics committee on 18/12/2014 (Reg. No. D148502044). Patient and relatives had been explained about the surgical procedure involved with the post-operative protocol. The informed written consent was obtained. The previous results and prediction were based on 2D but in the recent past 3D assessment has started. In this context, the dynamic tissue movement is relevant as shown in the Video no. 1

1.3. Inclusion and Exclusion Criteria for Patient Selection

The patients reported with facial asymmetry, maxillary and mandibular prognathism and retrognathism were included in the study and systemically compromised, drug or alcohol abuse, psychologically ill, current or past radiotherapy and patients who are not willing to enroll in the study for proper follow up were excluded.

2. METHODOLOGY

2.1. Assessment Procedure: Points to be Measured

2.1.1. Nasolabial Angle

It is the angle constructed between Columella lobular junction (Cl), Subnasale (Sn), and Upper Lip (UL) [6] (Fig. 1A).



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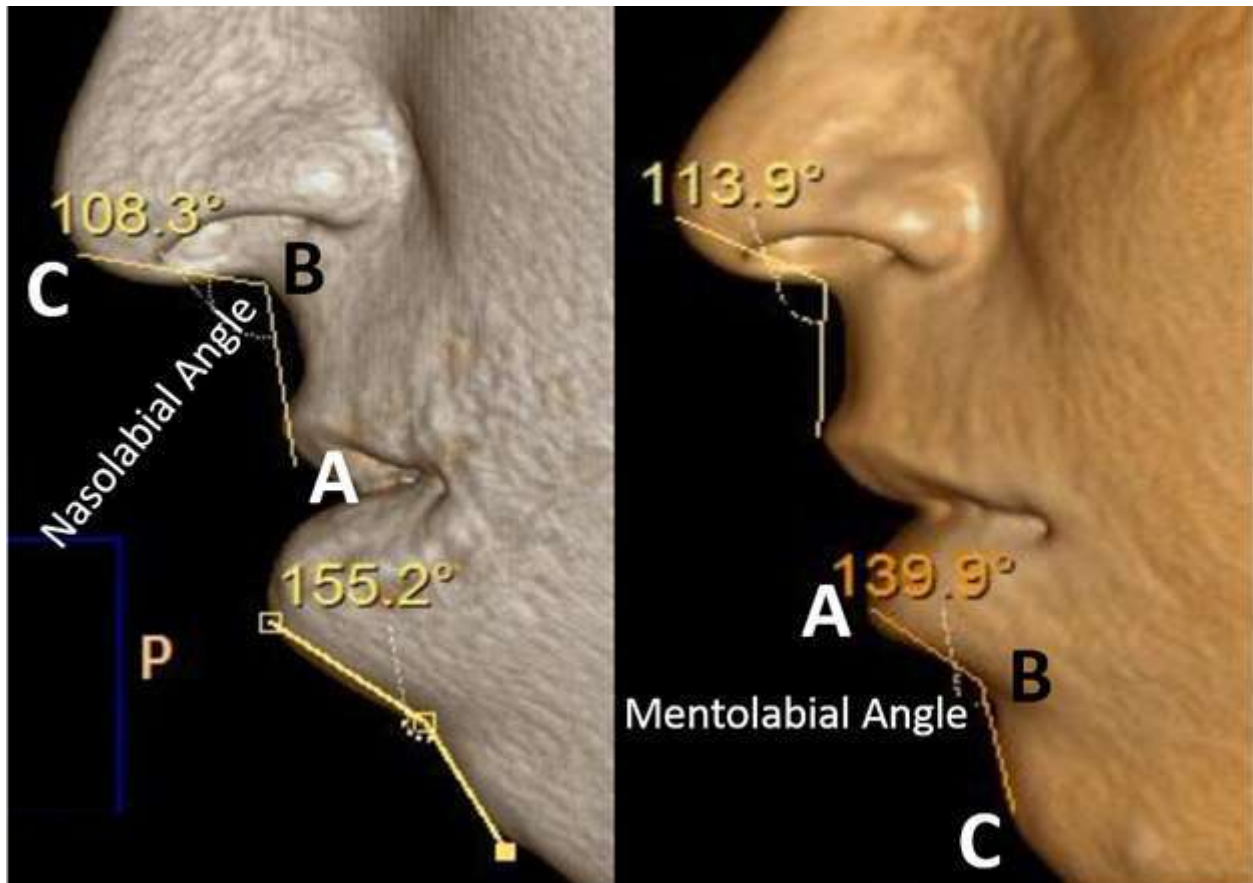


Fig. (1). Pre and post-operative close up profile 3DCT picture showing assessment of nasolabial and mentolabial angle.

- a. UL- Upper Lip- It is the most anterior point of the vermillion border of Cupid's bow of upper lip [7].
- b. Sn- Subnasale- It is the point at which columella meets with an upper lip in sagittal plane [7]
- c. Cl- Columella lobular junction- the junction between UL and Sn [7].

2.1.2. Mentolabial Angle

Angle constructed among Lower Lip (LL), Soft Tissue B Point (B), and Soft Tissue Pogonion (Pog) [6] (Fig. **1B**).

2.1.2.1. LL- Lower Lip

It is the most prominent point of the vermillion border of Cupid's bow of lower lip [7].

2.1.2.2. Pog- Soft Tissue Pogonion

It is the most prominent point of the chin [7].

2.1.2.3. B - Soft Tissue B Point

It is the most concave point of the curve between LL and Pog [7].

2.2. Lip Width

It is the distance between Cheilion of the Right Side (Rt.Ch) and the Cheilion of the Left Side (Lt.Ch) [6] Fig. (2).

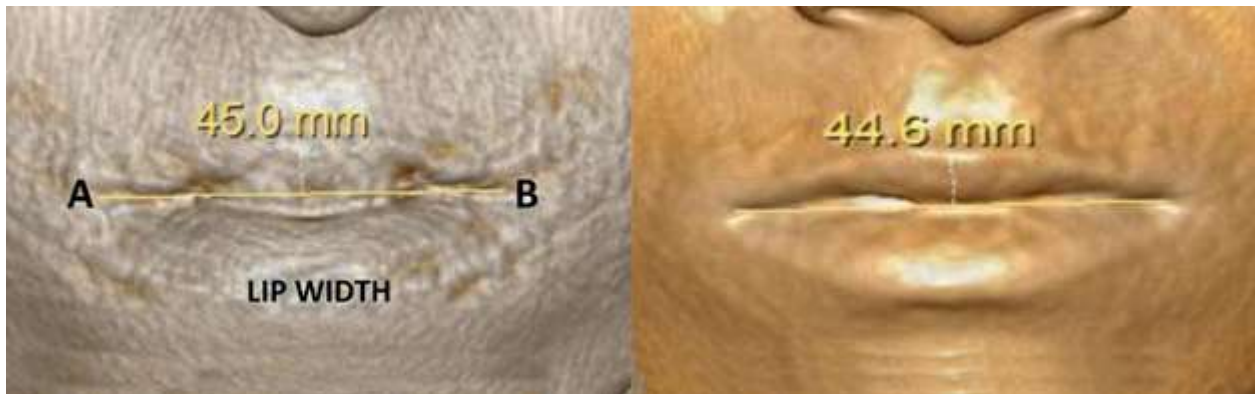


Fig. (2). Pre and post-operative close up frontal 3DCT picture showing assessment of lip width.



Fig. (3). Pre and post-operative profile view of the patient – right side.

2.2.1. Rt.Ch- Right Chelion

It is the most lateral extent of the outline of lip on the right side [7].

2.2.2. Lt.Ch- Left Chelion

It is the most lateral extent of the outline of the lip on left side [7].

Three dimensional computed tomography (3DCT) scan data obtained from the sleep apnea study center as pre and post-operative data for analysis of all involved patients which have been taken one week prior to surgery and post-operatively after one year for sleep apnea assessment. Two angular variables and one linear variable were measured (Figs. 1 & 2). 3D scans were performed with a Phillips Brilliance iCT 256 slice whole-body CT scanner by Gregard Phillips Amsterdam, Netherlands. Each patient required forty seconds of exposure for one scan. Voxel size was set at 0.45 mm for the sagittal, coronal and axial images and each scan contained 555 slices with bony and soft tissue reconstructive images. Each data set was imported directly into Dicom® PMS D view (Figs. 1 & 2). The scans were analyzed and the linear (in millimeters) and angular (in degrees) measurements were done *via* Dicom software. Frontal

view and Profile view of subjects were measured in pre and post-operative 3DCT. Other relevant figures like pre and post-operative patients photographs (Fig. 3), lateral cephalograms (Fig. 4) and three dimensional computed tomography bony window (Fig. 5) are also illustrated.



Fig. (4). Pre and post-operative lateral cephalogram of the patient.



Fig. (5). Pre and post-operative close up profile 3DCT picture showing bony window.

2.3. Statistical Analysis

The observations were tabulated using Microsoft Excel (Table 1). Descriptive statistics were used to interpret the data. All statistics were calculated using SPSS ver. 20.0 with mean, SD, and range for analysis.

3. RESULTS

Changes in the nasolabial angle after maxillary advancement: Two patients underwent maxillary advancement of 4 mm and 5mm in which the nasolabial angle was increased by 5.6° and 10.7°, respectively. So, the mean advancement in the maxilla was 4.5mm and the mean difference was 8.15°. Hence, 1mm forward movement of the maxilla is an increase in the nasolabial angle by 1.81° (Table 2).

Table 1. Cases records including procedure, movement and changes in perioral soft tissue changes Amo- anterior maxillary osteotomy, Sao - Su apical osteotomy, Bssso - Bilateral sagittal split osteotomy, Genio. - Genioplasty, Adv - Advancement, Sback - Setback, NLA- Nasolabial angle, MLA- Mentolabial angle, LW- Lip width, mm. - millimeter.

S.No.	Le fort		Amo		Bssso		Sao	Genio	Pre op NLA (°)	Post op NLA (°)	Pre op MLA (°)	Post op MLA (°)	Pre op LW (mm.)	Post op LW (mm.)
	Adv	Set back	Set back	Adv	Set back	Adv	Set back	Adv						
1	-	2mm	-	2mm	-	-	-	2mm	119.5°	109.6°	131.5°	106.9°	43.0	40.8
2	4mm	-	-	-	4mm	-	-	-	108.3°	113.9°	155.2°	139.9°	45.0	44.6
3	-	-	-	5mm	-	-	-	-	122.2°	123.1°	113.3°	132.6°	45.2	44.6
4	-	3mm	-	-	-	2mm	-	-	101.0°	96.7°	133.3°	119.1°	50.4	48.2
5	-	3mm	-	-	-	2mm	-	-	103.5°	99.4°	152.0°	131.8°	44.3	41.9
6	5mm	-	-	-	6mm	-	3mm	-	112.3°	123.0°	138.9°	130.8°	36.9	39.9
7	-	-	-	-	4mm	-	-	-	108.1°	108.6°	156.6°	133.1°	42.3	46.4
8	-	-	2mm	-	-	-	2mm	-	106.5°	100.7°	128.9°	113.5°	46.5	44.7
9	-	-	-	8mm	-	-	-	-	117.9°	118.9°	100.1°	131.3°	46.0	49.3
10	-	3mm	-	4mm	-	-	-	-	129.3°	117.8°	106.9°	117.0°	42.9	47.5

Table 2. Pre and post-operative differences in nasolabial angle after orthognathic procedures.

Procedure	Movement	Pre op	Post op	Difference
Max adv	4mm	108.3	113.9	Inc by 5.6
Max adv	5mm	112.3	123.0	Inc by 10.7
Max sback	2mm	119.5	109.6	Dec by 9.9
Max sback	3mm	101.0	96.7	Dec by 4.3
Max sback	3mm	103.5	99.4	Dec by> 4.1
Max sback	2mm	106.5	100.7	Dec by 5.8
Max sback	3mm	129.3	117.8	Dec by 11.5

Max – Maxillary, Adv – Advancement, Sback – Setback, Inc – Increase, Dec – Decrease, mm – millimeter, op- operative.

3.2. Changes in Nasolabial Angle After Maxillary Setback

A total of five patients have undergone maxillary setback of 2 mm to 3 mm in which the nasolabial angle has decreased by 4.1 to 11.5°, respectively. So, the mean setback in the maxilla was 2.6 mm and the mean difference was 7.12°. Hence, 1mm movement of maxilla setback is a decrease in the nasolabial angle by 2.73° (Table 2).

3.3. Changes in Mentolabial Angle after Mandibular Advancement

Among six patients, three patients underwent mandibular advancement of 2 mm to 8 mm. In three patients, the mentolabial angle was decreased by 7.6° and in remaining three patients, mentolabial angle increased by 3.6° (Table 3).

3.4. Changes in the Mentolabial Angle After Mandibular Setback

Four patients underwent mandibular setback of 2 mm to 9 mm in which mentolabial angle decreased by 3.27° with 1mm mandibular backward movement (Table 3).

Table 3. Pre and post-operative differences in mentolabial angle after orthognathic procedures.

Procedure	Movement	Pre op	Post op	Difference
Mand adv	4mm	131.5	106.9	Dec by 24.6
Mand adv	2mm	133.3	119.1	Dec by 14.2
Mand adv	2mm	152.0	131.8	Dec by 20.7
Mand adv	5mm	113.3	132.6	Inc by 19.3
Mand adv	8mm	100.1	131.3	Inc by 31.2
Mand adv	4mm	106.9	117.0	Inc by 10.1
Mand sback	4mm	155.2	139.9	Dec by 15.3
Mand sback	9mm	138.9	130.8	Dec by 8.1
Mand sback	4mm	156.6	133.1	Dec by 23.5
Mand sback	2mm	128.9	113.5	Dec by 15.4

Mand – Mandibular, Adv – Advancement, Sback – Setback, Inc – Increase, Dec – Decrease, mm – millimeter, op- operative.

3.5. Changes in the Lip Width in Bi Jaw Surgeries

Four patients underwent maxillary setback and mandibular advancement in which the mean maxillary setback was 2.75 mm. and the mean mandibular advancement was 3.0 mm. The mean decrease in the lip width was 2.15 mm. Three patients were operated for single jaw surgery (mandible) and two patients underwent maxillary advancement and mandibular setback showed no significant relationship with change in lip width. One patient with the setback of both the jaws had a decrease in the lip width. There was a significant relationship between lip width change only in the maxillary setback and mandibular advancement surgery when operated together (Table 4).

4. DISCUSSION

The change in soft tissue morphology after surgical therapy is dependent on several factors like wound closure, lip morphology and post-operative swelling [8]. Assessment of soft tissue changes after surgical procedure requires minimum 6 months [9] to maximum 12 months [10]. Due to swelling, tissue redistribution, and functional adaption, long-term follow up is needed. The morphology of lip is also one of the determining factors [11]. Thick lips absorb a huge amount of bony advancement without any change in the soft tissue measurements. Dead space under the lip may absorb the first position of bony advancement before the soft tissue is affected in severe maxillary retrognathia [11].

Nasolabial angle is used to know the protrusion and retrusion of the maxilla in conjunction with the upper lip. It also helps in diagnosing the nasal tip projection. Nasal tip projection varies according to race, ethnicity, age and gender. Our study showed decreased nasolabial angle by 4.1° to 11.5° in maxillary setback of 2 mm to 3 mm (Table 2). similar to Rosen HM [11] where 12 patients after moving the maxilla anteriorly and superiorly with follow up of 9.8 months concluded that at least 12 months are required before all residual edema to dissipate and complete animation of upper lip to return. If maxilla is moved superiorly without anteroposterior movements, the upper lip comes forward and nasolabial angle becomes more acute and conversely, if the maxilla is moved downward, the lip moves posteriorly and nasolabial angle becomes more obtuse. The study showed 0.51:1 ratio change in nasolabial angle after maxillary advancement. The mean setback in the maxilla was 2.6mm and the mean difference was 7.12° in our study which showed a decrease in the nasolabial angle by 2.73° with 1mm maxillary setback which clearly indicates that the impaction of the maxilla is causing the decrease in nasolabial angle and making the nasolabial angle more acute post-operatively. In Patrick J Louis study [12], maxillary advancement with a Le Fort I osteotomy (8 ± 2.5 mm) with eight months of follow up showed a decrease in the nasolabial angle by 5° (-10° to +7°). But our study results are in contrast to this study; as we operated only 2 patients. These patients underwent maxillary advancement of 4 mm and 5 mm in which the nasolabial angle is increased by 5.6° and 10.7°, respectively. So, the mean advancement found in the maxilla was 4.5 mm and the mean difference in nasolabial angle was 8.15°. Our study showed that 1mm forward movement of the maxilla, there is an increase in the nasolabial angle by 1.81°. Our results are supported by a study of Takahiro Shoji [13] where they found an increase in the nasolabial angle and projection of the nasal tip after maxillary advancement. Our results were obtained after 12 months whereas Rosen and Patrick assessed the changes after 9.8 months and 8 months, respectively. So, our results are more settled as suggested by Rosen HM for a minimum of 12 months follow up for the changes.

Mentolabial angle is influenced by the position of lower lip, chin and inclination of mandibular incisor teeth. An

acute mentolabial angle may be a reflection of the dentoalveolar protrusion or an over-grown chin and in contrast, the obtuse mentolabial angle is because of dentoalveolar reclination or an undergrown chin [14]. Young –Kyun Kim *et al.* evaluated perioral soft tissue changes in 15 patients after mandibular setback surgery and found significant lip changes after 6 months of follow up. Lower lip protrusion was seen about 1.67 mm, soft tissue point B around 1.28 mm and pogonion around 1.61 mm [14]. Our study showed 2 mm to 9 mm of mandibular setback whereas, 8.1° to 23.5° decrease in mentolabial angle. So, the mean setback in the mandible was 4.75 mm and the mean difference was 15.57°. The mandibular setback of 1 mm showed a decrease in the mentolabial angle by 3.27° which made the mentolabial angle acute (Table 3).

The increase of nasolabial angle and decrease of mentolabial angle were in accordance with previous study results [15 - 17]. Our study showed an increase in mentolabial angle in 3 patients and decrease in 3 patients, which were in significant relationships with the movement of the hard issue but not significant in relation to the procedure performed. The mandibular movement in our study was 2 mm to 4 mm whereas a decrease in mentolabial angle was 14.2° to 24.6°. The mean advancement in the mandible was 2.6 mm and the mean difference of mentolabial angle was 19.8°. Hence, 1 mm forward movement of mandible showed 7.6° decrease in mentolabial angle. The patients who underwent mandibular advancement of 4 mm to 8 mm showed an increase of mentolabial angle by 10.1° to 19.3° with 5.6 mm mean advancement and 20.2° mean difference in mentolabial angle. Hence, 1 mm forward movement of mandible showed an increase in the mentolabial angle by 3.6°.

Lip width is equal to the interpupillary distance in a normal individual. Yu Jin Jung [6] evaluated the hard and soft tissue changes in 17 subjects; where more soft tissue changes are related to horizontal and anteroposterior aspects than in the vertical one using 3DCT. The changes in the lip width were also not significant with the various procedures of orthognathic surgery. But in our study four patients underwent maxillary setback (mean of 2.75 mm) and mandibular advancement (mean of 3 mm) which showed mean decrease in the lip width by 2.15 mm and single jaw surgery with mandible showed no significant correlation in the changes with lip width. (Table 4).

Table 4. Changes in lip width after orthognathic surgery.

Pt.no.	Maxilla	Mandible	Pre op	Post op	Difference
1	S 2mm	A 4 mm	43.0	40.8	Dec by 2.2
2	A 4 mm	S 4mm	45.0	44.6	Dec by 0.6
3	-	A 5 mm	45.2	44.6	Dec by 0.6
4	S 3mm	A 2 mm	50.4	48.2	Dec by 2.2
5	S 3mm	A 2 mm	44.3	41.9	Dec by 2.4
6	A 5 mm	S 9mm	36.9	39.9	Inc by 3.0
7	-	S 4mm	42.3	46.4	Inc by 4.3
8	S 2mm	S 2mm	46.5	44.7	Dec by 1.8
9	-	A 8 mm	46.0	49.3	Inc by 3.3
10	S 3mm	A 4 mm	42.9	47.5	Inc by 4.6

A – Advancement, S – Setback, Inc – Increase, Dec – Decrease, mm – millimeter, op- operative.

Evaluation of three-dimensional images, reproducibility, accuracy and availability of computed tomography proved to be the most reliable tool [18]. Researchers also proved that the soft tissue starts adapting from third month [18] and it takes more than a year to give desired results post-operatively [19], So we followed our patients after 12 months. Combinations of soft tissue remodeling, tissue relocation, hard tissue relapse, weight loss, and weight gain are important parameters to be considered for evaluation of postoperative changes in facial soft tissues [20]. In our study, we have not correlated that facts but taken the weight and built into consideration.

The activity of muscles in motion and sometimes at rest is a prime factor which should be considered for the success of treatment. [Video - 1] The patient at rest shows no incisal show but normal speech will show uneven lip movements and incisal exposure and even gummy smile. If the lip framework is not assessed properly then the patient and surgeon will not be satisfied even with orthognathic surgery. In such patients dynamic, the facial expression should be assessed properly for successful treatment. As an adjunct to the surgical procedure neurotoxins like Botox can be injected into a predetermined area to prevent gummy smile and satisfaction of the patient. Such procedures are minimally invasive, effective and innovative which can be used as an adjunct for true vertical maxillary excess with a hypermobile lip. In our few cases Botox was useful to reduce incisal show even after maxillary superior impaction. These views have been



supported by few of the published literature [21 - 23].

Three-dimensional computed tomography is an effective tool for investigating the 3D changes in hard and soft tissues simultaneously in terms of direction and amount of movement information that 2D radiographs and three-dimensional surface scanning systems cannot provide [20]. The reproducibility of landmarks is better with no superimposition of structures. Image quality is in high resolution. If a better algorithm to combine the 3D laser or optical surface scanning and computed tomography without distortion error is developed, it would be a great advancement for the clinical research and the analysis can be performed with a larger sample size. The dynamism of perioral soft tissues irrespective of hard tissue positioning is the prime consideration for successful outcome [21 - 23].

Our study sample size was limited to 10 patients in a single center. The study was not confined to a single procedure, even it didn't consider morphological factors and ageing changes. Even though our study consisted ten patients in a single center, we did 12 months follow up as recommended by published literature which is not available till date. Our study stands alone in this aspect. The study was not confined to the single procedure and it did not consider morphological factors and ageing changes. Our study suggests multicenter randomized control trials with long-term follow up to incorporate age changes.

CONCLUSION

The amount of maxillary and mandibular advancement and setback plays an important role in the post-surgical increase and decrease in nasolabial angle, mentolabial angle, respectively. 3DCT scan and dynamic videography or clinical assessment are of paramount importance to assess the changes in perioral soft tissues where the published literature is scarce. The study also showed that conventional thought in nasolabial angle changes after surgery is changing because of underlying factors, hypermobility of the lip should be considered for prediction. Adjunctive therapy like Botox can be used to camouflage the hypermobility of lip for the success of treatment.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Institutional ethics committee on 18/12/2014 (Reg. No. D148502044).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient for publication of this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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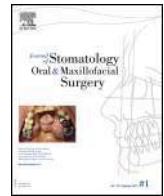


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Original Article

Role of extra oral monofocal distractor device in the correction of the facial asymmetry, sleep apnoea, and quality of life associated with TMJ ankylosis

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ABSTRACT

Objectives: The purpose of this study is to evaluate the role of primary osteo-distraction prior to ankylosis release in patients, diagnosed with sleep apnoea, facial asymmetry, and reduced quality of life secondary to temporomandibular joint (TMJ) ankylosis.

Methods: Ten patients in the age group of 13–40 years with TMJ ankylosis underwent primary osteo-distraction for mandibular advancement. They were evaluated pre- and post-operatively using radiographs, various questionnaires, and subjective evaluation of facial asymmetry, sleep apnoea, and quality of life (QOL).

Results: All the ten patients showed significant improvement in their sleep apnoea symptoms with a mean of 6.20 ± 1.39 ($P < 0.05$). The mean advancement of the mandible in all the ten patients (both bilateral and unilateral ankylosis) was 15.8 mm ($P < 0.05$). The quality of life showed marked improvement from very poor to very satisfactory ($P < 0.001$).

Conclusion: Primary mandibular distraction is an effective method of correction of facial asymmetry, sleep apnoea, and quality of life in patients with TMJ ankylosis.

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1. Introduction

Ankylosis of the temporomandibular joint (TMJ) occurring in early childhood results in severe micrognathia and facial asymmetry. These patients often have an associated deformity of the mandibular ramus and coronoid process, and also limitation of mouth opening and airway obstruction. The features of TMJ ankylosis are hypomobility of the mandible, retrognathia, micrognathia, dental malocclusion, change in the cant of the maxillary

occlusal plane, airway embarrassment and recurrent upper airway tract infections [1,2]. It also leads to a cosmetic deformity which might lead to overwhelming quality of life [3]. Also, the presence of retruded mandible and micrognathia in these patients create a narrowing of the oro-pharyngeal space with mechanical obstruction to respiration, more so in supine position and during sleep. This process forms complex syndrome of apnoeic episodes with significant reduction in the mean oxygen saturation levels during sleep and secondary cardiac and respiratory problems [1,3,4].

The correction of facial deformities following TMJ ankylosis remains a difficult and challenging problem in oral and maxillofacial surgery, various techniques for treating this problem have been described but with no uniformly successful results. Expected complications may vary from relapse, loss of vertical height of the affected ramus, foreign body reactions and reankylosis [5].

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Distraction osteogenesis has recently become a main stay for the treatment of craniofacial syndromes with mandibular hypoplasia including TMJ ankylosis. Its success in lengthening the mandible opens new perspectives for interceptive therapy. It is the technique of gradual bone lengthening that allows the body's natural healing mechanisms to generate new bone for augmenting [5].

One of the main advantages of DO involves distraction of soft tissue along with lengthening of the bone. Because of the gradual and controlled movement of the bone, the surrounding soft tissues are 'recruited' or "stretched" simultaneously [6]. Numerous studies now demonstrate the active use of distraction in the management of compromised airway and sleep apnoea in the paediatric population [4,6–8].

Mandibular distraction has been used successfully to manage the facial deformities associated with TMJ ankylosis [1,8–11]. However the simultaneous use of mandibular distraction with ankylosis release has significant disadvantages: [1] improper outcome of distraction due to unpredictable vector management and [2] physical interference of the distraction process, to active post-operative physiotherapy after ankylosis release [1,8,11]. The use of genial distraction as a modality to overcome these difficulties may not be useful in the case of pediatric patients where there is influence of mixed dentition and the probability of further mandibular growth [1].

The researchers hypothesized that the use of extra oral monofocal distraction would result in the improvement in the sleep apnoea along with correction of the facial esthetics, and rectification of the quality of life in these patients. Hence the specific aim of the study is to manage the primary problems like retruded mandible which leads to sleep apnoea, cosmetic deformity, and decrease quality of life; these problems were treated with the advancement of the mandible by distraction osteogenesis followed by a second stage release of the TMJ ankylosis.

2. Materials and methods

A simple non-randomized observational, clinical, and prospective study was conducted with approbation of Department of Oral and Maxillofacial Surgery for a period of 5 years in Meenakshi Ammal Dental College and Hospital. All procedures performed in study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and the regional Ethical Review Board of Institution approved the study. The procedure was explained to all the patients and informed consent was obtained from all participants included in the study. Those who were not ready and failed to report according to the set criteria were excluded from the surgery.

2.1. Inclusion criteria

A total number of 10 patients with TMJ ankylosis (unilateral or bilateral) having severe hypoplastic mandible were selected for this study and treated in our centre. Patients age ranges from 13–40 years were included in the study. Among these patients, all the patients presented with complaints of snoring during sleep, episodes of night-time awakening, and day-time somnolence were included.

2.2. Exclusion criteria

All patients with severe bone disorders, handicapped patients, subjects with any underlying systemic disease or compromised

immunity, patients who were unable to provide informed consent to the maxillofacial surgeon at the time of procedure are omitted.

In the present study, patients were treated by two-stage protocol:

- extra oral monofocal distraction done unilaterally or bilaterally for correction as indicated;
- release of TMJ ankylosis after three months post-consolidation of distracted bone.

The diagnostic work-up was done for all the patients; it includes clinical examination, radiographic presentation that includes: initial pre-operative assessment was done by using panoramic view, lateral cephalometry, postero-anterior cephalometry, computed tomography. Similarly, serial documentation and assessment of the amount of distraction after the period of consolidation was done after 3 months by using panoramic view, postero-anterior cephalometry, lateral cephalometry, computed tomography scan. Standard lab investigations were done for all the participants. Written informed consent was taken from all the subjects. In order to control the bias in the study, a single operator had performed all the surgeries under general anesthesia along with standard aseptic provisions and protocol.

The following primary outcome variables have been evaluated:

- amount of mandibular lengthening:
 - o for bilateral TMJ ankylosis: length required to distract the bone on both sides to improve the SNB angle in lateral cephalogram. A uniform standardized serial lateral cephalograms were taken for all the bilateral TMJ ankylosis cases in order to check the changes in SNB angle in the pre-distraction to the post-distraction after consolidation period. (Fig. 1),
 - o for unilateral TMJ ankylosis: grummons analysis in posterior-anterior cephalogram to check for facial asymmetry correction and length required to distract the mandible for asymmetry correction. (Fig. 2). A uniform standardized postero-anterior cephalograms were taken to rule out mandibular morphology correction according to Grummon's analysis in unilateral TMJ ankylosis in order to compare the deficit of the affected side of mandibular growth to the non-affected side. The amount of correction is calculated by measuring the area of triangle formed by three points in the postero-anterior view, such as condyilion, antegonial notch, and menton;
- evaluation of sleep apnoea:
 - o the patients were evaluated pre- and post-operatively with questionnaires. The questionnaire included queries related to presence of snoring, awakening episodes during sleep and day-time somnolence. The patients were also questioned regarding the activity during the day using Epworth Sleep Scale i.e.:
 - a) 0–10: normal range in healthy adults,
 - b) 11–14: mild sleepiness,
 - c) 15–17: moderate sleepiness,
 - d) 18 or higher: severe sleepiness;
 - o a comparison of pre- and post-operative evaluations of questionnaires were done and recorded. Multiple recordings were done for the patients and the mean was calculated for reliability. Lateral cephalograms were taken serially in order to observe airway changes from pre- and post-distraction changes after consolidation period i.e. after 3 months to 6 months;
- quality of life post-distraction osteogenesis and release of TMJ ankylosis:
 - o at the end of the treatment, patients were asked to complete the form on questionnaire, which we adapted from similar validated questionnaires that are routinely used by other surgical specialties [12]. It consists of 8 components that cover


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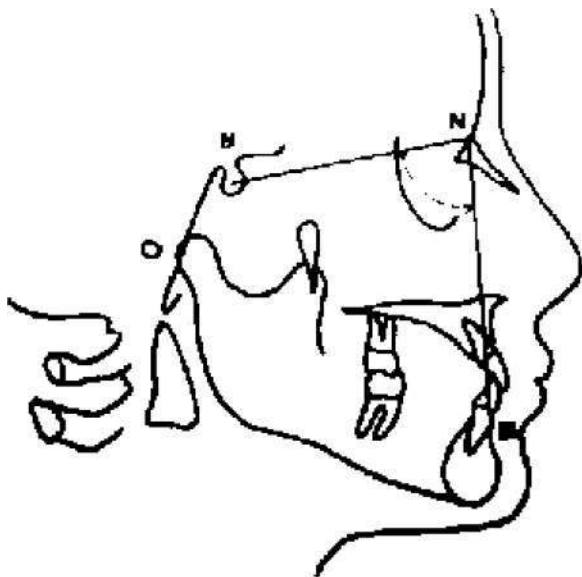


Fig. 1. Depicts the evaluation of SNB angle.

the physical, functional, and psycho-social aspects of patients' lives, and each component is graded from 1 to 5. A Score of 1 to 5 was rated according to the response of the patients while 1 represents the worse and 5 regarded as better quality of life (QOL).

All the patients underwent a thorough radiographic evaluation using lateral and frontal cephalograms. They were evaluated for oro-pharyngeal airway patency using McNammara's airway analysis and by Grummon's analysis for planning the quantum and vector of distraction. A monofocal external pin fixated distraction device was used for the procedure. The distraction

was performed with a latency period of 4 days and activation for the required quantum of movement and consolidation for 8 weeks. The post-operative assessment was carried out at the end of the consolidation period. All patients underwent the second stage of surgical release of ankylosis release followed by physiotherapy and rehabilitation 1 month after consolidation. All the patients were followed for a period of 5 years in order to check the recurrence or relapse of the treatment. (Figs. 3-11)

Management of ankylotic mass was done by using the modified Kaban's protocol as suggested which includes: aggressive resection (Gap Arthroplasty with resection of deformed condyle mass between the roof of glenoid fossa and ramus to create a gap of 1.5 cm to prevent re-ankylosis); Ipsilateral coronoidectomy on affected side; Contra lateral coronoidectomy when steps 1 and 2 do not result in maximum incisal opening greater than 35 mm; lining of the TMJ with temporalis muscle with or without fascia to maintain the vertical height of the resected ankylotic mass, and early mobilization of jaws and aggressive physiotherapy done after 7 to 10 days for 15 days

All the patients were followed for 15 days, 1 month, 3 months, 6 months, 1 year, and 5 years after ankylotic mass released in order to check the mouth opening and stability of the distracted mass.

The results of the clinical examination were recorded on a specific form. The data was subjected to statistical analysis using simple descriptive statistics, paired *t*-test, Wilcoxon's signed rank test, and SPSS version 20 (Chicago, Illinois, USA) software as per data requirements. The level of significance was concluded at $P < 0.05$.

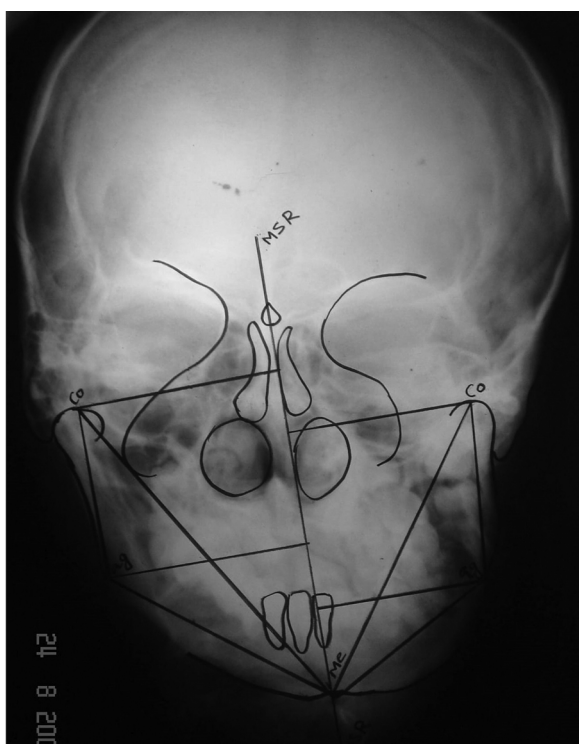


Fig. 2. Illustrates the measurements of Grummon's analysis.

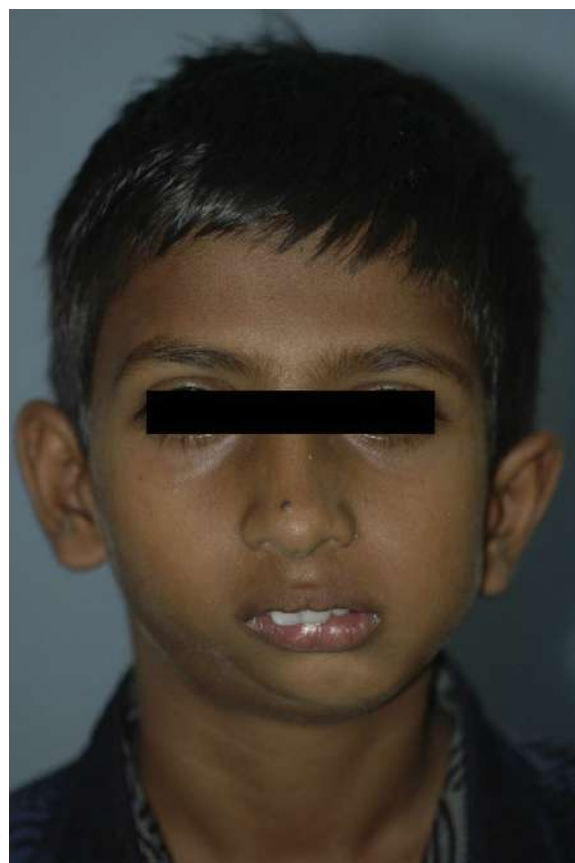


Fig. 3. Pre-operative frontal photograph demonstrating gross facial asymmetry associated with unilateral ankylosis.

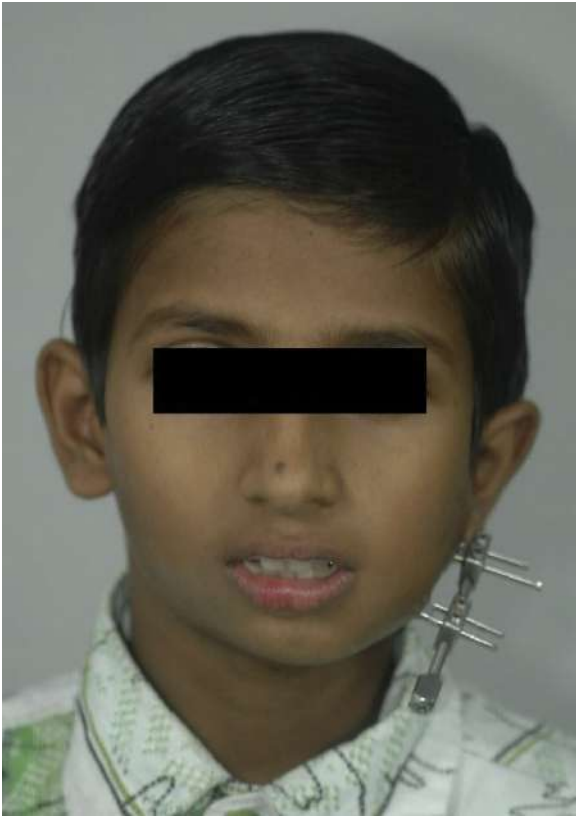


Fig. 4. Frontal photograph showing distractor in situ with unilateral ankylosis.



Fig. 6. Pre-operative frontal photograph demonstrating gross facial asymmetry associated with bilateral ankylosis.



Fig. 5. Post-operative frontal photograph after stage 2 demonstrating good facial symmetry and optimal mouth opening.



Fig. 7. Frontal photograph showing distractor in situ in bilateral ankylosis.


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Fig. 8. Post-operative frontal photograph after stage 2 demonstrating good facial symmetry.



Fig. 10. Pre-operative lateral cephalogram demonstrating constricted pharyngeal airway.



Fig. 9. Frontal photograph demonstrating optimal mouth opening.



Fig. 11. Post-operative lateral cephalogram demonstrating remarkable improvement in the pharyngeal airway.


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3. Results

Out of the ten patients, 9 patients were below 25 years, one patient was 39 years (a mean age of 25 years) and 7 patients were having unilateral ankylosis and 3 were having bilateral ankylosis. The amount of distraction was achieved in all the bilateral ankylosis patients were evaluated using lateral cephalogram pre- and post-distraction by noticing the changes in the SNB angle i.e. a pre-operative and post-operative mean of 61.66 ± 3.21 and 68.33 ± 3.51 ($P < 0.05$) which was found to be statistically significant. Furthermore, the values were recorded in millimeters pre- and post-operatively which were found to be statistically significant ($P < 0.05$). (Table 1) Similarly, for the remaining 7 cases of unilateral TMJ ankylosis patients the amount of distraction was calculated on postero-anterior radiograph by using Grummon's analysis pre- and post-operatively with a mean of 83.14 ± 5.98 and 91.15 ± 5.08 which was found to be statistically significant ($P < 0.05$). (Table 2)

For the sleep apnoea patients, all the patients showed cessation of snoring, decrease in their night time awakening episodes, and day-time somnolence which was calculated using Epworth Sleep Scale pre- and post-distraction osteogenesis with a mean of 15.3 ± 3.77 and 6.20 ± 1.39 which was to be statistically significant ($P < 0.05$). (Table 3)

All the post-operative lateral cephalograms showed a significant increase in the oro-pharyngeal airway as shown in Figs. 11.

With respect to quality of life, patients reported a significant improvement ($P < 0.05$), after operation. Overall, they thought that their QoL had improved from very poor before the operation to extremely good or good after-wards ($P < 0.001$; highly significant) (Table 4).

With regards to the mouth opening after release of ankylotic mass among 10 patients; 9 patients maintained the 35 mm of mouth opening which includes two bilateral and seven unilateral TMJ ankylosis. However, for 1 case of bilateral TMJ ankylosis, we could achieve only 25 mm of interincisal opening despite we followed the internationally accepted protocol of TMJ ankylosis as

suggested by Kaban et.al which was found to be statistically insignificant.

4. Discussion

The main aim of this study is to evaluate the effect monofocal extraoral distraction osteogenesis of mandible on improvement of facial esthetics by doing distraction osteogenesis, obstructive sleep apnoea, and quality of life following TMJ ankylosis.

The results of this study confirm the hypothesis that there is a significant rectification of facial esthetics, obstructive sleep apnoea, and quality of life in the patients with TMJ ankylosis after distraction of the mandible with extraoral monofocal distractor. In cases of bilateral TMJ ankylosis, the amount of distraction achieved post-operatively was 68.33 ± 3.51 ($P < 0.05$) and in cases of unilateral TMJ ankylosis it was 91.15 ± 5.08 ($P < 0.05$). In all the ten cases TMJ ankylosis with sleep apnoea, there was significant improvement in the airway and Epworth Sleep Scale ($P < 0.05$). Furthermore, there was significant improvement of quality of life among these patients' pre- and post-operatively.

Ankylosis of the mandible is a serious and most disabling condition. Impairment of speech, difficulty with mastication, poor oral hygiene, facial asymmetry, and mandibular micrognathia invariably result in much physical and psychologic disability, particularly in young children with complete inability to open the mouth [2].

The degree of deformity depends on the patient's age at the onset of the TMJ ankylosis and its subsequent duration. The morbid anatomy in such patients can be summarized as replacement of the involved joint by a mass of abnormal and irregular osseous tissue. Markedly enlarged and thickened coronoid processes [13].

The destruction of the growth centre and limited mobility of the mandible, gross changes in mandibular shape and size, as well as the surrounding functional matrix (reported by Kelvin Moss in 1969).

Table 1

For bilateral ankylosis: the changes in SNB angle calculated.

	Pre-distraction	Post-distraction after consolidation period and ankylosis release	Mandibular lengthening done	
			Right side	Left side
Case 1	58	65	10 mm	15 mm
Case 2	64	72	15 mm	12 mm
Case 3	63	68	10 mm	6 mm
Mean	61.66 ± 3.21	68.33 ± 3.51	11.66 ± 2.88	11 ± 4.58
P-value	0.017 ^a		-	

There is statistically significant difference present between the pre- and post-treatment SNB angle.

^a Statistically significant ($P < 0.05$) (paired *t*-test).



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Table 2

For unilateral ankylosis.

Diagnosis	Pre-distraction	Post-distraction after consolidation and ankylosis release	Mandibular lengthening done
Case -4, left side TMJ ankylosis	82%	95%	Left side-21mm
Case -5, right side TMJ ankylosis	88%	97%	Right side-14mm
Case-6, left side TMJ ankylosis	94%	95%	Left side-20mm
Case-7, right side TMJ ankylosis	80%	90%	Right side-10mm
Case-8, left side TMJ ankylosis	80%	90%	Left side-13mm
Case-9, left side TMJ ankylosis	82%	94%	Left side-18mm
case-10, right side TMJ ankylosis	76%	82%	Right side-16mm
Mean	83.14 ± 5.98	91.15 ± 5.08	-
P-value	0.001 ^a		-

There is statistically significant difference present between the percentage of correction in pre- and post-treatment.

^a Statistically significant ($P < 0.05$) (Paired *t*-test).

Table 3
For sleep apnoea patients Epworth Sleep Scale.

	Pre-distraction	Post-distraction after consolidation and ankylosis release
Case -1	20	08
Case -2	20	07
Case -3	21	09
Case -4	15	05
Case -5	15	06
Case -6	12	05
Case -7	14	06
Case -8	11	05
Case -9	11	05
Case-10	14	06
Mean	15.3 ± 3.77	6.20 ± 1.39
P-value	< 0.001 ^a	

There is statistically significant difference present between pre-distraction and post-distraction ESS Score. Within normal range after treatment.

^a Statistically highly significant ($P < 0.001$) (Paired t-test).

Mandibular hypoplasia which is seen in ankylosis can also be an isolated, non-syndromic finding or a morphologic component of some under lying craniofacial condition (e.g. Pierre Robin sequence, Treacher Collins syndrome, Nager syndrome, and so forth). Functional consequences of severe mandibular hypoplasia include acute and chronic upper airway obstruction, obstructive sleep apnea, speech difficulties, impaired feeding, and poor quality of life. Although surgery for early mandibular advancement is a viable option in the management of infants and children who have severe micrognathia, it is rarely the first line treatment modality. This is because most patients with congenital, non-syndromic micrognathia will undergo substantial catch-up growth early in the postnatal period. This obviates the need for any surgical intervention the total magnitude of that catch-up growth in the long term is often not enough to eliminate the need for comprehensive orthodontics and orthognathic surgery later in life to normalize their facial profile and occlusion.

Bone regeneration by distraction has become an accepted method of treatment of congenital and acquired mandibular hypoplasia. With this technique, significant skeletal and soft tissue enlargement in the hypoplastic area can be obtained in a short period without the need for bone grafting. Since the first clinical report in 1992 by McCarthy et al, several large series have been published. Distraction osteogenesis was defined by Ilizarov as a method of endogenous tissue generation in which new bone is mechanically induced within the gap between opposing bone surfaces that are gradually separated.

According to, Spagnoli DB et al, 2006 Spagnoli and Gollehon [14] and Sadakah et.al, 2006 [5] distraction osteogenesis was

Table 4
For quality of life.

	How TMJ problems have limited daily activities		How TMJ problems have limited social life		How often patients felt low or depressed because of TMJ problems		Quality of life	
	Before	After	Before	After	Before	After	Before	After
Mean	3.95	1.56	4.4	1.60	3.85	1.64	4.17	1.78
Z-Score	-3.60		-3.58		-3.58		-3.58	
Effect size	0.58		0.58		0.58		0.58	
P-value	0.000095		0.00012		0.000095		0.00095	

Limits to social life: 1: none, 2: slight, 3: moderate, 4: quite a lot, 5: extreme.
Effect of TMJ problems on mood: 1: never, 2: seldom, 3: quite often, 4: very often, 5: always.
Quality of life: 1: extremely good, 2: good, 3: neither poor nor good, 4: poor, 5: very poor.

preferred, as orthognathic surgery has relapse risk in severe mandibular deficiencies requiring lengthening of the mandible more than 8–10 mm after ankylosis release. Lengthening of the ramus was needed even after conventional orthognathic surgery procedures to over the defect of the pterygoid muscle which usually does not adapt to the elongation of the ramus. However, during distraction osteogenesis, active histiogenesis occurs in different tissues including gingiva, blood vessels, ligaments, cartilage, muscles and nerves. These adaptive changes in the soft tissues decrease the relapse risk and allow the treatment of severe facial deformities.

Today, the use of distraction osteogenesis is considered a useful treatment option for the correction of specific facial skeletal deformities. Growing numbers of clinicians have advocated the use of distraction of the jaws through published reports that largely consist of isolated case reports or small case series. Distraction of hypoplastic mandible in ankylosis can be done in two ways i.e. distraction prior to release and distraction after release of ankylosis. The advantage of the former out weights the disadvantage of the later. In cases of distraction prior to release the proximal segment is relatively stable and resistant to displacement due to ankylosis. Thus resulting and favoring a controlled and guided resultant vector to move the distracted mandible forward. The barrier that could forbid the forward movement of distracted mandible improper occlusal intercuspation of teeth can overcome by placing bite blocks.

Rubio Bueno et al, 2000 [15] have used internal and external monofocal distraction devices to correct mandibular hypognathia in hemi facial microsomia and they reported pain in the ipsilateral TMJ i.e. on the site of distraction during the activation period of distractor device; the authors attributed this to distraction forces pushing the condyle up into the glenoid fossa. However, in our study, we have not encountered any episode of pain in the TMJ during the distraction period. This perhaps attributed towards the advantage of using extraoral monofocal device for distraction.

Ayoub AF et al, 2002 Ayoub et al. [3] studied the positional and morphologic changes of the mandibular condyle after mandibular distraction osteogenesis in skeletal class II patients, and concluded that most of the condyles were displaced in an upward and backward direction in the glenoid fossa, and the amount of displacement is correlated with the amount of mandibular lengthening [5]. In the current study, release of the TMJ ankylosis was postponed to the 2nd stage of surgery after distraction so that the immobile joint represented a fixed point that would push the mandible forward, rather than backward. This helps to correct the asymmetrical mandible to restore the midline. Surgical treatment objective to bilateral and unilateral ankylosis cases for asymmetry correction resulted in satisfactory outcome to prediction. The overall relapse percentage is 3.5% in 6 month follow up which is compared to the immediate post-operative [16–22].

Numerous complications occurred as a result of distraction osteogenesis either through technical error or uncontrollable problems, even though the distraction is well executed. Generalized complications such as wound infections, hematomas, pin-site infections, and undesirable scarring through the insertion site are all potential problems. Complications related to the osteotomies are similar to those when the osteotomies are performed for non-distraction purposes. Damage to tooth roots occurred while osteotomizing which is unavoidable due to severe occlusal discrepancy. In children during early stages of dentition, tooth damage is a particular risk because of the presence of multiple unerupted tooth buds [16–18,23,24].

Technical problems that are faced which are related to the devices themselves include, loosening of the devices attachment to the bone or the attachment of the device to the pins or screws that will lead to early loss of distraction forces. All patients underwent surgical ankylosis release after successful correction of asymmetry.



The mean post-operative mouth opening achieved is 34 mm (pre-operatively 9 mm) and all the patients underwent post-operative physiotherapy. None of the patients had any sign of fracture or green stick- fracture at the distracted segment during the second stage of surgery or physiotherapy. All the patients are in follow up after second surgical protocol [25–30].

The primary problem of sleep disturbance and nocturnal desaturation during sleep (NDS) in our group of patients shifted the focus to the correction of the mandibular hypoplasia before the release of the joint. In this group of patients, mandibular distraction was performed prior to release of the joint and showed excellent results in the management of the sleep disorder. The patients were reviewed and recalled after 3 months and checked for consolidation of the distracted bone. The patients then underwent release of the ankylosis followed by rehabilitation [31–35].

Patients have found it easier to talk, yawn, and eat after joints have been released and distracted, and they can start enjoying a normal diet. This correlated with our findings that all patients could eat a normal diet after operation whereas 10 had had to take a liquid diet before it. Most had less pain and better mouth opening. The disease no longer limited normal daily activities and they were able to enjoy their social lives, which improved their mood and gave them a better quality of life. Sanovich et al, 2014 [24] also reported a moderate and significant improvement in the quality of life of patients who had had total replacements [10].

5. Conclusion

This technique of extra oral monofocal primary mandibular distraction followed by ankylosis release has shown good and predictable results in the management of the facial esthetics, sleep apnoea, and quality of life without compromising the outcome of the distraction process or the ankylosis release.

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Disclosure of interest

The authors declare that they have no competing interest.

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Review Article

Recent Advancements in Immediate Loading and Single Piece Implants in Full Mouth Rehabilitation

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ABSTRACT:

Dental implants are one of the most commonly procedures employed these days for prosthetic rehabilitation of missing teeth. Most of the implant surface modifications showed good osseointegration results. Regarding biomolecular coatings, which have been recently developed and studied, good results were observed in animal experiments. Immediate loading had similar clinical outcomes compared to conventional loading and can be used as a successful treatment because it has the advantage of reducing treatment times and providing early function and aesthetics. In the present review, we have highlighted some of the important aspects of the recent advancements in immediate loading and single piece dental implants in full mouth rehabilitation.

Key words: Immediate, Implants, Recent, Rehabilitation

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INTRODUCTION

Originally, dental implants were considered as “last resort” for treatment of the edentulous patients. As implant dentistry progressed, the original Brånemark protocol required long healing periods of several months for osseointegration to take place before beginning fabrication of the definitive prosthesis. Dentists consequently became profoundly aware of time-dependent relationship between form and functional changes in the masticatory system. Such knowledge helped nurture the development of new materials and knowledge about the relationships between esthetics, occlusion, and patient's personalities.¹ Although a favorable treatment outcome often was achieved, few patients were not able to tolerate removable complete dentures. This failure is neither an indictment of one's professional skills nor necessarily a condemnation of the patient's response to the clinician's efforts. There is a growing need of patients to be rehabilitated with a fixed, implant-supported prosthesis immediately after surgery, not only to minimize patient discomfort but also to restore functionality and esthetics quickly so that patients

can return to their normal routine within a short period of time.²

Immediate loading dental implants

There are three basic approaches to replace a missing tooth or teeth including removable dental prosthesis, fixed dental prosthesis, and dental implants. Each alternative has its own benefits and shortcomings. It is important to consider the patient's financial, medical, and emotional condition for the best treatment.³

Most advanced way to replace missing teeth is dental implant which is designed to replicate the natural tooth root and crown of the natural tooth. This procedure preserves the gingival mucosa and bone with no damage to adjacent teeth. Conventional procedure for implant placement involves extraction of offending tooth, waiting 2–4 months for extraction socket to heal, insertion of implant, and again waiting for 3–6 months for integration of implant with surrounding bone; after this procedure, another surgery is necessary to expose the implant and to place a prosthetic abutment. Taking into consideration the prosthetic treatment, the patient had to wait up to 8–12 months for a lost tooth to be replaced. Because of these

shortcomings related to conventional technique, strategies were developed to substantially shorten the entire treatment by placement of implant immediately after extraction of tooth followed by immediate loading of implant with prosthesis.⁴

Immediate loading versus conventional (delayed) loading

According to many previous studies, many researchers believed that after implantation in the jaw for a future prosthesis, titanium implants should be left submerged to undergo a healing process before they are capable of functional loading. This healing process, which is called osseointegration, could be completely achieved in a period from 3 to 6 months. The reason for the delayed loading was to avoid micro-movement on the implant, which could interfere with the healing process. If this situation occurs, connective tissue can develop at the interface between the implant surface and the bone. The result would be failure of the implant due to not being able to resist the masticatory forces.³

Following the progressive development of technologies and the wide spread of implantation in dentistry, more recent research has focused on the mechanism of bone healing. It has provided a better understanding of osseointegration. It was suggested that it would be possible to reduce the period between implantation and the placement of a prosthesis.⁴

RECENT ADVANCEMENTS

Implant surface

Modification of the implant surface has been studied and applied to improve biological surface properties favoring osseointegration. The surface roughness of implants has been increased by various methods such as machining, plasma spray coating, grit blasting, acid etching, sandblasted and acid etching (SLA), anodizing, and biomimetic coating. The key factor in implant osseointegration is surface roughness, which shows increased osteoblast activity at 1 to 100 μm of the surface roughness compared to a smooth surface. It is believed that rough surfaces have better osseointegration than smooth surfaces, but the results of the research have been diverse and it is not clear that multiple treatments provide better predictive results.⁵

The machined implant surface is the first-generation implant surface design with a turned surface implant. Plasma spray coating generally forms a thick layer of deposition such as hydroxyapatite (HA) and titanium by spraying a material dissolved in heat on the surface of the implant. Grit-blasting is a process of spraying particles onto the surface of the implant using ceramic material or silica. Sand, HA, alumina or titanium dioxide (TiO₂) particles are used and acid etching is performed to remove the remaining blasting particles. Acid-etching is the roughening of the titanium implant surfaces using strong acids such as hydrofluoric acid (HF), nitric acid (HNO₃), and sulfuric acid (H₂SO₄) or combinations of these acids. SLA is acid etching after sandblasting with 250–500 μm large grit particles. Anodizing is the dielectric breakdown of the TiO₂ layer by applying an

increased voltage to generate a micro-arc. This process forms a porous layer on the titanium surface.⁶

Sinus lifting

In the immediate time period after maxillary posterior tooth extraction, initial decrease in alveolar width is by resorption and/or loss of buccal bone. With continuous bone remodeling, absence of stimulation, loss of bone height, and density leads to an increase in antral pneumatization. The maxillary sinus pneumatization is caused by progressive hollowing out of alveolar process of apical aspect mediated by osteoclasts and by increase in positive intra-antral pressure. In such a situation, the residual vertical bone height is decreased making standard implant placement difficult.⁵

To adapt, circumvent, and treat this local physiological as well as anatomical limitation; maxillary sinus floor elevation has become an important preplacement procedure in dental implant treatment planning. Various methodologies have evolved to increase the thickness of maxillary sinus floor. The treatment goal of all such procedures is to increase residual bone height. Few of the technique involve simple, minimal elevation of maxillary sinus membrane, Schneiderian membrane, while other include placement of various type of grafts including allografts, autografts, bone morphogenetic proteins, and hydroxyapatite crystals. The factors that contribute to survival rate of sinus augmentation and dental implant placement are still the subject of discussion.⁷

Short implant

In an atrophic alveolar ridge, there are many anatomical limitations (maxillary sinus, nasal floor, nasopalatine canal, inferior alveolar canal) that make placement of a standard implant difficult. To overcome these limitations and vertical bone deficits, additional surgical procedures, such as guided bone regeneration, block bone grafting, maxillary sinus lift, distraction osteogenesis, and nerve repositioning, are performed to place a standard implant. However, the procedure is sensitive, challenging, costly, and time-consuming and increases surgical morbidity and causes many complications such as sinusitis, infection, hemorrhage, nerve injury, and gait disturbance.⁸

Short implants are considered to be simpler and more effective by reducing the likelihood of such complications, patient discomfort, procedure costs, and procedure times in rehabilitation of the atrophic alveolar ridge. The term of a short dental implant is subjective, and there is no clear criteria for the length of a short dental implant. Some articles defined 10 mm or less as the criterion of a short dental implant, and some defined less than 10 mm as a short dental implant. Some defined the short implant as 8 mm or less. Implant companies have recently offered short implants of less than 8 mm. In this paper, a short dental implant was defined as less than 8 mm, which is similar to other papers.⁹

Alternative techniques

Despite the reliability and efficiency of various sinus augmentation techniques, there is still a high rate of complications and complexity for such procedures. With



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the advances in technology and improvements in design and manufacture of implants, some alternative concepts suggested implantation without sinus augmentation could be possible.¹⁰

The use of a tilted (angulated) implant in the posterior maxilla was suggested to avoid sinus augmentation. In this study, an evaluation was made to compare the efficiency between tilted and axial implants with no sinus grafting. After 5 years of follow-up, the implant success rate was 95.2% (survival: rate 100%) for the tilted implants and 91.3% (survival rate 96.5%) for the axial implants. The average marginal bone loss was 1.21 mm for the tilted implants and 0.92 mm for the axial ones.¹¹

Zygomatic implants offer another option treatment modality to sinus augmentation. Almost similar to trans-sinus tilted implants, zygomatic implants are long implants that pass through the sinus or laterally to the sinus. The difference was the anchorage position. While the tip of a trans-sinus tilted implant is positioned in the bone between the anterior sinus wall and the nasal cortical bone, a zygomatic implant will anchor itself into the zygomatic process for stability.¹²

Advanced dental implant placement techniques

The virtual planning for the precise placement of dental implants using CT scanning, rapid printing and prototyping, optical scanning, and CAD CAM milling can now be utilized in a unified manner. As a result of this progress from the digital technology, surgeons have improved diagnosis, with more accurate implant placement, and superior long term results. CT guided dental implant surgery allows decreased operating time, flapless procedures, and decreased postoperative pain and swelling, and immediate temporization. The development of CT scanning from fan beam to spiral methods has resulted in the development of in office Cone Beam CT (CBCT) scanners with decreased radiation dosage which are now widely available as standup, lie down, sitting and mobile CBCT units. CBCT has become an important in office or scanning center based dental imaging technology, providing powerful diagnostic capabilities and practical applications. Software planning for dental implant placement allows preoperative diagnosis, precise planning and trajectories, and the fabrication of rapid printed surgical drill guides. New technologies of CAD CAM milling, optical scanning, and modular implant fabrication will allow further advances in this rapidly developing aspect of dental implant treatment. In addition to the decision of choosing the optimal 3D data between the pre-extraction or post-extraction tooth for fabrication of a customized implant, the intactness of the tooth must also be taken into account, particularly in the root area.¹³

¹⁴ Teeth that need to be replaced by implants are commonly damaged or even already extracted; thus, it is suggested that recreating a 3D model based on the contralateral tooth could be a suitable option. Additionally, the concept of using 3D data of the tooth without extraction could achieve better accuracy because there was no damage to the tooth by the elevator or dental forceps. With the ongoing development of new technology in 3D

and CAD/CAM, it is predicted that customized implants could be the promising future of implant dentistry as an alternative to conventional implant designs. However, more clinical trials are needed to evaluate the effectiveness of this approach.¹⁵⁻¹⁸

Nanotechnology-based implants

Nanotechnology approaches require novel ways of manipulating matter in the atomic scale. Currently, extensive research on techniques to produce nanotechnology-based implants are being investigated. Nanotechnology-based trends for dental implants consist on surface roughness modification at the nanoscale level to promote protein adsorption and cell adhesion, biomimetic calcium phosphate coatings, and the incorporation of growth factors for accelerating the bone healing process.¹⁹⁻²²

Most attempts to get nanoroughness have used processing methods like lithography and surface laser-pitting, but only a few studies have reported modifications to the roughness as well as the chemistry at the nanometer scale in a reproducible manner. Other technique is the deposition of nanoparticles like biomimetic calcium phosphate, alumina, titania, zirconia, and other materials to coat Ti surfaces. The surface of Ti dental implants can also be coated with bone-stimulating agents such as growth factors (transforming growth factor- β , bone morphogenetic proteins [BMPs], platelet-derived growth factors and insulin-like growth factor [IGF]-1 and 2) and antiresorptive drugs (bisphosphonates) in order to enhance the bone healing process locally. In one study, a Ti machine smooth implant was compared to a Type-1 collagen coated Ti implant and a Type-1 collagen-BMP-2 coated implant. The results of this animal study showed greatest peri-implant bone formation within the grooves of the endosseous screw for the collagen-BMP implant when compared to the collagen-coated implant. In this example, both collagen and BMP-2 serve as bioactive molecules. In addition to adding biomolecules which promote bone growth, molecules such as bisphosphonates which prevent bone resorption may also be added.²³⁻²⁶

CONCLUSION

Implant dentistry has evolved into the mainstream of restorative practices all over the world. Maintenance of bone after tooth loss to improve or maintain facial esthetics and improved retention, function, and performance of removable restorations are only some of the advantages for the edentulous patient. Long-term clinical trials and more predictive studies are required for better exploration of this field of dentistry.

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Original Research Article

Effect of acidic pH on microhardness and microstructure of TheraCal LC, Endosequence, mineral trioxide aggregate, and Biodentine when used as root repair material

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Abstract

Introduction: The aim of this study was to investigate the microhardness and microstructural features of newer tricalcium silicate materials: TheraCal LC, mineral trioxide aggregate (MTA), Biodentine (BD), and Endosequence Root Repair Material (ERRM) putty, after exposure to acidic environments in comparison with distilled water.

Materials and Methods: A total of 80 extracted single-rooted premolars were collected. All the selected specimens were sectioned vertically, and cavities were prepared on the root surface. Specimens were divided into four groups of 20 each, i.e., Group 1: ($n = 15$) MTA (ProRoot, Dentsply Tulsa Dental, Tulsa, OK, USA), Group 2: ($n = 15$) BD (Septodont, France), Group 3: ($n = 15$) ERRM putty (Brasseler, USA), and Group 4: ($n = 15$) TheraCal LC (Bisco Inc Schaumburg). Materials were placed into prepared cavities. About 10 specimens per each group were exposed to butyric acid buffered at a pH level of 5.5 for 7 days at 37°C, and 10 specimens from each group were exposed to distilled water serving as a control group. The surface microhardness was measured after exposure to either acid or distilled water. Scanning electron microscope was used to observe the internal microstructure morphology. Two-way analysis of variance was applied to evaluate the Knoop microhardness value (KHN).

Results: Results showed that the microhardness values of the materials were significantly higher in the neutral environment of butyric acid at pH 7.4 when compared to those in the acidic condition of pH 5.4 for all groups ($P < 0.001$). TheraCal LC had higher microhardness values than BD, MTA, ERRM putty at 5.5 pH levels ($P < 0.001$).

Conclusion: The microhardness values of TheraCal LC, BD, ERRM Putty, and MTA were reduced in an acidic environment, which resulted in these materials having more porous and less crystalline microstructures. TheraCal LC seems the most suitable material for application to an area of inflammation where a low pH value may exist.

Keywords: Biodentine; butyric acid; endosequence root repair putty; microhardness; microstructure; mineral trioxide aggregate; TheraCal LC

INTRODUCTION

The outcomes of endodontic procedures are influenced by the chemical and physical properties of the materials used.

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An ideal root repair material should be biocompatible, nonresorbable, radiopaque, dimensionally stable, and insoluble in tissue fluids and should have sufficient sealing property. Over the years, various root repair materials, such as amalgam, Super-EBA, Intermediate Restorative Material, glass ionomer cement, and calcium phosphate cement, were used.^[1]

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Mineral trioxide aggregate (MTA), a calcium silicate-based hydraulic cement, has shown promising results over the past years.^[2] Despite the various beneficial properties of MTA such as biocompatibility, sealing ability, and antibacterial effects, it has a few disadvantages such as difficulty in handling and long setting time.^[3]

To overcome the disadvantages of MTA, a calcium silicate-based material, biodentine (BD) (Septodont, Saint Maur Des FOSSES, France) was introduced.^[4] BD consists of calcium carbonate, zirconium oxide, tricalcium silicate, and a water-based liquid-containing calcium chloride which is used as setting accelerator and water-reducing agent. BD with increased physical properties and reduced setting time claimed to be used as a dentin restorative material as well as root repair material.

It has been reported that the hardness, pushout bond strength to dentin, and sealing ability of MTA and BD were decreased after placing in an acidic environment.^[5,6]

Hence, to overcome the above drawbacks, a new tricalcium silicate material Endosequence Root Repair material putty (ERRM; Brasseler, Savannah, GA) has been introduced.^[7] According to the manufacturer, it is composed of calcium silicates, monobasic calcium phosphate, zirconium oxide, tantalum oxide, proprietary fillers, and thickening agents.^[8] The material is biocompatible, hydrophilic, insoluble, radiopaque, and aluminum free. It is having high pH and working time of nearly 30 min. ERRM is available in a moldable putty form to facilitate placement in clinical situations with physical properties comparable to MTA and BD.

TheraCal LC (Bisco Inc, Schaumburg, IL, USA) is a new light-cured resin-modified calcium silicate-filled material designed for direct and indirect pulp capping. It consists of approximately 45% wt mineral material (type III Portland cement), 10% wt radiopaque component, 5% wt hydrophilic thickening agent (fumed silica), and approximately 45% resin. It also shows physiochemical bonding to dentin, good sealing abilities, and it is well tolerated by immortalized odontoblast cells.^[9]

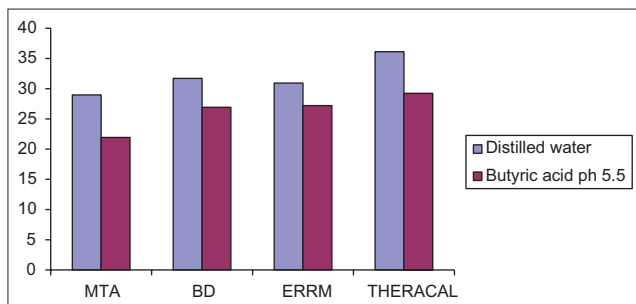


Figure 1: Bar diagram showing mean surface microhardness (KHN) of different root repair and butyric acid of pH 5.4

An acidic pH value, mostly as a result of bacterial-induced local metabolic acidosis or tissue inflammation, might probably affect the physical and chemical properties of calcium silicate-based material.^[10] As of now, no studies have been conducted regarding the effect of acidic pH comparing TheraCal LC, endosequence putty root repair with MTA and BD.

Hence, the aim of the present study is to evaluate the microhardness and microstructural changes of ERRM putty, TheraCal LC, MTA, and BD after exposure to the acidic environment.

MATERIALS AND METHODS

A total of 80 extracted human mandibular premolar teeth were selected. Selection criteria included the presence of a single root canal, mature apex, the absence of root filling, and without fractures or caries. After extraction, the teeth were kept in 5% sodium hypochlorite for 30 min to eliminate residual soft tissues. The collected samples were then vertically sectioned buccolingually using low-speed diamond disc under water coolant. On the cut section of

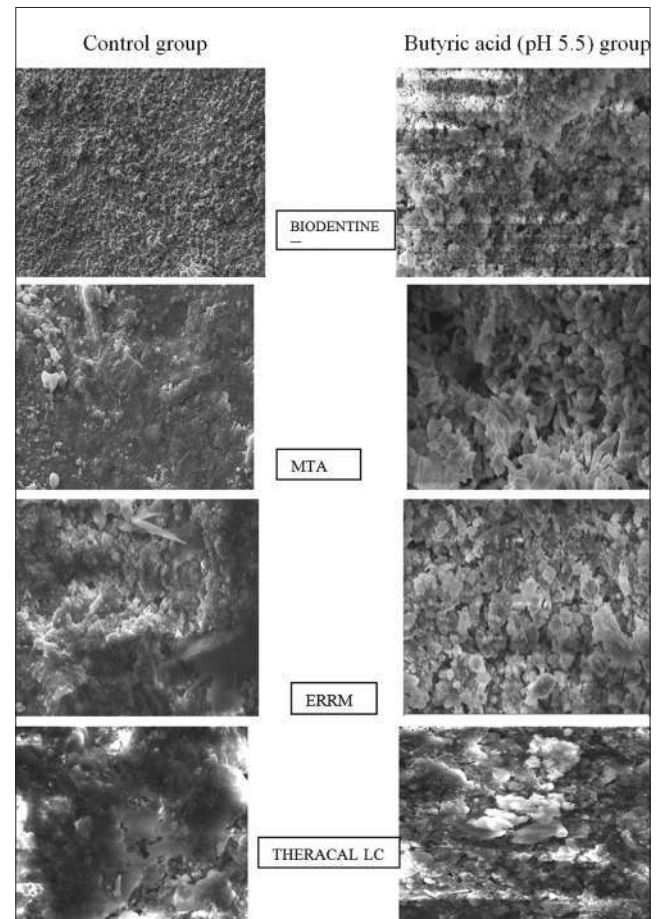


Figure 2: Scanning electron microscope images showing structural topography of all the 4 root repair materials exposed to butyric acid at pH 5.5 (left) and control group (right)

each specimen, a size of 2 mm × 2 mm dimension cavity marked at the junction of a cervical and middle third of the root surface to standardize the size of perforation. The cavities were then prepared using high-speed super-torque handpiece from the inner root surface of the vertically sectioned specimen to outer surface. The significance of vertical sectioning of the tooth structure is to simulate the clinical situation of root perforation.

All the collected samples ($n = 80$) were randomly divided into four groups such that 20 specimens per each group ($n = 20$).

- Group I ($n = 20$): One gram of Proroot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) was mixed with 0.35 ml sterile distilled water and placed into the prepared cavities manually using similar sized hand condenser
- Group II ($n = 20$): BD (Septodont, Saint Maur des Fosses, France) which was available in capsule and liquid form was triturated using amalgamator and packed into prepared cavities of the samples
- Group III ($n = 20$): Endosequece root repair putty (ERRM (Brasseler, Savannah, GA) which was available in premixed paste form, placed directly into prepared cavities using sterile cement carrier under manual pressure
- Group IV ($n = 20$): TheraCal LC (BiscoInc, Schaumburg, IL, USA) which was dispensed into the prepared cavities from the syringe and light cured with a light-emitting diode light-curing unit for 20 s per increment. All the samples were allowed to set at 37c in 100% relative humidity for 7 days.

Each group of 20 specimens was divided into two subgroups according to experimental liquids: (a) exposure to distilled water and (b) exposure to buffered butyric acid (pH = 5.5). Each subgroup was submerged in a petri dish, with its experimental solution, i.e., distilled water and butyric acid (pH = 5.5). Soaked gauze was placed on the bottom surface of samples. The top surface of the samples was covered with moist gauze and placed in an incubator at 37c in 100% relative humidity for about 7 days. The solution-soaked gauze pieces were replenished daily to ensure a constant pH.

Microhardness measurement

After 7 days, all the specimens were removed from the different solutions. They were washed and gently dried with air spray. The Knoop microhardness test was performed using microhardness tester (FM-100, Futuretech, Kawasaki, Kanagawa Prefecture, Japan) with the diamond-tipped tool. A full load of 100 g was applied for 10 s at room temperature. For microhardness, two indentations were made per specimen using Knoop diamond indenter. Knoop hardness (HK) was calculated according to an equation where C_p is the correction factor related to the shape of the indenter (0.070279), P is the test load (kgf), and L is the length of the longer diagonal (μm).

$$HK = \frac{\text{load (Kgf)} P}{\text{impression } C_p / \text{area (mm)} L^2}$$

The data were analyzed using two-way analysis of variance (ANOVA) followed by the *post hoc* test. The significance level was set at $P < 0.05$.

Surface analysis with scanning electron microscope

Each specimen from all subgroups was selected for microstructural surface morphology analyses, under scanning electron microscope (SEM). Magnification of ×10,000 was used to evaluate the different topographic microstructure of different set cements.

The rationale of the present study is to evaluate the microhardness and microstructural changes of ERRM putty, TheraCal LC, MTA, and BD after exposure to the acidic environment.

RESULTS

The mean surface hardness ± standard deviation of MTA, BD, Endosequence Root Repair Material putty (ERRM), and TheraCal LC were summarized in Table 1. The data were statistically analyzed using two-way ANOVA. For multiple comparisons, *post hoc* Tukey test was performed. Results showed that all the specimens exposed to butyric acid (pH = 5.5) had significantly lower microhardness values than those exposed to distilled water ($P < 0.05$). TheraCal LC and Endosequence putty showed significantly higher microhardness than MTA and BD exposed to the acid solution [Figure 1].

Scanning electron microscope analysis

The internal microstructure of the four materials exposed to butyric acid or distilled water at various pH levels was observed by SEM. The specimens exposed to pH 5.5 displayed more pores than those stored in distilled water for MTA and ERRM Putty, whereas the BD and TheraCal LC showed less microchannels than MTA and ERRM putty under same magnification [Figure 2].

Table 1: Mean ± standard deviations of surface microhardness (Knoop microhardness) of different root repair materials after 7-day setting in distilled water and butyric acid at pH 5.5

Groups ($n=20$)	Mean ± SD	
	Subgroup A	Subgroup B
	Distilled water ($n=10$)	BA pH 5.5 ($n=10$)
Group I MTA	28.97 ± 1.04	21.93 ± 1.01
Group II BD	31.71 ± 0.87	26.93 ± 0.88
Group III ERRM putty	30.93 ± 0.93	27.21 ± 0.59
Group IV TheraCal LC	36.12 ± 1.21	29.24 ± 0.68

MTA: Mineral trioxide aggregate, BA: Butyric acid, BD: Biodentine, SD: Standard deviation, ERRM: Endosequence root repair material

DISCUSSION

Despite of advances in endodontic therapy, root perforation complicates the treatment and deprives the prognosis if not properly managed. Irrespective of location or etiology, root perforation, which is a communication between root canal system and external tooth surface has to be sealed with a material having good physical properties and biocompatibility. In the present study, the effect of acidic environment on the microhardness and microstructural changes of tricalcium silicate materials such as MTA, BD, Endosequence Putty, and TheraCal LC was evaluated.

Microhardness test is not only a measure of strength or resistance to deformation but also it is influenced by the crystal structural stability and has an inverse relationship with porosity. In the present study, HK test was used to evaluate the microhardness as it is insensitive to bulk properties of the cement.^[11]

In the current study, the properties of MTA, BD, Endosequence putty, and TheraCal LC were evaluated after exposure to butyric acid at a pH level of 5.5. This study attempted to simulate the actual clinical environment during inflammation using butyric acid at pH 5.5, a byproduct of anaerobic bacteria metabolism.^[12]

When considering the clinical situation, the acidotic metabolism of butyric acid may suppress the bone mineralization activity of alkaline phosphatase. Lee *et al.*^[13] stated that pulpal and periapical inflammation typically lowers the tissue pH near the involved tooth to around 5.5.

The present study showed that exposure of the cements to an acidic environment significantly lowers the microhardness value than the control group. These results are consistent with the studies conducted by Bolhari *et al.*^[14,15] and Wang *et al.*^[10] A low pH could potentially inhibit the setting reaction, affect adhesion, or increase the solubility of calcium silicate-based materials, which could affect the mechanical properties of the material including the surface microhardness. TheraCal LC is an example of newly developed, ready-to-use tricalcium silicate materials, and due to its adherence property to a moist substrate, it can be used as root repair material. In the present study, it has shown significantly higher microhardness than MTA, BD, and Endosequence putty in acidic environment.

A substantial change in the microstructure of BD, Endosequence, TheraCal LC, and MTA occurred after exposure to butyric acid at pH of 5.5 compared to distilled water. A needle-shaped structure and cubic crystals-shaped structures of BD were observed. On the other hand, the microstructures of WMTA appeared more eroded with laminated cross-stratified structures and pore formation

after exposure to acidic pH. These results were in accordance with the studies conducted by Elnaghy *et al.*^[4] and Namazikhah *et al.*,^[12] who stated that WMTA might be more sensitive to acidic pH environment. Namazikhah *et al.* reported that the higher porosity of MTA revealed that the environment associated with bacterial colonization that could facilitate the passage of microorganisms or their metabolic products into the periapical tissues.

SEM finding of ERRM putty shows fewer ettringite crystals and high level of porosity due to the low pH environment that inhibited crystallization in hydration reaction of this material which was in agreement with results of Wang *et al.*^[10,15] ERRM materials contain calcium phosphate and tantalum oxide (a radiopacifier) but lacked aluminum. The absence of aluminate phase may result in fewer formed ettringite crystals which interlock cubic crystals. Another possible explanation could be the setting time accelerator in ERRM interfere with the hydration reaction of the cements especially at low pH values when the crystalline structures of the hydrated cement appeared less cohesive.^[16]

TheraCal LC is a resin-modified material composed of tricalcium silicate and zirconium oxide. In contrast to other materials, it is light-cured and the initial strength of material is gained immediately because of which it showed more resistance to acidic environment by forming more stable crystals and less microporosities and microhardness value. Camilleri *et al.*^[17] stated that there is no intermediate calcium hydroxide was formed as a by-product of hydration as it does not include water for material hydration. In the present study, TheraCal LC is one of the best used under inflammatory conditions, as it is a light curable material the site of perforation repair and depth of cure has to be considered. Therefore, further *in vivo* and *in vitro* research should be done to assess TheraCal LC as root repair material.

CONCLUSION

Within the limitations of this *in vitro* study, microhardness and surface microstructure of MTA and Endosequence root repair putty were highly altered in acidic environment than TheraCal LC and BD. Microhardness of TheraCal LC and BD were statistically higher than MTA and Endosequence putty in acidic solution. Neutral and alkaline solutions may enhance biologic properties of the biomaterials while acidic solution negatively influenced them.

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Conflicts of interest

There are no conflicts of interest.

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Case Report

Unusual root canal morphology of mandibular first premolar and its management: A rare case report

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Abstract

Clinicians proceed the canal exploration according to the literature so far reported. At times, there are apparent variations in the teeth. These discrepancies should be thoroughly surveyed and piled up to date. One such rare complexity of canal morphology was discussed in the present case. A 48-year-old female patient referred to the endodontic clinic for full-mouth rehabilitation. On intraoral hard-tissue examination, decay was noted in multiple teeth which were confirmed by an orthopantomogram and endodontic treatment plan was suggested. The patient was prepared for the access opening of right mandibular first premolar (tooth #44) before which endodontic treatment of first and second quadrants was completed. The cone-beam computed tomography (CBCT) images revealed that tooth # 44 had two separate roots and four distinct root canals (mesiobuccal, mesiolingual, distobuccal, and distolingual). Root canal treatment was completed in two visits. Postoperative CBCT was advised to confirm the obturation of all the canals.

Keywords: Cone-beam computed tomography; root canal anatomy; variations; Vertucci

INTRODUCTION

Knowledge of root canal anatomy and morphology is imperative to locate, clean, and shape root canal systems and seal them. While any single factor cannot be related to clinically better outcomes, all these factors seem to work in close relation to each other.^[1]

The complexities of the root canal systems of human teeth have been published by several authors.^[2,3] Although some variations have been reported to be very rare, an astute clinician must be able to recognize such a variation when encountered clinically. One tooth which is often considered simple, while in reality houses a very complex anatomy is the mandibular premolar. High flare-up and failure rates have been reported with root canal treatment in these

group of teeth.^[4] One possible explanation could be the extreme variations in root canal morphology in these teeth.^[5]

To the best knowledge of the authors, the occurrence of two canals with four orifices and four apical foramina in a mandibular first premolar has not been reported thus far. This case report presents the nonsurgical endodontic management of a mandibular right first premolar with two roots and four distinct root canals which were diagnosed using cone-beam computed tomography (CBCT).

CASE REPORT

A 48-year-old female patient was referred to the department for full-mouth rehabilitation due to multiple carious lesions and spontaneous pain in the right lower back teeth region. Clinical and radiographic examination revealed dental caries involving the pulp in teeth #11, #12, #13, 14, #21, #22, #23, #24, #44, #45 [Figure 1a]. Teeth #11, #44, and #45 were tender on percussion. Pulp

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sensibility test with an electric pulp tester (API Pulp Tester, India) showed negative response in teeth #11, #12, #13, #14, #21, #22, #23, and an exaggerated response with lingering pain in #44 and #45. Based on the clinical and radiographic findings, teeth #44 and #45 were diagnosed as symptomatic irreversible pulpitis with symptomatic apical periodontitis while #12, #13, #14, #21, #22, and #23 were diagnosed as pulp necrosis and #11 as pulp necrosis with symptomatic apical periodontitis. Root canal treatment was advised for the aforementioned teeth.

Following local anesthesia (Lignox), under rubber dam isolation (Coltene, Whalident), access cavity was prepared in #44 and #45. During access cavity preparation in #44, vague outlines of three canals were seen (mesiobuccal, mesiolingual, and distal). The trunk of the pulp chamber seemed to trifurcate giving rise to three separate canals. Working length (WL) was determined using an electronic apical locator (Propex pixi, Dentsply) and an intraoral periapical radiograph was also taken for confirmation of the presence of three canals [Figure 1b]. The endometric radiograph revealed an extra canal that was distally placed [Figure 1c]. However, the canal could not be confirmed clinically and hence not explored. The mesiobuccal, mesiolingual, and distal canals were first negotiated with a size 10 K file (Dentsply) and prepared up to an apical size of 25 in a step-back approach. During root canal preparation, 3% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid (EDTA) (SmearClear, Kerr) was used as the irrigant. The access cavity was filled with intracanal medicament and temporarily restored with Cavit (3M, ESPE).

To confirm the diagnosis of the presence of a fourth canal and identify its location, a CBCT scan was advocated following an informed consent. A three-dimensional image of the mandibular first premolar was obtained at a setting of 70 KV of tube voltage and a tube current of 10 mA for 10.8 s. The CBCT images revealed that tooth #44 had two separate roots and four distinct root canals (mesiobuccal, mesiolingual, distobuccal, and distolingual) [Figure 1g and h].

At the second visit using the CBCT images as the guidance, the fourth (distobuccal) canal was located under $\times 3.5$ following refinement of the access cavity using tapered, diamond-coated ultrasonic tips (Tün Endo Ultrasonics, USA). WL was established [Figure 1d]. The fourth canal was instrumented with size 06, 08, and 10 K-Flexo hand files. Cleaning and shaping was completed in all canals using Hero Shaper (Micro Mega) rotary instruments, up to an apical size 30/0.04. Canals were filled with 5.25% NaOCl and sonic activation for 30 s at 10,000 rpm (EndoActivator, Dentsply). Obturation was done using backfill technique (Obtura II) and restored with composite [Figure 1e]. Postoperative CBCT was taken to confirm the quality of treatment procedure [Figure 1i and j]. Under

asymptomatic conditions, porcelain fused to metal crown was placed after 15days [Figure 1f].

DISCUSSION

The endodontic literature so far revealed a high percent of cases with more than one canal in mandibular premolars [Table 1].^[6-9] In a review of Blaine *et al.* (2013) which included eight studies found the majority of the mandibular first premolar had a single root in (97.9%). Two roots were found in 1.8% of the teeth studied. Three-rooted (0.2%) and four-rooted (0.1%) varieties were quite rare.^[6] Therefore, it infers a racial predisposition for the presence of two or more canals in mandibular first premolar.

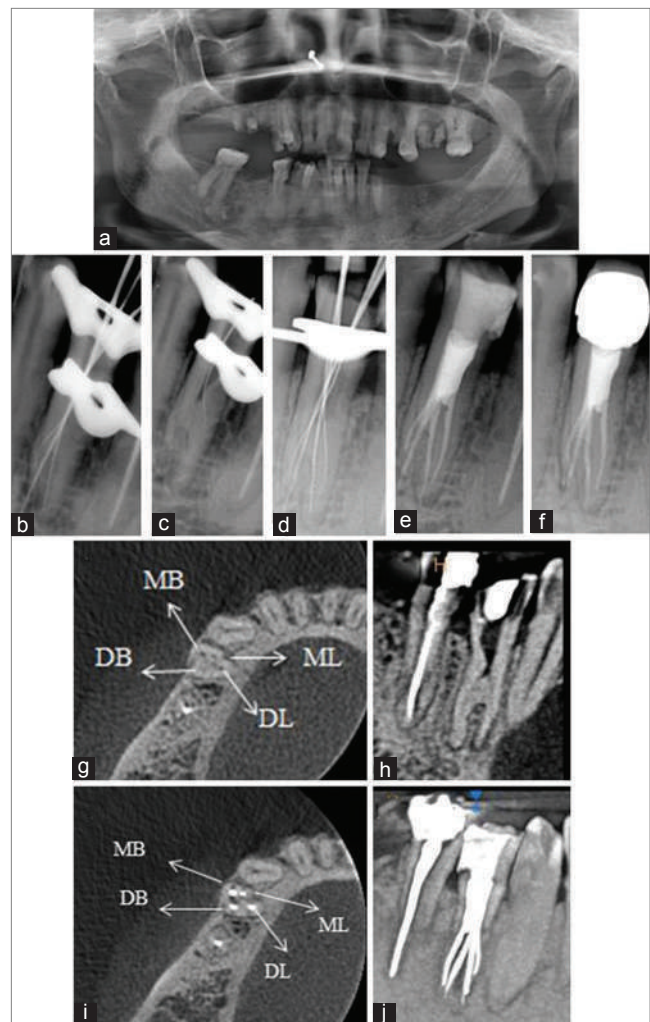


Figure 1: Mandibular right first premolar (#44): (a) Preoperative orthopantomograph. (b) Working length intraoral periapical radiograph, (c) Intraoral periapical radiograph reveals to have an extra fourth canal, (d) Working length intraoral periapical radiograph, (e) Postoperative intraoral periapical radiograph. (f) Postoperative intraoral periapical radiograph with crown, (g and h) Preoperative cone-beam computed tomography scan axial view and sagittal view, (i and j) Postoperative cone-beam computed tomography scan axial view and sagittal view

Table 1: Cases reported by authors with varied canal morphologies

Number of roots	Root canals	Diagnostic method	Country	Authors
1 Root	2 Canals	R/G	USA	England <i>et al.</i> 1991
	3 Canals	R/G	Jamaica	Nallapati 2005
	3 Canals	R/G	Brazil	De Almeida-gomes <i>et al.</i> 2006
	2 Canals	R/G	India	Shenoy <i>et al.</i> 2013
	3 Canals	R/G	India	Daneshvar F <i>et al.</i> 2015
2 Roots	3 Canals	R/G	Iran	Vyapaka Pallavi <i>et al.</i> 2015
	3 Canals	R/G	India	Moayedhi and Lata 2002
	Nil	R/G	USA	Milano <i>et al.</i> 2002
	3 Canals	R/G	India	Poorni <i>et al.</i> 2010
	2 Canals	R/G	India	Kararia <i>et al.</i> 2012
3 Roots	3 Canals	3d CBCT	India	K Balakasireddy <i>et al.</i> 2015
	3 Canals	R/G	China	Chan <i>et al.</i> 1992
	3 Canals	Extraction	USA	Fischer and Evans 1992
	3 Canals	Micro	Britain	Cleghorn <i>et al.</i> 2008
	3 Canals	CT After Extraction	India	Kakkar and Singh 2012
N/L	3 Canals	R/G	Germany	Hulsmann, 1990
N/L	3 Canals	R/G	Australia	Australia Yang, 1994
N/L	R/G	R/G	China	Du Et <i>et al.</i> 2013
N/L	4 Canals	R/G	China	Liu XY <i>et al.</i> 2015
4 Roots	4 Canals	R/G	India	Vaghela and Sinha 2013

The present case with two roots and four distinct canals was not reported in the literature so far. It is highly possible that endodontic treatment results in failure if the third and fourth canal had not been recognized in this present case. During access cavity preparation, vague outlines of three canals were noticed (mesiobuccal, mesiolingual, and distal). It was in accordance with Rodig and Hulsmann, who stated that the presence of a triangle-shaped pulp chamber is the characteristic of mandibular premolars with three root canals, in which the distance from the distobuccal to the lingual orifices was at a peak.^[10]

While estimating the WL radiographically, a fourth canal was suspected. Outline of the orifice was modified to a “h” shape to locate the fourth canal which was recommended by Slowey who stated that is helpful to visualize this canal configuration as a lower case letter “h.”^[4]

In the case of the multiplicity of canals, means of magnification and illumination are of high value. This case was dealt under $\times 3.5$ to confirm the configuration of four canals which was not proportionate. Hence, the clinical photograph of the access cavity was not reproduced. As the division of the four canals was from the middle third, not coronally, the direct photograph using the camera was also obscured.

Exploring the canals is one of the important missions to be accomplished by an endodontist. Many advancements were put forward to effectuate the task, one of which was ultrasonics which has enhanced the quality of treatment

and represents an important adjunct in the treatment of difficult cases. It also aids in refining the access, locating the orifices and calcified canals, and removal of attached pulp stones. Summing up the microscopic visualization with ultrasonic instruments can be the safe and effective combination to achieve optimal results.^[11]

Radiographs reproduce two-dimensional image of a three-dimensional object resulting in overlapping of images. As they are of restricted value in cases with complex root canal anatomy, a preoperative CBCT was advocated. The CBCT images displayed two roots, mesial and distal (mesiobuccal, mesiolingual, distobuccal, and distolingual) with four distinct root canals. Hence, this case may be considered as Type 14 as per Gulabivala’s classification.^[12] However, CBCT stand in need only where conventional imaging systems do not yield sufficient information that it helps in the appropriate canal definition. Endodontists should see to that ALARA (as low as reasonably achievable possible) principle is followed.^[13]

With all the methods established, the protest is still to penetrate, reach, and kill bacteria, which are well-known to protect themselves from the dentinal mud, their own secretions, and biofilms. Ample of irrigation and disinfection aids in the success of root canal therapy. In the present case, 5.25% (NaOCl) and 17% (EDTA) were active irrigants. Passive irrigation was done with EndoActivator. The EndoActivator system has been reported to provide inmost penetration of an irrigation solution to all areas of the endodontic space, and vigorously clean debris from lateral canals, remove the smear layer, and dislodge clumps of simulated biofilm.^[14]

Warm vertical compaction (system B) was done for obturation with backfilling technique (Obtura II) which had shown to provide an excellent apical plug with great adaptation of gutta-percha to root canal walls till the apex. The use of the Obtura II technique is beneficial in cases with irregular canals. The conversion of the softened gutta-percha to the canal walls had shown to be significantly better than lateral compaction by cold gutta-percha point.^[15]

CONCLUSION

The significant accomplishment of the root canal is influenced by the multiple factors such as through cognition about the anatomy, constructive diagnosis with the help of advanced diagnostic tools, and application of skillful equipment in the completion of an uneventful treatment. An endodontist should anticipate the phenomenal aberrations in the morphology of mandibular premolars. The use of CBCT facilitates the diagnosis of such complex variegations.



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This case report details the endodontic management of sparse case of mandibular first premolar with two roots and four canals which was ended with a favorable outcome due to the satisfactory execution of the preferred constituents.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Apical Extrusion of Debris in Mesio Buccal Root of Maxillary Molars with Five Rotary File Systems

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ABSTRACT

Introduction: Flare-ups in endodontics are multifactorial and may cause severe discomfort to the patient. Apical extrusion while preparing root canal space in any form such as debris, bacteria or irrigants has been found to be associated with many flare-up events and may even lead to treatment failure.

Aim: To quantitatively evaluate apically extruded debris with five rotary instrumentation systems in mesio buccal root of maxillary molars.

Materials and Methods: A total of 120 human maxillary first molars extracted between January and March of 2017 in oral and maxillofacial surgery department at Drs. Sudha and Nageswara Rao Siddhartha Institute of Dental Sciences, Gannavaram, Andhra Pradesh, India, were included in the current study. Only the Molars with a three rooted pattern were selected and mesio buccal root was used to evaluate apical extrusion. An experimental apparatus for evaluation of extruded debris as previously described by Myers and Montgomery was fabricated. All samples were divided into six groups (n=20 each) and instrumentation was done with five rotary file systems (K3XF, ProTaper NEXT, HyFlex CM, Revo-S, and FLEXICON) and HAND K FILES which served as control. The

weight of the micro tubes in the apparatus before and after the instrumentation was calculated using an electronic weighing balance with a minimum sensitivity of 10^{-4} and weights were compared to quantify the apical extrusion. Statistical analysis was analysed with the ANOVA and multiple comparison (Post-hoc-Dunnnett and Tukey) tests.

Results: It was found that all the instrumentation systems showed a considerable amount of apical debris extrusion. HAND K FILES were associated with most debris extrusion compared to all rotary files ($p < 0.01$). FLEXICON file system showed lowest debris extrusion among all experimental groups. However, there was no statistically significant difference between K3XF and FLEXICON file systems ($p = 0.312$).

Conclusion: The rotary systems extruded less debris compared to HAND K FILES. However, it was observed that there was no statistically significant difference between apical extrusion of K3XF and HyFlex CM, K3XF and FLEXICON X7, HYFLEX CM and Revo-S file systems. Among all the rotary groups, FLEXICON X7 and ProTaper NEXT files showed lowest and highest apical extrusion respectively. Design of rotary endodontic instruments may have a greater impact on their innate apical extrusion potentials.

Keywords: Design of rotary files, Endodontic flare-ups, Flexicon X7, Maxillary molars, ProTaper NEXT

INTRODUCTION

Endodontic therapy enjoys a higher success rate in recent times with innovations in cleaning and shaping systems. The principle behind these systems is that Ni-Ti rotary instruments help in easier, faster and efficient cleaning and shaping. However, many instrumentation related factors might cause post-treatment complications, were not eliminated completely even in these rotary file systems and apical extrusion is one of them. Apical extrusion is considered as an important factor responsible for flare-up and post-operative pain or even failure of endodontic healing [1,2]. An update on apical extrusion potentials of recently introduced rotary systems with newer Ni-Ti technologies is much needed for better endodontic therapy. Among many rotary files that were introduced recently, K3XF (Sybron Endo, Kerr Dental, USA), PROTAPER NEXT (Dentsply, Tulsa Dental Products, Tulsa, OK, USA), HYFLEX CM (Coltene/WhaledentInc; USA), Revo-S (Micro-Mega, Sanavis Group, USA) and Flexicon (Edgeendo, Canada) have demonstrated improved mechanical properties and shaping abilities [3-7].

PROTAPER NEXT file system (DentsplyMaillefer, Ballaigues, Switzerland) introduced in the year 2013 is the next generation of Protaper universal files and are designed with variable tapers and an off-centered rectangular cross section. This design makes it possible to completely prepare root canals using fewer instruments than the number required by the ProTaper Universal. A newer

NiTi technology known as M-wire was employed for PROTAPER NEXTfiles which increased flexibility and offered greater resistance to cyclic fatigue of the instruments [8].

K3XF files (Sybron endo), introduced as successors of K3 files in the year 2011 are made of R-phase nickel-titanium alloy which increased cyclic fatigue and flexibility. Improvements in cross-sectional design such as third radial land and reduced radial land with blade support in combination with newer revolutionary R-phase NiTi technology ensured better performance of these files [9].

Hyflex files (Coltene Whaledent.), a new revolution in rotary endodontics (2012) are machined from a wire (termed CM wire) with double fluting, symmetrical cross-section, variable pitch, non-cutting tip, negative rake angle. Hyflex files by the virtue of their NiTi technology have shape memory and thus have an excellent canal centering ability enabling possibility to negotiate even canals with a greater curvature [10].

Revo-S NiTi instrument system (Micro-Mega, Besancon, France), was introduced as an asymmetric cross-section which has more flexibility and less stress. A customised three instrument preparation technique including both symmetric and asymmetric instruments facilitated penetration by the snake-like movements and offered a root canal shaping adapted to the biologic and ergonomic imperatives with improved debris elimination [11].



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Recently in the year 2015, FLEXICON X7 (Edgeendo, Canada) files that are made of an annealed heat treated NiTi alloy brand named Fire Wire™, have been introduced. According to the manufacturer, Fire Wire Ni-Ti yields performance enhancing durability that provides incredible flexibility so that X7 files enhance and expedite the endodontic treatment [12].

To date, there appear to be very few studies which evaluated the apical extrusion potential of these file systems and thus the present study was aimed at comparative evaluation of apically extruded debris with these five rotary instrumentation systems.

MATERIALS AND METHODS

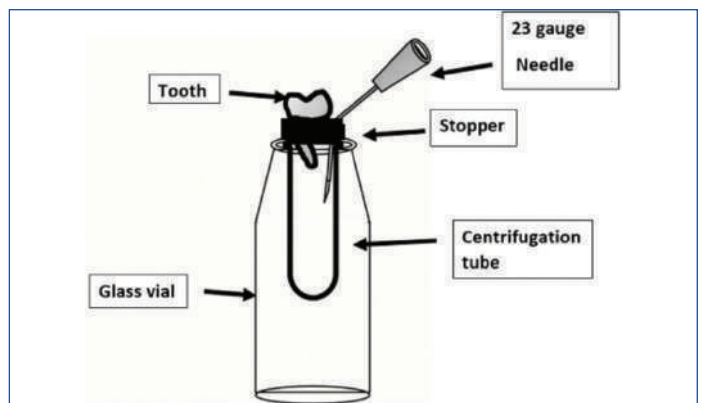
An in vitro experimental study was conducted on 120 mesio buccal roots of human maxillary first molars, which were extracted due to periodontal considerations between January and March 2017. The study was performed in the Department of Conservative Dentistry and Endodontics, Drs. Sudha and Nageswara Rao Siddhartha Institute of Dental Sciences, Gannavaram, Andhra Pradesh, India, after obtaining an Institutional Ethical Clearance (OC No./IEC/02/2014). The sample size was calculated using Raosoft online sample size calculator and the power of study set was 80% [13]. The criteria for teeth selection included the presence of a distinct three rooted pattern with canal curvature of mesio buccal root between 10° to 20°, the absence of root fractures and cracks under stereo microscope at 4X magnification; absence of internal and external resorption or calcification and completely formed apex. Each tooth was radiographed in buccolingual and mesiodistal directions to categorise them and detect any possible obstructions. The teeth in which the apical minor constriction was gauged larger than 20 size hand file and a second mesio buccal canal were excluded from the study. After a thorough screening, a total of 120 teeth were considered for inclusion in the study.

Preparation of Specimens

Access cavities were prepared using endo access and endo-Z burs (Dentsplymailefer, Tulsa). After access cavity preparation, distobuccal and palatal roots were amputated with a diamond disc (Wuxi xiangsheng industrial and trading co) mounted on a micromotor (Hero dental products, India). The canal orifices of amputated roots were sealed with Glass Ionomer cement (GC FUJI IX). Later the entire root surface of mesio buccal root was coated with three coats of nail varnish to prevent microleakage through accessory canals and other discontinuities in the cementum. A 10 size K-File (Mani inc, Tochigi, Japan.) was passed 1 mm beyond the apical foramen through nail varnish to ensure uniform apical patency for all the experimental samples. The working length was determined simultaneously while creating apical patency by keeping the tip of instrument 0.5 mm short of the apex which was further confirmed by taking a radiograph.

Debris Extrusion Apparatus

The method for the collection of apically extruded debris was adapted from a previous study conducted by Myers GL and Montgomery S (1991) [14]. 120 centrifugation micro tubes (Tarsons, India) were taken and their stoppers were detached. The initial weight of individual tubes was taken using single pan analytical balance with 10⁻⁴ sensitivity (Shimadzu ATX224). An average of three readings for each sample was considered to avoid numerical error. With the help of a heated instrument, a hole was created in the center of the rubber stopper to fix the tooth. After creating a hole, the root was pushed into the stopper of centrifugation tube up to the cervical area and subsequently sealed with cyanoacrylate glue (Anabond Darien). The stopper was attached to the respective tube and a 23 gauge needle was inserted into it to equalise atmospheric pressure [Table/Fig-1]. This entire assembly was transferred to a transparent glass vial and subsequently, the vial was covered with aluminium foil for the purpose of isolation and blinding to avoid bias.



[Table/Fig-1]: Schematic representation of apparatus used for evaluation of debris extrusion.

GROUPS

All the samples were randomly assigned to six groups containing 20 teeth each, based on the file systems used for root canal preparation as mentioned below.

- Group 1:** Root canal preparation was done using Hand-K files (Mani inc.).
(This was considered as a control group.)
- Group 2:** Root canal preparation was done using K3XF files. (Sybron endo.)
- Group 3:** Root canal preparation was done using PROTAPER NEXTfiles (Dentsply Mailefer.)
- Group 4:** Root canal preparation was done using HYFLEX CM (Coltene Whaledent.)
- Group 5:** Root canal preparation was done using Revo-S files (Micro-Mega)
- Group 6:** Root canal preparation was done using FLEXICON X7 files (Edgeendo, Canada).

All the samples in their respective groups were prepared according to the manufacturer's instructions [Table/Fig-2] [15-20]. Glide path for all the rotary groups was created with a #15 K-File. All the rotary files were used as per manufacturer's specific RPM and torque. Each instrument was used in a short in and out motion for a maximum of four times per instrument. In all the groups except Group-1, a corresponding file with tip diameter #25 with a 6% taper was used as a finishing file while in Group-1 the finishing file was a size #25 k file (Mani Inc.) with 2% taper. The rubber dam was applied on to the tooth to prevent any possible contact of irrigating solution to the exterior of centrifugation tubes while irrigating.

Irrigation Protocol

All the canals were irrigated in between instrumentations and also after completion of instrumentation by using a 30 gauge side vented irrigation needle. A total of 5 mL bidistilled water was used per sample. After completion of instrumentation, stopper was removed and apically extruded debris attached to root tip was washed into Eppendorf tube with 1 mL of bidistilled water.

Drying of Samples

After completion of the root canal instrumentation, stopper assembly was removed from centrifugation tube. In order to facilitate evaporation of the irrigant, all the samples were placed in a hot air oven (BTI, India) at 140°C for five hours.

Measurement of Extruded Debris

After ensuring complete evaporation of the irrigant the final weights of individual tubes were measured using the same analytical balance as before [Table/Fig-3]. An average of three consecutive weights

Group	Mode of preparation	Sequence of instrumentation	Torque (NCM)/RPM
Group1 (control) (HAND K FILES)	Crown down	Coronal flaring with Headstrom files till 16 to 18 mm → coronal flaring size 2,3 & 4 GG drills → creation of apical seat and final apical finishing with size 25 k-file [15]	NIL
Group 2 (K3XF)	Crown down	0.12/#25 (orifice shaping) → 0.10/#25 (1/3 rd of working length) → 0.08/#25 (2/3 rd of working length) → 0.06/#25 (till working length) [16]	2.8/450
Group 3 (PROTAPER NEXT)	Crown down	SX (orifice shaper) → X1 0.04/#17 (till working length) → X2.06/#25 (till working length) [17]	2/300
Group 4 (HYFLEX CM)	Crown down	0.08/#25 (orifice shaper) → 0.04/#20 (apical enlargement/working length) → 0.04/#25 (apical enlargement/working length) → 0.06/#20 (middle part shaping/working length) → 0.06/#25 (apical finishing/working length) [18]	2.5/500
Group 5 (REVO-S)	Crown down	Endo flare (orifice shaper) → SC1 0.06/#25 (coronal 1/3 rd of working length) → SC2 0.04/#25 (apical shaping till working length) → SU 0.06/#25 (apical finishing till working length) [19]	2.5/400
Group 6 (FLEXICON)	Crown down	X7 0.06/#25 (Till resistance is felt) → X7 0.04/#17 (till working length) → 0.06/#20 (till working length) → 0.06/#25 (apical finishing till working length) [20]	2.5/350

[Table/Fig-2]: Strategy of root canal preparation and sequence of instrumentation in all the study groups [15-20].

was obtained for each tube and amount of extruded debris was calculated by substituting in the following formula and results were subjected to statistical analysis.

Extruded debris=final weight of centrifugation tube-initial weight of centrifugation tube



[Table/Fig-3]: Micro-tubes showing extruded debris after evaporation of irrigating solution.

STATISTICAL ANALYSIS

The results were analysed using SPSS version 22.0. (Statistical Package for Social science, IBM Corporation) software. All the groups were analysed for overall significance values using ANOVA (Analysis of Variance) test (confidence interval was F=65.39) [Table/Fig-4]. whereas, comparison with control group (GROUP-1) and other Intergroup comparisons were done utilising Post-hoc

Dunnnett's test and Post-Hoc Tukey's tests respectively with a level of significance set at $p < 0.01$ [Table/Fig-5,6].

Group	Apical Extrusion of Debris (mg)						
	N	Minimum extrusion	Maximum extrusion	Mean	SD	Median value	p-value
1 HAND K	20	6.9	59.1	41.3	13.8	42.35	<0.01
2 K3XF	20	.3	13.9	6.1	4.0	5.55	<0.01
3 PTN	20	2.5	40.0	20.4	8.8	19.15	<0.01
4 HYFLEX	20	.7	21.6	8.3	6.2	8.20	<0.01
5 REVO-S	20	3.4	19.3	9.2	4.3	8.25	<0.01
6 FLEXICON	20	1.1	14.8	5.5	3.7	5.1	<0.01

[Table/Fig-4]: The mean values and standard deviation of apically extruded debris of all groups.

Test: ANOVA; F-value=65.39; $p < 0.01$; highly significant

Comparison between groups	p-value
1 HAND K vs 2 K3XF	<0.01
1 HAND K vs 3 PTN	<0.01
1 HAND K vs 4 HYFLEX	<0.01
1 HAND K vs 5 REVO-S	<0.01
1 HAND K vs 6 FLEXICON	<0.01

[Table/Fig-5]: Comparison of Control group (Hand-k files) with all other experimental groups using Post-Hoc Dunnnett's test with a level of significance set at $p < 0.01$.

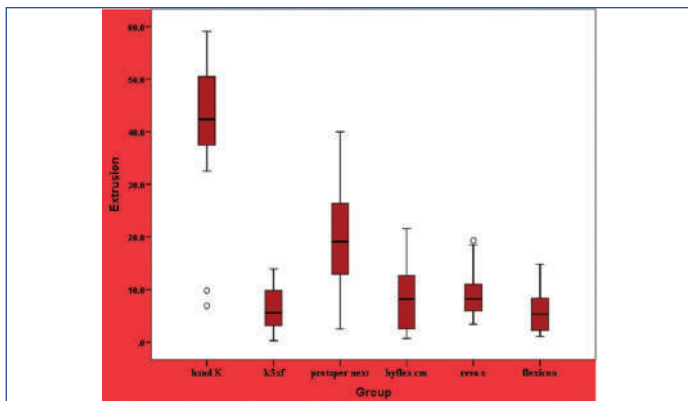
Comparison between groups	p-value
2 K3XF vs 3 PTN	<0.01
2 K3XF vs 4 HYFLEX	0.095 NS
2 K3XF vs 5 REVO-S	0.01
2 K3XF vs 6 FLEXICON	0.312 NS
3 PTN vs 4 HYFLEX	<0.01
3 PTN vs 5 REVO-S	<0.01
3 PTN vs 6 FLEXICON	<0.01
4 HYFLEX vs 5 REVO-S	0.80 NS
4 HYFLEX vs 6 FLEXICON	<0.01
5 REVO-S vs 6 FLEXICON	0.003

[Table/Fig-6]: Inter-Group Comparison of all groups with rotary systems by using Post-Hoc Tukey's test with a level of significance set at $p < 0.01$.

RESULTS

Under the experimental conditions of the current in vitro study, the results showed that all instrumentation systems caused apical extrusion of debris. However, highest extrusion was observed in Group-1 (HAND K FILES) and least was observed in case of Group-6 (FLEXICON) [Table/Fig-4]. Mean debris extrusion of Control group was compared with other experimental groups utilising Post-hoc Dennett's test where the values were compared with 5% (0.05) level of significance. This comparison revealed that there was a highly significant difference in extrusion between the control group and all other experimental groups. An Intergroup comparison for experimental groups utilising Post-hoc Tukey's test revealed a statistically significant difference in extrusion between all Groups except between Group-2 (K3XF) and Group-4 (HYFLEX CM), Group-2 (K3XF) and Group-6 (FLEXICON), Group-4 (HyFlex CM) and Group-5 (Revo-S).

Debris extrusion values are represented in the box plot diagram which showed more deviation in extrusion values as observed in Group-1 (Hand-K files) and Group-3 (PTN) followed by Group-4 (Hyflex-CM) and Group-2 (K3XF), whereas least is observed in Group-6 (FLEXICON). Two outliers are seen in Group-1 (Hand-K files) and one outlier is observed in case of Group-5 (Revo-S) [Table/Fig-7].



[Table/Fig-7]: Box-plot diagram representing the distribution of apical extrusion of debris of all groups.

Based on middle quartile values the order of debris extrusion is as follows:

[GROUP-1 (HAND K)] > [GROUP-3 (PTN)] > [GROUP-5 (REVO-S)] > [GROUP-4 (HYFLEX CM)] > [GROUP-2 (K3XF)] > [GROUP-6 (FLEXICON)]

DISCUSSION

During mechanical preparation, dentin chips, remnants of pulpal tissue, irrigating solutions, and microorganisms and their by-products are often transported through the apical foramen and introduced into the periapical tissues causing postoperative inflammation/infection, pain, flare-ups, and consequently, delay apical healing [1,2].

Key factors that are responsible for apical extrusion can be categorised under: i) natural physical factors, such as anatomy of apical constriction, root dentin hardness, quantity and momentum of flow of irrigant in the root canal and the position of the tooth whether in the upper or lower jaw which may be affected by gravity; ii) Mechanical factors, such as the selection of the final apical size of instrument, instrumentation techniques, designs of instruments, rotation speed of the file and movements of the hand of operator during preparation [21]. As most of the natural factors are not under the control of an operator, a balancing approach towards various mechanical factors that might govern and aid in minimising apical extrusion should be considered. Standardisation was achieved by selection of a narrow root, the inclusion of samples with similar initial apical diameter, canal curvature in the range of 10° to 20°, confining apical preparation to 0.25 mm with 6% taper for all samples and a common irrigation system with canal preparation done by a single operator. This leaves us with a conclusion that it is the design of the instrument and instrumentation system that is most responsible for this mode of apical extrusion in the present study.

At present NiTi instruments are available on hand as well as engine driven preparation techniques and are 2-3 times more elastic than stainless steel files due to their very low modulus of elasticity, NiTi files have shown higher resistance to torsional fracture. According to the structural characteristics of these files, their use is likely to reduce the extrusion of debris from the apical end [9]. Manufacturers have designed new nickel-titanium rotary files with different parent metallurgical phases and they differ greatly in their design which may influence the amount of apically extruded debris through the apical foramen.

Maxillary molars were chosen for the current study as the apical diameter of selected mesio buccal root is small and it helps in achieving reliable results as most of the working part of the file is in contact with canal walls which may not be possible with single-rooted teeth with larger apical diameters and wide canal geometry as previously reported by Kirchoff AL et al., [22]. Arias A et al., while establishing predictive models to evaluate post-operative pain found that the incidence of more post-operative pain was associated with maxillary molars which can be a complication due to apical extrusion [23].

Generally accepted method of Myers and Montgomery et al., [14] was used to collect the debris extruded apically with minor modifications to make it more simple, practical and affordable. As suggested by Tanalp et al., [24] the usage of Sodium hypochlorite and lubricants like EDTA were avoided in the current study as their extrusion might cause biased weights when measured and irrigation was strictly restricted to 5 mL of bi-distilled water which leaves no residue when it evaporated [25].

In current study, results suggested that control group i.e., Hand-K files is associated with greater apical extrusion of debris compared to other rotary groups. This observation is in accordance with previous studies [24, 26-29]. Previously Lousi SB et al., suggested that a file without rotatory motion acts like a piston in tube which results in extrusion of a greater amount of debris which could be the reason for this observation whereas, continuous rotation seemed to improve coronal transportation of dentine chips and debris by acting like a screw conveyor [26].

PROTAPER NEXT showed second highest debris extrusion in the current study. The preparation technique associated with these files does not resemble a pure crown down technique as canal preparation till working length is achieved with initial instruments itself. Results in present study are in accordance with Uzunoglu E et al., [30]. It was hypothesised that larger apical taper of instruments working at the apical area (X1 and X2) while using PROTAPER NEXT might be the reason for more extrusion. Though the off-centred design of PROTAPER NEXT was supposed to aid in augering more debris out of the canal, a greater taper of instruments at the apical 3 mm which might remove more dentin and possibly the number of files instrumented till working length which might push more debris apically when the file is moved apically in a linear fashion.

Revo-S system is a unique combination of files with both symmetric and asymmetric cross-section. An asymmetric cross-section helps in more debris elimination in a coronal direction. Though the same feature was incorporated in PROTAPER NEXT system, the differences in the extrusion of debris could be due to differences in the apical taper of both systems and difference in the files that are used till working length. Kocak S et al., demonstrated in a previous study that REVO-S extruded lesser debris compared to ProTaper Which they attributed to an asymmetrical cross section of the instrument design an off-centered design [31].

HYFLEX CMfile system was found to be associated with more debris extrusion next to Revo-S. Elmsallati EA et al., in a previous study showed that the short pitch design extruded less debris than the medium and long ones [21]. Capar ID et al., proposed that the unwinding of the spirals of HyFlex instruments is well known during root canal preparation which results in lengthening pitch and thereby more debris extrusion [32]. The results of the current study are in accordance with Kocak S et al., who hypothesised that physical properties of CM system could be the reason for these observations [31].

K3XF file system has a variable pitch to prevent the screwing-in effect and presence of radial lands to aid in debris elimination, along with increasing variable helical flute angle from tip to handle which helps to dislodge the dentin chips from working area and carried coronally to the orifice. These observations are in accordance with previous studies done by Ghogre P et al., and Zan R et al., [33,34].

FLEXICON X7 file system showed least apical extrusion in the current study. This newer file system has a similar cross-sectional design as K3XF files including radial lands, variable pitch and increasing helical angle which supports efficient removal of debris in coronal direction. However, there is no statistically significant difference between apical extrusion patterns of these two files. To date, there is not enough published data to emphasise the design features of FLEXICON files that could affect the apical extrusion of debris and further research is required in this regard. It is noteworthy that both HYFLEX CMand FLEXICON X7 files belongs to a newer category

of files having a property of deformation memory with differences in cross-sectional design.

Differences in the amount of apical extrusion, when compared to previous studies, were due to variations in selected teeth, file systems and irrigation protocols followed. The results of the current study showed significant differences in mean apical extrusion values with different rotary file systems by the virtue of their cross-sectional design. Further, it was also observed that there is no exact correlation between the number of files used and corresponding extruded debris for each system. On the other hand, it is still unclear that whether a specific NiTi technology can directly influence innate apical extrusion potentials of these rotary systems and has to be evaluated in further studies.

LIMITATION

The limitation of the study was the inability to simulate apical resistance to extrusion which exists in the natural tooth due to the presence of periapical tissues. Further studies are required to evaluate apical extrusion patterns of these file systems under the presence of apical resistance.

CONCLUSION

Under the limitations of this in vitro study, FLEXICON X7 and K3XF files extruded less apical debris compared to all other groups. Highest debris extrusion was associated with PROTAPER NEXT files. There is no statistically significant difference in the apical extrusion patterns of Group-2 (K3XF) and Group-4 (HYFLEX CM), Group-2 (K3XF) and Group-6 (FLEXICON), Group-4 (HyFlex CM) and Group-5 (Revo-S) file systems. Knowledge of these factors may help in reduction of apical extrusion while cleaning and shaping which facilitate proper endodontic therapy; prevent flare-ups and help to obtain satisfactory results.

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Unveiling the Link between Prostatitis and Periodontitis

Abstract

Background: One of the important tumor markers having critically important applications in every aspect of treating men with prostatic illness is prostate-specific antigen (PSA), formed by prostate acini's epithelial cells. Where prostate is affected by inflammation or malignancy, the PSA levels rise to/and above 4 ng/ml. This study analyzes the interlink between different severity of periodontitis and prostatitis by assessment of PSA antigen levels and periodontal clinical parameters. **Materials and Methods:** In this study, 100 chronic prostatitis patients diagnosed to also have periodontal diseases were divided into four batches on the basis of the nature of prostatitis and levels of periodontal clinical attachment. The grouping was as: group 1A – clinical attachment level (CAL) <3 mm and mild prostatitis, Group 2A – CAL ≥3 mm and mild prostatitis, and Group 1B – CAL <3 mm and moderate-to-severe prostatitis, Group 2B – CAL ≥3 mm and moderate-to-severe prostatitis. Readings of CAL, probing pocket depth, bleeding on probing, plaque index, and gingival index (PI and GI) were recorded, followed by calculation and assessment of PSA values and correlation of periodontal parameters, respectively. **Results:** An important and affirmative correlation ($r = 0.5549$, $P < 0.05$) was seen between PSA and CAL scores at significance level of 5%, and also between PSA and probing depths (PD) scores at 5% ($r = 0.5315$, $P < 0.05$), indicating that PSA and CAL scores, as also PSA and PD scores are mutually dependent. The similar positive correlation was seen between PSA with PI ($r = 0.3231$, $P < 0.05$) and GI ($r = 0.3567$, $P < 0.05$) scores, respectively, at 5% level of significance, which shows PSA is also mutually dependent on PI and GI scores. **Conclusion:** Patients with of grades, moderate-to-severe prostatitis as well as periodontitis were found having higher PSA levels. The clinical readings of periodontal parameters were significantly higher in patients with moderate-to-severe prostatitis which shows a pathological link between the above two.

Keywords: Periodontitis, prostate carcinoma, prostate-specific antigen levels, prostatitis

Introduction

Periodontitis is an inflammatory condition of the tissues supporting the teeth caused by certain particular micro-organisms or clusters of such microorganisms, leading to the continuous destruction of the periodontal ligament and the alveolar bone with an increase in probing pocket depth, pocket formation, recession, or both. It may affect varying number of teeth with varying progression.^[1] The emergence of host response paradigm to explain the pathogenesis of periodontal disease has expanded our knowledge to a better understanding of the disease. Research findings, of late, show potential links between periodontitis and systemic disorders, such as diabetes, coronary heart disease and lung disorders, stroke and pregnancy-related adverse conditions.

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These findings together with the surfacing of a new discipline of periodontal medicine, as a domain, has led to many developments in remedial and beneficial approaches about managing periodontitis.^[2]

Of the various systemic disorders inflicting human beings, prostatitis is one of the disorders that has shown to have a relationship with periodontitis, the former being a common inflammatory condition below the age of 50. Prostatitis clinically presents with painful urination, difficulty in emptying the bladder, frequent tendency to urinate, pain in penis, testicles, even during or after ejaculation, with associated fever and chills.^[3] Prostate-specific antigen (PSA), a glycoprotein produced mainly by the epithelial cells along the acini and ducts of the prostate gland, is a biological or tumor marker for conditions such as benign prostatic hyperplasia, prostate cancer, and the screening of which has proven to be

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beneficial in the confirmatory diagnosis of substantial numbers prostate cancer patients.^[4] The serum PSA levels are normally very low; however, when the normal prostatic structural design is disrupted, like prostatic disease, inflammation or trauma, it permits entry of more PSA to the systemic circulation. PSA level below 4.0 ng/ml is normal and above 0.35 ng/ml/year has a relatively higher risk of death from prostate cancer, unlike those who had a PSA volume <0.35 ng/ml/year.^[5]

Chronic prostatitis etiology depends on multiple host immuno inflammatory factors, likely to be channeled via pro-inflammatory cytokines, such as interleukin (IL-1 β) and tumor necrosis factor- α (TNF- α). Increased ratio of pro-inflammatory to anti-inflammatory cytokines imbalance has been concerned with the pathogenesis of periodontitis as well as prostatitis. Similarity in the etiopathogenesis of these denotes a possible relationship between the two which may be apparent with the elevated PSA values in circulation.^[6] This study evaluates any link between periodontitis and PSA levels in chronic prostatitis patients, and compares the serum PSA levels with periodontal clinical parameters which include CALs, probing depths (PD), plaque index and gingival index (PI and GI), bleeding on probing between the study groups.

Materials and Methods

Patients who attended, Government General Hospital, Guntur presenting with signs and symptoms of lower urinary tract infections and diagnosed with prostatitis or elevated PSA levels PSA (4 ng/ml) together with chronic periodontitis were assigned in the study. Permissions as dictated by the ethics and protocols were obtained after sufficient explanations and clarifications to the patients about the treatment procedures and those who were willing to participate were selected for the study, as allowed by the Institute's Ethical Committee.

Inclusion criteria were that patients aged 30–60, with ≥ 12 teeth, with elevated serum PSA levels ≥ 4 ng/ml, who did not undergo oral prophylaxis in the previous 6 months. Those with any history of systemically compromised conditions such as myocardial infarction, stroke, and organ transplant during the previous 6 months were not covered in the study.

One hundred prostatitis patients with periodontal problems were considered, of which 83 patients presented with the benign condition and 17 turned out to be malignant. They were further divided into four groups on the basis of prostatitis conditions and the level of periodontal clinical attachment. The groups were Group 1 A– clinical attachment level (CAL) <3 with mild prostatitis, Group 2 A–CAL ≥ 3 with mild prostatitis, Group 1B–CAL <3 with moderate or severe prostatitis, and Group 2 B–is CAL ≥ 3 mm with moderate or severe prostatitis. Five milliliters of intravenous blood was collected from the

antecubital vein of each patient, and the samples were sent for determination of serum PSA levels. Prostate needle biopsy of prostate gland had been taken and sent for histopathological examination.

Estimation of prostate-specific antigen

The PSA chemiluminiscent immunoassay test, works on the principle of a solid phase enzyme-linked immunosorbent assay, using a rabbit anti-PSA antibody directed against intact PSA for solid phase immobilization (on the microtiter wells). The sample reacts with monoclonal-anti PSA antibody conjugate for 15 min at 37°C–40°C resulting in molecules getting sandwiched between the solid phase and enzyme-linked antibodies. The wells are then water-washed to remove unbound labeled antibodies and substrate reagent is added and incubated for 5 min.

Assay procedure

Serum is separated by adding ethylenediaminetetraacetic acid after collecting blood sample. Following which, 13 ml of serum is removed and the sample is placed in a well; later 50 ml of tracer reagent is added to that well. Swirl the plate for 60 s, and incubate the well for 45 min at 22°C–26°C. Decanting and washing are done five times with wash buffer, after which reagents are added in the ratio of 1:1 to the sample well, and incubated for 5 min following which the results of PSA will then be displayed on the screen.

Periodontal examination

After getting patients consent, periodontal examination was carried out using UNC-15 Probe (University of North Carolina-15), and assessment of periodontal parameters included measurement of probing depth from the gingival margin to the base of sulcus, measurement of CAL from the cement-enamel junction to the base of pocket, GI of Silness and Loe (1963), PI of Loe and Silness (1967), Bleeding index of Ainamo and Bay (1975).

Statistical analysis

Descriptive statistical analysis was carried out in the present study. For intragroup comparison, Kruskal–Wallis test was used. The Mann–Whitney test was used to help analyze the specific sample pairs for significant differences. Intergroup comparison was done with one-way analysis of variance. The relationship between PSA scores with all clinical parameters was done using Pearson's correlation coefficient technique. Difference in means as statistically significant was analyzed using Student's *t*-test.

Results

The age of the patients ranged between 40 and 75 years, with the mean age of 57.63 \pm 13.65 in Group 1A and 60.50 \pm 0.71 in Group 1B, 63.75 \pm 8.03 in Group 2A, and in Group 2B, it is 63.97 \pm 8.60 [Table 1]. The percentage of moderate/severe prostatitis patients with malignancy were 49% and with nonmalignancy are 51%. A significant difference was

observed between mild, moderate, and severe types of prostatitis in relation to the status of malignancy ($P < 0.05$), as shown in Table 2. Table 3 shows the mean GI and PI scores in all the four groups. The mean bleeding index score in all the groups are depicted in Table 4 and mean CAL and probing depth scores of the groups are represented in Table 5. No difference was observed among four Groups 1A, 1B, 2A, and 2B with respect to PSA scores ($P > 0.05$), showing that the PSA scores are very similar in four groups [Table 6].

Table 1: Mean and standard deviation age of study samples by four groups (1A, 1B, 2A, and 2B)

Groups	Age (mean±SD)
Group 1A	57.63±13.65
Group 1B	60.50±0.71
Group 2A	63.75±8.03
Group 2B	63.97±8.60

SD: Standard deviation

Table 2: Distribution of samples by types of prostatitis and status of malignancy

Status of malignant	Mild (%)	Moderate/severe (%)	Total
Malignant	0 (0.00)	15 (100.00)	15
Nonmalignant	16 (50.00)	16 (50.00)	32
Total	16 (34.04)	31 (65.96)	47
χ^2 with Yates's correction		9.2533	
P		0.0023*	

* $P < 0.05$

Table 3: Comparison of four groups (1A, 1B, 2A, 2B) with respect to gingival index and plaque index scores by Kruskal–Wallis analysis of variance test and Pairwise comparison by Mann–Whitney U test

Groups	GI scores		PI scores	
	Mean±SD	Sum of ranks	Mean±SD	Sum of ranks
Group 1A	1.63±1.27	121.00	1.59±1.23	122.00
Group 1B	1.40±0.50	34.00	1.56±0.69	40.00
Group 2A	1.37±0.47	121.50	1.35±0.42	122.50
Group 2B	1.89±0.53	851.50	1.83±0.49	843.50
H		11.6201		10.6321
P		0.0088*		0.0139*

Pair-wise comparison by Mann–Whitney U-test

1A versus 1B	0.7940	0.6015
1A versus 2A	0.7527	0.9164
1A versus 2B	0.0134*	0.0134*
1B versus 2A	1.0000	0.7940
1B versus 2B	0.2278	0.3764
1A versus 2B	0.0067*	0.0103*

* $P < 0.05$. SD: Standard deviation; GI: Gingival index; PI: Plaque index

The mean PSA in mild prostatitis patients is 3.23 ± 2.04 , whereas in moderate-to-severe prostatitis is 18.47 ± 27.887 .

Table 4: Comparison of four groups (1A, 1B, 2A, 2B) with respect to bleeding index scores by oneway analysis of variance test and Pairwise comparison by Tukeys multiple *post hoc* procedures

Groups	BI (mean±SD)
Group 1A	0.80±0.31
Group 1B	1.00±0.00
Group 2A	0.97±0.06
Group 2B	1.00±0.00
F -test	5.3210
P	0.0033*

Pair-wise comparison by Tukeys multiple *post hoc* procedures

1A versus 1B	0.2040
1A versus 2A	0.0458*
1A versus 2B	0.0017*
1B versus 2A	0.9918
1B versus 2B	1.0000
1A versus 2B	0.9417

* $P < 0.05$. SD: Standard deviation; BI: Bleeding index

Table 5: Comparison of four groups (1A, 1B, 2A, 2B) with respect to clinical attachment level and probing depth scores by oneway analysis of variance test and Pairwise comparison by Tukey's multiple *post hoc* procedures

Groups	Mean±SD	
	CAL scores	PD scores
Group 1A	1.90±0.61	3.26±0.55
Group 1B	2.28±0.03	3.72±0.01
Group 2A	3.61±0.87	3.49±0.71
Group 2B	5.69±1.34	3.76±0.96
F	27.5763	0.8025
P	0.0000*	0.4994

Pair-wise comparison by Tukey's multiple *post hoc* procedures

1A versus 1B	0.9759	0.9016
1A versus 2A	0.0251*	0.9516
1A versus 2B	0.0002*	0.4693
1B versus 2A	0.4722	0.9849
1B versus 2B	0.0014*	0.9999
1A versus 2B	0.0004*	0.8547

* $P < 0.05$. SD: Standard deviation; CAL: Clinical attachment level; PD: Probing depth

Table 6: Comparison of four groups (1A, 1B, 2A, 2B) with respect to prostate-specific antigen scores by one-way analysis of variance test

Groups	PSA (mean±SD)
Group 1A	1.75±1.24
Group 1B	4.23±0.32
Group 2A	4.73±1.53
Group 2B	19.46±28.60
F -test	1.8265
P	0.1566

PSA: Prostate-specific antigen; SD: Standard deviation

A significant difference was observed between mild and moderate/severe types of prostatitis with respect to PSA scores ($P < 0.05$), indicating that PSA scores are significantly higher in moderate-to-severe prostatitis as compared to mild prostatitis [Table 7].

The mean CAL in mild prostatitis patients is 2.75 ± 1.14 whereas in moderate or severe prostatitis is 5.47 ± 1.54 . A significant difference was observed between mild and moderate/severe types of prostatitis with respect to CAL scores ($P < 0.05$) [Table 8]. There is no significant difference while comparing the CAL <3 and ≥ 3 groups, with respect to PSA scores ($P > 0.05$) indicating that the PSA scores are similar in <3 CAL and ≥ 3 CAL groups [Table 9]. Table 10 shows the distribution of samples by type of prostatitis, malignancy, and CAL. The correlation coefficient values obtained among the four groups, i.e., (1A, 1B, 2A, and 2B) were 0.532, 1.00, 0.684, and 0.558 with values of $P = 0.175, 0.01, 0.06,$ and 0.002 , respectively. From Table 11, it can be inferred that statistically significant correlation between prostate specific antigen levels and clinical attachment level scores was seen in Group 1B, i.e. CAL <3 with moderate or severe prostatitis, and Group 2 B, i.e. CAL ≥ 3 mm with moderate or severe prostatitis, when examined among all the four groups.

A significant and positive correlation ($r = 0.5549, P < 0.05$) was observed between PSA and CAL scores at 5% level and also between PSA and PD scores at 5% ($r = 0.5315, P < 0.05$), showing that the PSA and CAL scores, as well as PSA and PD scores, are dependent on each other. Furthermore, a significant and positive correlation was observed between PSA and PI ($r = 0.3231, P < 0.05$) and GI ($r = 0.3567, P < 0.05$) scores at 5% level showing that the

PSA is also dependent on PI and GI scores. A nonsignificant and positive correlation ($r = 0.1331, P > 0.05$) was observed between PSA and BI scores at 5% level showing that the PSA and BI scores are not dependent on each other [Table 12].

Histological examination reveals that mild prostatitis usually appears as a localized process involving a small number of ducts or acini. The lumina are distended and filled with secretion mixed with inflammatory cells among which neutrophils were predominant [Figure 1]. Whereas in severe prostatitis, a localized process involving a large number of ducts or acini are present with the lumina distended and filled with secretion, mixed with large areas of inflammatory cells composed of lymphocytes, plasma cells and giant cells [Figure 2]. Microscopically, prostatic carcinoma composed of small-to-medium-sized glands with irregular outline and inner smooth surface. Individual cells show large, irregular hyperchromatic nuclei, few cells showing nucleoli [Figure 3].

Table 10: Distribution of samples by prostatitis, malignancy, clinical attachment level

Attribute	Category	n (%)
Prostatitis	Mild	16 (34.05)
	Severe	31 (65.95)
Malignancy	M	15 (31.91)
	NM	32 (68.09)
CAL	<3	10 (21.28)
	>3	37 (78.72)

CAL: Clinical attachment level

Table 11: Correlation between prostate-specific antigen levels and clinical attachment level scores across four groups (1A, 1B, 2A, 2B)

Group	Pearson correlation coefficient value	Significance (P)
1A	0.532	0.175
1B	1.000	0.01*
2A	0.684	0.06
2B	0.558	0.002*

*Correlation is statistically significant

Table 12: Relationship between prostate-specific antigen scores with all clinical parameters by Karl Pearson's correlation coefficient technique

Parameters	Relationship between PSA scores with		
	Correlation coefficient r (X, Y)	t	P
CAL	0.5549	4.4742	0.0001*
PD	0.5315	4.2086	0.0001*
GI	0.3567	2.5612	0.0139*
PI	0.3231	2.2901	0.0268*
BI	0.1331	0.9011	0.3723

* $P < 0.05$. PSA: Prostate-specific antigen; GI: Gingival index; BI: Bleeding index; PI: Plaque index; PD: Probing depth; CAL: Clinical attachment level

Table 7: Comparison of types of prostatitis with respect to prostate-specific antigen scores by t-test

Types of prostatitis	Mean±SD	t	P
Mild	3.2375±2.0422	-2.1715	0.0352*
Moderate/severe	18.4787±27.8877		

* $P < 0.05$. SD: Standard deviation

Table 8: Comparison of types of prostatitis with respect to clinical attachment level scores by t-test

Types of prostatitis	Mean±SD	t	P
Mild	2.7571±1.1439	-6.1870	0.05*
Moderate/severe	5.4705±1.5461		

* $P < 0.05$. SD: Standard deviation

Table 9: Comparison of <3 clinical attachment level and ≥ 3 clinical attachment level groups with respect to prostate-specific antigen scores by t-test

CAL	Mean±SD	t	P
<3	3.2450±1.867	-1.6942	0.0971
≥ 3	18.2754±27.9676		

SD: Standard deviation; CAL: Clinical attachment level

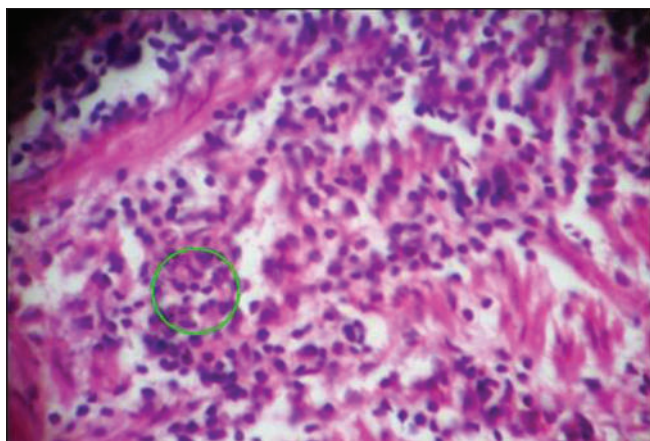


Figure 1: Histological section showing mild prostatitis with inflammatory cells ($\times 100$)

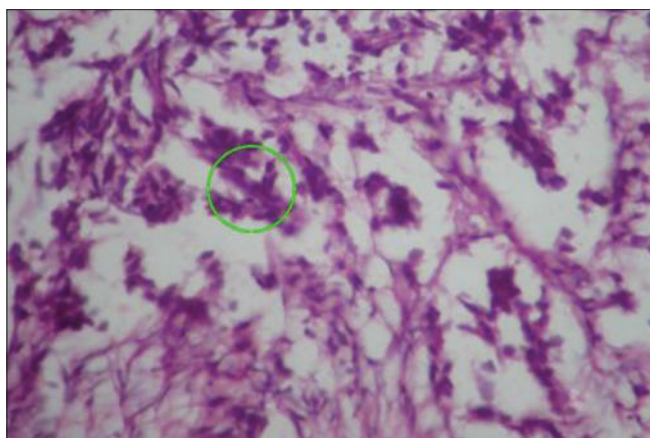


Figure 2: Histological section showing severe prostatitis with inflammatory cells, lymphocytes and plasma cells ($\times 100$)

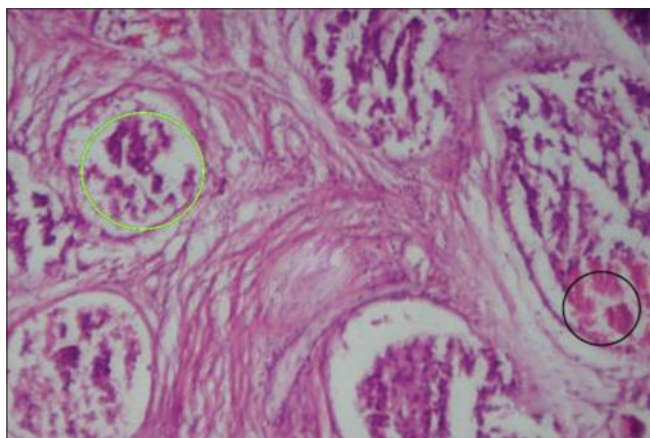


Figure 3: Histological section showing prostate carcinoma with hyperchromatic nuclei and necrosis ($\times 100$)

Discussion

The association between periodontitis and prostatitis is yet to be established. The role played by pro-inflammatory cytokines in the pathogenesis of periodontitis and prostatitis has already been recognized. There is substantial evidence

to establish that periodontal disease leads to amplified levels of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α which were found to be prominent in the serum of men with prostatitis. Thus, a conclusion can be drawn that periodontitis indirectly contributes to prostatitis by increasing inflammatory response to prostatitis.^[7,8] There is another possibility of inflammation of prostate gland resulting in increased PSA levels in the circulation. Periodontium, a distant nonprostatitis source of PSA may also be considered responsible for the increase in serum PSA levels.^[6]

Park *et al.* carried out a study evaluating the optimal baseline PSA level at different ages to estimate the prostate cancer risk (CaP). It was inferred that optimal PSA value that distinguishes the threat of CaP was 2.0 ng/mL for 50- to 69-year-old. Patients having a baseline PSA level higher than the optimal value had a 27.78-fold increase of prostate cancer risk.^[9] Retrospective studies by Lee *et al.* have evaluated the correlation between bone-metastasis serum PSA accumulation in diagnosed prostate cancer (Pca) through a bone scan and concluded that bone scans might be required in men with serum PSA between 10 and 20 ng/mL.^[10]

Along with periodontal parameters like CAL, others like the GI and PI values have also been seriously taken into account and these values have also shown a positive correlation with PSA values. Similarly, when gingival, PI scores were compared between the groups, it was raised in 2B group (CAL ≥ 3 mm with moderate/severe prostatitis) in comparison to other groups, implying that PSA values go up with severe periodontitis and severe prostatitis. Joshi *et al.* studied the link between periodontal disease and PSA levels in chronic prostatitis patients and concluded that patients having comorbidity of CAL ≥ 2.7 mm and moderate/severe prostatitis had higher levels of PSA than those with one of these conditions.^[6]

The present study was also in confirmatory with the findings of Kandirali *et al.*, who showed, higher PSA levels in patients, suffering from moderate-to-severe prostatitis in comparison with those who suffered from no/mild condition of the same. They also proved the affirmative association between the serum PSA levels and the assertiveness of inflammation of prostate glands, through the histopathological findings from the biopsy.^[11]

The rationale for the enhanced volume of serum PSA was related to breach in the epithelial integrity of the cells of prostate glands, rather than increased production of the same as proven by Hasui *et al.*^[12] Wayne Kuzner accomplished that amplified PSA levels in prostatitis patients were due to response to the inflammatory reaction which disrupts glandular epithelium and causes leakage of PSA into the blood stream.^[13]

Furthermore, studies by Morote *et al.* showed that serum PSA levels were directly related to the prostate volume and

not the extent or type of inflammatory infiltrate.^[14] This study was in unison with Nadler *et al.* who confirmed that prostate volume and inflammation are the most important factors contributing to serum PSA elevation in men devoid of any clinically diagnosed prostate cancer.^[15] Because of this disparity in the increased levels of PSA levels, it was assumed that there could be a distant possible nonprostatitis basis of PSA such as periodontium which is responsible for the increasing serum PSA levels.^[14] Diamandis conducted immunological and molecular techniques to demonstrate the presence of PSA protein in various nonprostatic tissues, also its proportionality with steroid hormones and its receptors, and showed that PSA regulation is under the control of steroid hormones and their receptors, thus throwing light on the realization that PSA could also be formed by normal, hyperplastic, and even malignant cells.^[16]

The limitations of the study are portrayed by the lack of supportive evidence which fails to provide a causal affiliation to periodontitis and high serum PSA levels, due to the size of the sample and manner of the cross-sectional study, in spite of deducing a favorable association between periodontitis and prostatitis. The prostate needle biopsy sample being non-representative of the pathology of the whole gland was not dependable for a confirmatory diagnosis. Furthermore, an estimation of prostate volume by transrectal ultrasonography was not done in this study. Hence, longitudinal studies with larger sample size are required to establish association between periodontitis and prostatitis. Substantial efforts are underway to improve the reliability of the study by aiming principally at minimizing the false-positive test results, thereby improving the specificity of the test. Alwithanani *et al.* have conducted a study to assess the changes in serum PSA and inflammatory cytokine levels such as CRP and IL-1 β after nonsurgical periodontal treatment in men with chronic periodontitis, and concluded that the periodontal treatment improved prostate symptom score and lowered PSA value in men afflicted with chronic periodontitis.^[17] In the 12 years longitudinal study conducted by Lee *et al.* in South Korea to assess the relationship between periodontal disease and prostate cancer, using data in the National Health Insurance Service–Health Examinee Cohort, have inferred that periodontal disease is significantly and positively associated with prostate cancer.^[18]

Conclusion

Patients with moderate/severe prostatitis and the same grades of the severity of periodontitis were found to have higher PSA levels. The clinical parameters of periodontitis were also found to be significantly increased in patients with moderate-to-severe prostatitis, revealing a probable pathological link between the two.

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Conflicts of interest

There are no conflicts of interest.

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Case Report

Periodontally accelerated osteogenic orthodontics: Novel perio-ortho interrelationship

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Abstract:

Periodontally accelerated osteogenic orthodontics (PAOO) is a clinical procedure that incorporates selective corticotomy, particulate grafting, and application of optimal orthodontic forces. It reduces treatment time, increases stability of teeth, and prevents relapse of orthodontic tooth movement (OTM). The present case report highlights the technique and principles of PAOO for rapid OTM.

Key words:

Corticotomy, orthodontic tooth movements, periodontal accelerated osteogenic orthodontics, piezoelectric surgery

INTRODUCTION

Malpositioned teeth are responsible for esthetic and occlusal aberrations in many adults. Patients frequently avoid orthodontic treatment because of its long duration. Orthodontic movements lead to compression of periodontal ligament which leads to remodeling of alveolar bone. Moreover, orthodontic tooth movement (OTM) is a periodontal phenomenon as all periodontal tissues are involved. It is difficult to maintain periodontal integrity on long-term orthodontic movement.^[1] Since orthodontic treatment takes a longer duration, there are chances of gingival inflammation, root resorption, decalcification, and dental caries.^[2] Hence, reducing the treatment time by increasing the rate of tooth movement is considered as an appropriate goal for successful orthodontic treatment.^[2]

A novel treatment procedure to maintain periodontal integrity and to shorten the treatment time had been introduced by Dr. William Wilcko (orthodontist) and Dr. Thomas Wilcko (periodontist) known as periodontally accelerated osteogenic orthodontics (PAOO) popularized as Wilckodontics.^[1,3]

Piezoelectric bone surgery is a new technique developed by Vercellotti.^[4] The principle behind piezosurgery is, when an electric energy is applied, ceramic discs contract and expand, which causes ultrasonic transduction, and ultimately these vibrations are transferred to the insert of the drill that is applied on mineralized tissues such as bone.^[5] These vibrations are in the range of 25–29 kHz that cut only the hard tissues

and frequency of above 50 Hz will cause damage to soft tissues.^[6] Piezosurgery provides precise, clean, and smooth cutting with excellent visibility.

G-Graft is the combination of crystalline hydroxyapatite and bovine collagen.^[7] Osteoconductive materials such as hydroxyapatite or tricalcium phosphate will act as osteoinductive material when used in combination with collagen; with this adjunct effect, G-Graft results in earlier bone regeneration and high dense mature bone. Studies also have shown that combination of type 1 collagen and hydroxyapatite has synergistic effect which accelerates the osteogenesis.

This case report is an attempt to highlight the technique and principles of PAOO for rapid OTM along with the use of piezo surgery for precise incisions.

CASE REPORT

A 28-year-old female patient was referred to the Department of Periodontics, for corticotomy

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procedure for retraction space closure. Although the alignment was almost completed in anterior region, retraction space was not closed. The reason was that the tooth movement was beyond the envelope of motion which led to failure of retraction space closure. This is the perfect indication for PAOO procedure.

Intraoral examination revealed Angle's Class I molar relation. The patient was on arch expansion in maxilla [Figure 1a]. After profound anesthesia, a full-thickness mucoperiosteal flap was then elevated extending 3–4 mm beyond the mucogingival junction. With the help of piezosurgical inserts under cold saline irrigation, vertical grooves were placed in the inter-radicular space, midway between the root prominences in the alveolar bone from the distal surface of extraction space on one side to the distal surface of extraction space on the other side. The vertical cuts extend 2–3 mm from the alveolar crest to approximately 2 mm beyond the apices of the roots. At the apical aspects of the roots, semilunar corticotomy cuts were made to join these vertical cuts [Figure 1b]. Perforations were made in the alveolar bone over the radicular surfaces with a round bur.

After the placement of the corticotomy cuts, required amount of G-Graft (particle size 0.9 mm–1.9 mm) was placed in the vertical corticotomy sites [Figure 1c]. The decorticated bone helped to retain the graft material. The flap was adapted to normal position without tension and suturing was done by nonresorbable silk suture. The sutures were left in place for 10 days [Figure 1d].

Capsule Novamox 500 mg was given thrice daily for 5 days as an antibiotic. Tablet Ultracet was prescribed twice daily for 5 days as analgesic. The patients were instructed not to brush the operated area for 1 week and to rinse the oral cavity twice a day with chlorhexidine (0.2%) mouthwash daily. Sutures were removed after 10 days postoperatively. Orthodontic

treatment was started within 2 weeks after surgery. 250 g of force was applied on both sides and appliance activation was done every 2 weeks.

The retraction space was closed within 4 months after surgery which is possible only with PAOO [Figure 1e]. Bone density changes were evaluated before and after surgery through computed tomography (CT). CT scan was used for evaluating the changes in the alveolar bone thickness as well as bone density before and after the surgical intervention and retraction of anterior teeth. For the assessment of alveolar bone thickness, contiguous 0.6-mm slice-thickness CT scans were obtained for the maxillary incisors at 120 kV and 175 mA, with the window width set at 2000, 1500 Hounsfield Units. For each tooth, the density of the labiolingual alveolar plates was measured to the nearest 0.2 mm. The same measurements were repeated between 6–7 months after incisor retraction was completed (depending on the case). The evaluation of radiographic density (HU) was done by Siemens Healthcare GmbH, Henkestr. 127 91052 Erlangen, Germany. Postsurgery CT demonstrated an increase in the bone density after 6 months of surgery. The CT image has shown the change in the bone density after the procedure, that is, from 405 to 456 HU [Figure 2a and b].

DISCUSSION

According to the American Association of Orthodontists, the comprehensive orthodontic period ranges between 18 and 30 months. It depends on the severity of malocclusion, any associated bony deformities, and treatment options. The treatment period for nonextraction and extraction case varies between 21–27 and 25–35 months, respectively. Major drawbacks of longer treatment time are increased risk for caries, periodontal diseases, and root resorption. Hence, the prime goal for orthodontists is to reduce the treatment span so that it alleviates the drawbacks of longer treatment time and also improves the patient satisfaction.

Various approaches are there to reduce the treatment span. One among them is administration of parathyroid hormone, thyroxin, Vitamin D3, and prostaglandins. Local/systemic administration of these biologic factors resulted in local pain, severe root resorption, and drug-induced side effects. In order to overcome these effects caused by the drugs, trend turned toward mechanical approach to accelerate the tooth movement. Mechanical approaches include electric current, magnets, laser beams, mechanical vibration, and ultrasound. Apart from this, the technique which has received maximum

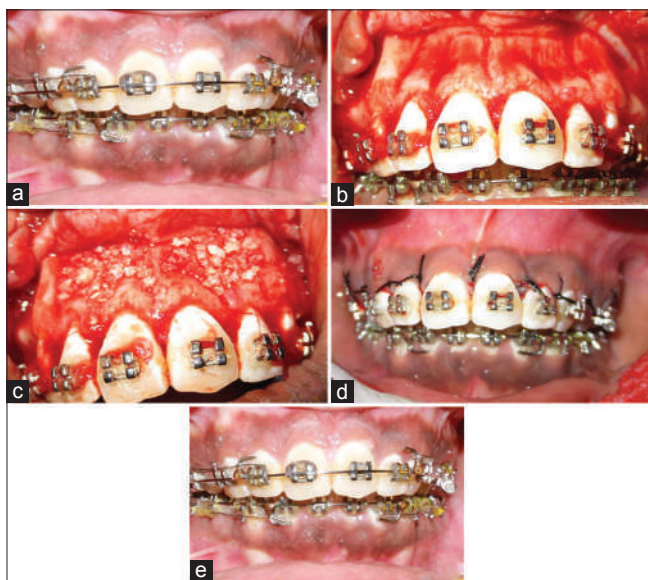


Figure 1: Preoperative image (a); Mucoperiosteal flap elevation and corticotomy (b); Particulate grafting with G-Graft (c); Suturing was done with 3-0 Silk (d); Postoperative image after 6 months (e)

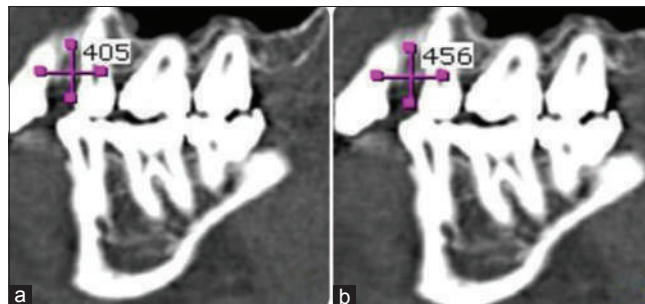


Figure 2: Preoperative image of bone density (a); Postoperative image of bone density after 6 months (b)

attention is the surgical manipulation of bone using dental distraction, alveolar surgery to underline interseptal bone, and corticotomies. These approaches focus mainly on the micro movements of the alveolar bone, thereby reducing the tissue resistance.

Orthodontic relapse is the most common posttreatment complication regardless of the method used. To overcome this, surgically facilitated orthodontic treatment (SFOT) procedures have become popular. Though the conventional OTM and SFOT have got advantages and disadvantages in their own way, patients must be aware of all treatment alternatives.

PAOO is one of the SFOT procedures. PAOO is a clinical procedure that combines selective decortication, alveolar augmentation, and application of orthodontic forces. It is based on regional acceleratory phenomena (RAP), the concept first described by Herald Frost in the year 1983. RAP was described as a local response of the tissue to a noxious stimuli resulting in accelerated regional regeneration process. The principle of RAP is to accelerate the repair process and to attain functional recovery during bone remodeling. The bone injury caused by piezo incision triggers bone remodeling which is the part of RAP. Demineralization of bone leads to faster movement of tooth adjacent to the decortication site. Particulate grafting leads to increase in the bone thickness which decreases relapse of OTM and increases postorthodontic stability. In this case, retraction was started 2 weeks after surgery and the retraction space was closed within 4 months as recommended by Wilcko *et al.*^[1]

CT was preferred in this study for evaluating the bone density before and after the surgical intervention. This was supported by a similar study done by Vercellotti and Podesta.^[8] In this case, piezo electric surgical insert was used to stretch the incision for examination of dental and osseous topography, similar to a study which was done by Dibart *et al.*^[9] The use of CT and a piezo inserts for placing vertical incisions was in contrary to a study by Jofre *et al.*, in which they have used metal markers for the placement of vertical incisions.^[10]

There have been several reports regarding adverse effects on the periodontium after corticotomy, ranging from no problems to slight interdental bone loss and loss of attached gingiva to periodontal defects observed in some cases with short interdental distance, but no such changes were observed in the present case report. Subcutaneous hematomas of the face and the neck have been reported after intensive corticotomies. However, no such complications were reported in this case. In this case report, activation of appliance was started within 2 weeks after surgery to take the advantages of RAP, which is limited duration effect. In the present case report, G-Graft was used for particulate grafting which showed increase in radiographic bone density after 7 months of surgical procedure. According to Murphy *et al.*, no objective data exist comparing one grafting material with another in terms of superiority.^[2]

The present case report confirms the dental CT scanning utility in diagnostic imaging of the buccal, palatal, and interdental bones. Bone density measurements by CT scanning offer more precise results compared to radiologic evaluation. Therefore,

bone density measurement by this method can offer more valuable data than other methods. Studies have shown that alveolar corticotomies not only accelerate the orthodontic treatment but also provide the advantage of increased alveolar width to support the teeth and the overlying structures.

A large sample size with long-term follow-up is required to assess the full effect of PAOO in accelerated orthodontic movement.

CONCLUSION

PAOO reduces the treatment time when compared to conventional orthodontic treatment. PAOO increases the bone volume posttreatment and covers the vital surfaces of the root, which contribute to the postorthodontic stability as well as repair of alveolar dehiscence. The periodontist should consider an appropriate technique according to the alveolar topography to avoid complications and to assist accelerated OTM. With the increasing esthetic demands and a desire for rapid treatment outcome, PAOO technique can be an attractive treatment alternative.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understand that her name and initial will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Research Paper

Remineralizing potential of commercially available pediatric dentrifices: An *In vitro* study

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ABSTRACT

Background: Remineralizing agents are found to be effective in the remineralization of early enamel caries and the remineralized enamel crystallites are more resistant to decalcification and also have the same orientation as the original enamel crystallites. Although child formula fluoridated dentrifices are used safely by young children their remineralizing capability remains questionable.

Aim: To evaluate the remineralizing potential of three commercially available pediatric dentrifices on artificial carious lesions in primary teeth.

Materials and methods: A total of fifty sound human primary teeth were coated with nail varnish leaving a window of 2 mm × 4 mm on buccal surface and were subjected to demineralization for a period of 96 h and then sectioned. Out of the hundred sections obtained, ninety sections are equally divided into three groups with thirty in each group: Group I (Colgate Spider man), Group II (Kids bunny), Group III (Kidodent) subjected to remineralization respectively for 10 days using pH cycling model. The sections were then evaluated under the stereomicroscope for the remineralization values using MICAP image analyser software.

Results: Statistical analysis was done by using ANOVA test which showed that all the three dentrifices showed remineralization with artificial carious lesions. Among the three test groups, Group I showed higher remineralization potential compared with the other two pediatric dentrifices which was statistically significant ($p < 0.05$).

Conclusion: All the three dentrifices demonstrated remineralization of carious lesions by virtue of decrease in lesion depth.

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1. Introduction

In the oral cavity delicate balance exists between de/remineralization of the enamel surface and the interruption of this balance leads to dental caries which is considered as the most common disease of childhood worldwide.

Research has demonstrated that it is pH of saliva rather than sugar considered as the discriminating factor for cariogenic plaque biofilms. When the pH of the saliva falls below 5.5 (critical pH), the process of enamel demineralization begins which results in the loss of calcium and phosphates from the surface and subsurface enamel creating a white spot lesion [1]. Many studies have shown that early carious lesions have a potential for remineralization, with an increased resistance to further acid challenge, particularly with the use of enhanced remineralization agents [2].

This new sight into caries provided the scope for promoting remineralization and fluoride is one such most substantial agents, among which dentifrices are considered as the common source of topical fluoride available for majority of children [3]. Myriad of dentifrice brands are available at the local supermarket that improves the oral health due to their cariostatic action and are generally used as a part of normal body hygiene procedures. Various fluoride compounds are tested for caries inhibitory properties among these sodium fluoride (NaF) and sodium monofluorophosphate are two fluoride agents most widely used in toothpaste [4]. Fluoride ions help in remineralization by the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralization [5].

Many investigators have studied the de/remineralization of enamel lesions in permanent teeth by conventional fluoridated and nonfluoridated dentifrices, and there are only a few studies that have tested the effect of low fluoride concentration dentifrices on primary teeth. So the aim of this *In Vitro* study was to evaluate and compare the de/remineralizing potential of different child formula dentifrices with a low fluoride concentration when applied to artificial caries lesions in primary tooth enamel.

2. Material and methodology

After obtaining institutional ethical committee approval, the present *In Vitro* study was conducted which included fifty sound extracted or naturally exfoliated human primary anterior teeth. With a mean lesion depth of 0.95 μm was taken from the literature review and Type I error rate of 0.05 and power of 0.8 the sample size was calculated. Carious, hypoplastic discoloured teeth and teeth with cracked areas and white spots were excluded from the study. The selected teeth were thoroughly cleaned and hand scaled to remove soft tissue debris, which were then stored in 0.1% thymol until the experimental procedure was initiated.

3. Artificial caries lesion formation

Teeth were dried and coated with acid resistant nail varnish leaving a rectangular window of 2 mm \times 4 mm on sound intact

labial surface. Then they were then immersed into a glass container containing 500 ml of demineralizing solution for a period of 96 h to produce artificial carious lesions. This demineralizing procedure was intended to produce a white spot lesion.

4. Demineralizing solution

1050 ml of distilled water was taken in a beaker and 2.2 g of calcium chloride was added to it. To this, 2.2 g potassium hydrogen orthophosphate, 3 g of acetic acid and 56 g of potassium hydroxide was added. The pH of this solution is maintained at 4.5 [10].

5. Sectioning and dividing the samples into groups

Teeth were then longitudinally sectioned using diamond disc mounted on a low speed hand piece in buccolingual direction, such that a part of the demineralized area and normal enamel is present in each section. A total of hundred sound sections were obtained, which further divided into three groups with thirty sections for each dentifrice group: Group I Colgate kids Spiderman[®] with 600 ppm of sodium fluoride, Group II Kidodent[®] with 500 ppm of sodium monofluorophosphate and Group III Kids bunny[®] with 500 ppm of sodium monofluorophosphate. Ten sections from the total sample were discarded due to damage occurring during sectioning and inadequate enamel thickness (chipping of enamel while sectioning).

6. Recording depth of demineralization

The teeth sections in each group were then studied under the stereomicroscope at 40 \times magnification to determine the depth of demineralization using MICAP Micro view software. The depth of demineralization was noted at three different points on the demineralized area and the average of these three values was considered as depth of demineralization. The demineralization depth was measured in micrometres from the surface of tooth to the maximum depth of demineralization. (Fig. 1).

7. Remineralizing solution

152 ml of distilled water was taken and 0.1665 g of calcium chloride, 0.108 g of sodium hydrogen phosphate and 11.25 g of potassium chloride was added to it. The pH of this solution is maintained at 7 [10].



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8. pH cycling

After obtaining the demineralized values the samples in each group were subjected to pH cycling model used by Ten Cate and Duijsters [6]. Each section in the test group was then



Fig. 1 – Tooth section showing demineralized enamel surface.

treated with respective dentifrice solution which was prepared by 3:1 ratio of deionized water to dentifrice in a pH cycling for a period of 10 days. Fresh slurry was prepared for all three dentifrices before each use.

9. Recording depth of remineralization

After the completion of pH cycling the sections in each group were again evaluated under stereomicroscope at 40× magnification for determining the lesion depth in the same way as measured for demineralization. (Fig. 2).

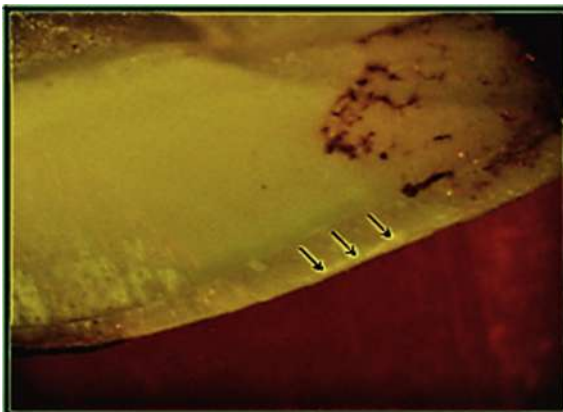


Fig. 2 – Tooth section showing remineralized enamel surface.

The entire procedure was performed by a single operator. However, to avoid bias second operator who was unaware of the prior results evaluated the samples randomly. As the inter examiner variability was not significant the scores given by the first investigator were only considered. The data obtained were tabulated and subjected to statistical analysis. Paired-t-test, one-way ANOVA and Bonferroni multiple comparison test, Student newmann Kleus test were performed.

10. Results

In the present study, remineralizing potential of three commercially available dentifrices were evaluated on artificial carious lesions. The mean lesion depth and standard deviations were calculated by Paired-t-test, and inter group comparison by one-way ANOVA and Bonferroni multiple comparison test. The p value was taken as significant when less than 0.05. The mean lesion depth of three groups after demineralization ranges from 68.26 μm to 71.39 μm . The mean lesion depths were not significantly different when compared between three groups after demineralization. The mean lesion depth of three groups after remineralization ranged 53.19 μm –76.0 μm . The difference in mean lesion depth is more in the Group I (16.39 \pm 10.92 μm) followed by Group II (8.21 \pm 12.72 μm) and Group III (6.83 \pm 11.15 μm). The three test groups showed decrease in mean lesion depth after remineralization as evident from the results observed by paired t-test with a statistically significant result is (P-value of 0.0062) seen in Group I whereas P > 0.05 is seen in other groups which indicates insignificant P-value (Table 1).

While the Intergroup comparison is made between test groups by using Bonferroni multiple comparison it showed insignificant p-value of 0.8617 for group I and II and similar results were obtained when comparing group II and III and for group III and I with p-value of 0.6680, 1.0 respectively (Table 2).

11. Discussion

Remineralization is the natural repair process for non-cavitated lesions, and relies on calcium and phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization [7].

Demineralization of tooth at an early stage can be reversed naturally by saliva with a natural buffering capacity, which contains calcium and phosphate ions, buffering agents, fluoride and other substances. The strategy behind the

Table 1 – Mean lesion depth after demineralization and remineralization and P-value.

Groups	Mean lesion depth after demineralization	Mean lesion depth after remineralization	Difference	P value
I	69.57 \pm 7.81 μm	53.19 \pm 9.35 μm	16.39 \pm 10.92 μm	0.0062*
II	68.26 \pm 6.69 μm	60.04 \pm 6.98 μm	8.21 \pm 12.72 μm	0.1879
III	71.39 \pm 6.52 μm	65.71 \pm 3.92 μm	6.83 \pm 11.15 μm	0.2083

*Statistically significant if P \leq 0.05.

When paired t-test was used to compare the three groups, statistically significant results were seen in Group I (P value 0.0062) whereas in other groups the P value was insignificant.

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Table 2 – Inter group comparison between the test groups.

Groups	P value	Inference
I–II	0.8617	NS
I–III	0.6680	NS
III–II	1.0000	NS

NS – Not Significant.

Multiple pair wise comparison between groups after remineralization were done by using Bonferroni multiple comparison test showed no statistically significant difference in mean lesion depths between any of the compared groups.

remineralization is to deliver ions directly to where and when they are mostly needed [8]. These remineralized crystals are acid resistant, being much less soluble than the original mineral [7]. Several mechanisms are available for aided remineralization. The most well-known concept is the delivery of topical fluoride, which has been proven to be a highly effective measure for prevention of caries [8].

Fluorides used for remineralization are available in various forms such as fluoride varnishes, fluoride mouth washes, fluoride gels and fluoridated dentifrices. The most common source of topical fluoride for the majority of children is fluoride dentifrices, as they have been accepted as one of the most important factors in the decline of dental caries [8]. Apart from its benefits, a controversy always arises with the fluoride toothpaste is ingestion and risk of fluorosis in children. Dose response relationship of fluoride dentifrices is important because dentifrices used for children contain between 250 and 500 ppm fluoride. Though the risk of fluorosis is low with small quantities of fluoride, they should be sensible against not reducing the cariostatic effects. Therefore the present *in vitro* study was planned to evaluate the efficacy of remineralization of fluoride dentifrices with low ppm of fluoride on artificial carious lesions in primary teeth.

In the present study the dentifrices Colgate kids Spiderman, Kidodent and Kids bunny were selected because they are readily available commercial pediatric dentifrices with low fluoride content that are commonly used by children.

In Vitro de-remineralization studies using pH cycling model provide a valuable tool to investigate the fluoride efficacy [9]. A 'pH cycling' is applied to an *In Vitro* experiment involving the exposure of specimens (enamel and/or dentine) to a combination of remineralization and demineralization solutions along with remineralizing agent. These experiments were designed to stimulate the dynamic variations in mineral saturation and pH associated with the natural carious process. According to TenCate and Duijsters, the best way to simulate the environment *In Vitro* is to apply pH cycling models that are equivalent to dynamics of dental decay [6]. The solution concentration and pH should be kept within the range that exists in the oral cavity. So, in our study freshly prepared demineralizing and remineralizing solutions are used to avoid the risk of the solutions becoming saturated and pH value was also checked prior to every use.

The results of the present study reveals that all tested dentifrice groups showed decrease in lesion depth after remineralization and this decrease in lesion depth in the test groups can be attributed to presence of fluoride intervention

in the form of dentifrices. Among the three test groups Group I showed more remineralization potential followed by Group II and Group III. However, the mean difference in the lesion depth before and after remineralization between the three test groups was statistically significant for Group I where as in other three groups it was statistically insignificant. Group I showed greater remineralization potential compared to other groups, the probable reason could be attributed to the greater the amount of fluoride content (600 ppm) and the type of the fluoride compound that is used (sodium fluoride).

The results of the present study are similar to the study done by Advani et al. (2014) [10] evaluated the remineralization potential of one regular dentifrice Colgate total (1000 ppm F) and two pediatric dentifrices that is Kidodent (458 ppm F) and Cheerigel (500 ppm F) following *In Vitro* pH cycling model. Their results revealed that Colgate total showed more remineralization potential compared to other two pediatric dentifrices which could be attributed to the high fluoride content. Likewise, another study was conducted by Ebrahim et al. [11] (2011) in which they evaluated the remineralization potential of four dentifrices: Crest (Adult type with 1100 ppm F), Crest (Children type with 500 ppm F), Poonah (500 ppm F) and Poonah without fluoride and the results revealed that Crest (Adult type) showed higher remineralization potential compared to others. In the above two study's authors have compared the adult dentifrices with pediatric dentifrices and their results showed higher remineralization potential with adult dentifrices, because of presence of high fluoride content. However, pediatric dentifrices should never be compared with adult dentifrices because of disparity in the fluoride content.

Studies have also proven that the remineralization potential of a dentifrice depends on the amount of fluoride and the type of the fluoride compound that is present in the dentifrice. Fluoride bioavailability is important for caries prevention and it is particularly dependent on the solubility of the fluoride containing compound and the adhesion of the fluoride compound to the surface. In dentifrices, fluoride formulations like sodium fluoride, sodium monofluorophosphate and amine fluoride are frequently used as the carrier for fluoride ions.

Beiswanger et al. [12] (1989) proposed that sodium fluoride provided consistently improved anti-carries efficacy relative to sodium monofluorophosphate. This was further supported by Rank (1991) who preferred sodium fluoride over sodium monofluorophosphate. The critical review by Stookey et al [13] (1993) demonstrated that sodium fluoride was significantly more effective than sodium monofluorophosphate in preventing caries and also recommended that sodium fluoride to be used as the active system in fluoridated dentifrice whenever feasible.

Sattler et al. [14] (1993) also demonstrated that in the presence of human saliva the bioavailability of fluoride from NaF containing dentifrices was significantly greater than from NaMFP containing dentifrices.

The present study result showed that fluoride ion from sodium fluoride was more effective as compared to sodium monofluorophosphate. This might be due to the reasons that in the present study the solution that was used for the remineralization of artificial carious lesions constitutes only inorganic components without any enzymatic system. Whereas, for the

sodium monofluorophosphate to be effective there should be presence of enzymatic system. This can also be attributed for the poor performance of sodium monofluorophosphate due to absence of enzymatic system in the inorganic components (remineralizing solution) used in this study.

Based on the results obtained from the present study it can be suggested that fluoride in low concentrations as in child formula dentifrices can effectively slow down the progression of a carious lesion and remineralize the demineralized lesion. In general while prescribing fluoridated toothpaste it is strongly recommended to take into consideration the patient's caries risk and fluoride toxicity. However certain limitations exist in this *In Vitro* study. The pH cycling model has not entirely simulate the oral conditions where the pH fluctuates frequently and also depends on individuals eating habit, oral hygiene practice, fluoride usage, composition and quality of saliva and plaque.

12. Conclusion

The following conclusions were obtained from the present study:

- All the three dentifrices in Group I [Colgate Spiderman (600 ppm NaF)], Group II [Kid's bunny (500 ppm MFP)] and Group III [Kidodent (500 ppm MFP)] demonstrated remineralization of carious lesions by virtue of decrease in lesion depth.
- Group I showed higher remineralization potential compared to other Group II and Group III because of high fluoride content and presence of sodium fluoride.

Conflict of interest

No conflicts of interest.

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Dermatoglyphic Analysis in Parents with Cleft Children: A Comparative Study

Abstract

Background and Objective: There were studies in the literature which showed the altered dermatoglyphics in the cleft children. But it would be beneficial if probable expression can be proved in prior generations, so that genetic counseling and other preventive measures can be undertaken. The aim of the study is to determine variations in dermatoglyphics of prior generations which would serve as bench markers to predict the occurrence of cleft in off springs. **Materials and Methods:** A total of 400 parents aged between 25–45 years were selected and divided into two groups - Group A consisted of parents with cleft children and Group B comprised of parents with at least 2 healthy children who met the inclusion criteria. Dermatoglyphic prints were collected by ink method and evaluated for pattern types, total ridge count and palm prints were evaluated for a-b ridge count, atd angle and asymmetry. **Results:** Significant difference was observed between Group A and B with reference to pattern types; TRC and a-b ridge count and atd angle. Absence of t point was variably noticed in the mothers of affected children. **Conclusion:** Dermatoglyphics can be used as tool to study the developmental instability of cleft anomalies and provides data to assess the genetic etiology of clefting.

Keywords: A-b ridge count, ATD angle, dermatoglyphics, nonsyndromic cleft lip/palate, pattern types, total ridge count

Introduction

The term dermatoglyphics is derived from the Greek word Derma – Skin and Glyphic – Carving, coined by Cummin and Midlo in 1926.^[1] It is an established fact that no two individuals, including monozygotic twins, have the same fingerprints and other details of dermal ridges. Thus, fingerprints are unique to each person and they are not altered during lifetime due to disease, age, or any reason.^[2] Owing to these facts, dermatoglyphics has been a useful tool in understanding basic questions in biology, medicine, genetics, and evolution, and it is the best and most widely used method of personal identification over the past 150 years.^[3]

The widespread medical interest on epidermal ridges has clearly witnessed the association between chromosomal aberrations and unusual dermal ridge presentations.^[4] Craniofacial disorders and syndromes known to be associated with

gross variation in dermatoglyphic pattern presentation, asymmetry and unusual expression of patterns such as dermal ridges, and craniofacial structures take their origin from fetal volar pads and develop from embryonic ectoderm.^[5] Morphogenesis of dermatoglyphic structures and organogenesis coincide with each other in time period and programmed with genetic expressions which are interrelated.^[6] Investigations suggest that both the dermal patterns and craniofacial organs are genetically governed structures yet influenced by intrauterine environment. Affliction of anyone of these will adversely affect the other.^[7] Nonsyndromic cleft lip and/or palate (NSCL/P) is a relatively common condition among the world's population, occurring 1 in 700–2000 live births and the etiology of such clefts is complex where interplay between genes and environment would contribute to the development of cleft. Some clefts are of nongenetic origin and their occurrence cannot be determined with genetic analysis.^[8] Some individuals have increased genetic liability for having a

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child with CL/P often fail to be recognized, as they do not have CL/P for themselves. This happens because incomplete penetrance and variable gene expressivity, but this developmental instability (DI) would have autosomal dominant inheritance pattern and manifests itself as cleft in their offspring's.^[9,10] Phenotypically, such DI may manifest through abnormal dermatoglyphic pattern presentation.^[11]

The correlation between cleft and dermatoglyphic alterations in the affected has been clearly demonstrated in the literature, this only gives insight into the association between craniofacial anomalies and dermatoglyphics, thus this is not helping us to predict the future probable occurrence of cleft. But in the literature, it has been clearly validated that in the instances of NSCL/P of genetic origin, the tendency for clefting starts in the previous generations itself. The phenomenon of incomplete genetic penetration most often associated with genetic NSCL/P is responsible for its nonoccurrence. This incomplete genetic penetration may phenotypically exhibit some alterations in dermal patterns.^[9]

Hence, this particular study was planned to determine the degree of DI which had been phenotypically expressed in the parents as dermatoglyphic variation. This may help in predicting the future risk of cleft in genetic NSCL/P cases. Thus, this is an attempt to determine the usefulness of dermatoglyphics in studying the genetic etiology of CL/P.

Material and Methods

In the present study, a total of 400 healthy individuals, that is, 200 couples (200 mothers and 200 fathers) aged between 25 and 45 years were included. They were divided into two groups based on the presence or absence of cleft in their offsprings. Group A – 100 couple were the study sample (Group A_M 100 mothers and Group A_F 100 fathers) having one or more cleft children and Group B – 100 couple were the controls (Group B_M 100 mothers and Group B_F 100 fathers) with at least two healthy children.

Individuals with congenital and acquired deformities of fingers and palms, any congenital malformations, skin diseases, history of maternal problems or diseases during pregnancy, and family history revealing cleft in the previous generations were excluded from the study.

Sample selection and obtaining dermatoglyphic data were accomplished during January 2015–June 2016. Group A sample was selected from various cleft rehabilitation institutes. Group B sample was selected from outpatient block, department of pedodontics and preventive dentistry.

Before starting up the study, permissions were obtained from selected institutional heads to carry out the procedure. Before the sample collection, informed written consent was obtained from the participants. Dermatoglyphic prints were collected by ink method (Inkredible Turbo Chrome Black; Hubergroup India Pvt. Ltd.) which included finger and palm prints.^[12]



Figure 1: Armamentarium

Rolled prints of each finger and palm were taken from each participant using ink method in which the digits and palms were inked by rolling them across the roller onto which black ink is coated. A sheet of paper is placed on top of a foam rubber pad on a flat, stable surface. The foam pad was used to feel the concavity of the palm. Then, the wrist was placed on to the bottom of the paper, and the rest of the palm and digits were pressed on to the paper [Figure 1].

Handprints were labeled by the side (right and left) and abbreviations were given to each finger (thumb – T, index finger – I, middle finger – M, ring finger – R, and little finger – L) [Figure 2].

Fingerprints

Fingerprints were evaluated for pattern types and total ridge counts (TRCs).

One of the three patterns was identified on each fingertip, namely, Arch (A), Loop (L), and Whorl (W). The categories were after Cummins and Midlo^[1] [Figure 3a-c].

TRCs were calculated by drawing straight lines between the center of a fingerprint pattern (core point) and the corresponding triradius [Figure 3d]. The term triradius refers to the area or the point that is surrounded by three different directional ridges, the angles of which are about 120°.

Arch has no triradius and belonged to the simplest pattern; the TRC of the arch is 0. The loop has one triradius and one core point, the total number of ridges between these two points has to be taken into consideration. The whorl has two triradii and one core point, the larger of which has to be taken into account [Figure 3e-g].

Palm prints

Palm prints were evaluated for a-b ridge count and ATD angle.

There are 5 triradii on each palm, 4 of which will be located on the distal palm just inferior to the 2nd, 3rd, 4th,

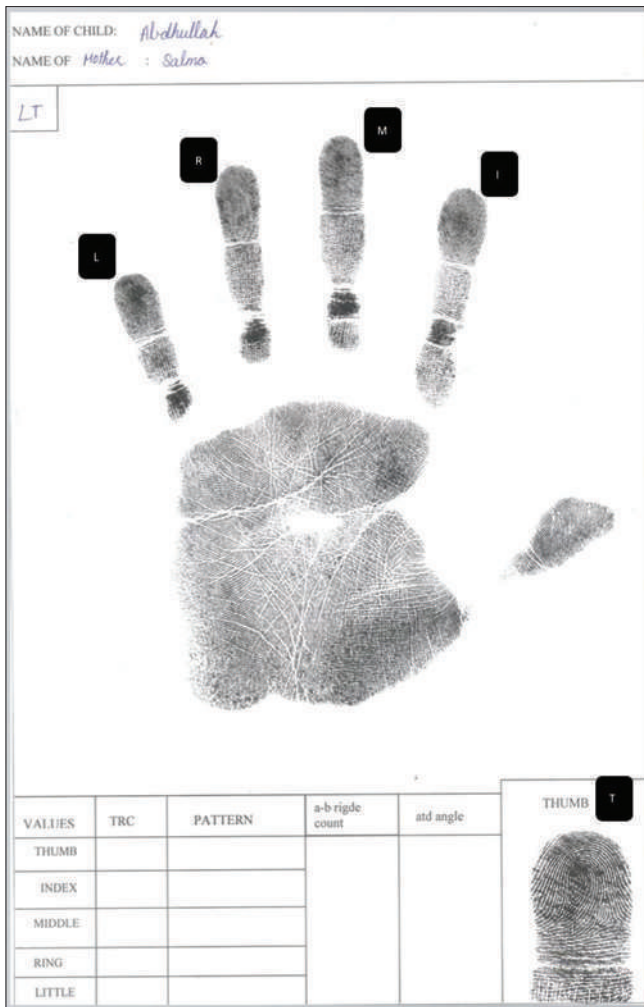


Figure 2: Handprint with labeling

and 5th fingers and were named as a, b, c, and d triradii, respectively. The remaining one will be t triradii, the location of which varied on the proximal palm at the junction of thenar and hypothenar areas [Figure 4A].

a-b ridge counts were captured by drawing straight lines between a and b triradii and counting the ridges that cut through them [Figure 4B].

ATD angle is measured by capturing the relative position of three triradii (a, t, d), by drawing two straight lines through a and t and d and t [Figure 4C].

All the measurements and counts were independently assessed and were randomly cross-verified by the trained investigators who were blind to the study.

Asymmetry of pattern types was determined by calculating the dissimilarity scores. These scores were calculated by assigning “0” when the fingerprint type was identical for the same digit on the right and left hands and “1” when the fingerprint the patterns were different. Finally, the scores over all five pairs of digits were summed. For each individual, the dissimilarity scores can range from

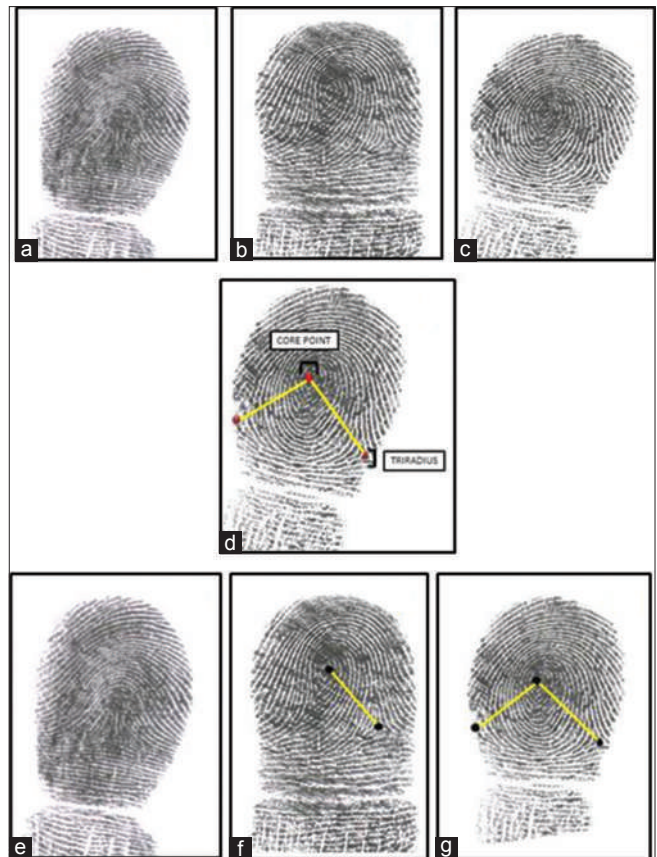


Figure 3: (a-c) Pattern Types – A: Arch; B: Loop; C: Whorl. (d) Core point, triradius point. (e-g) Total ridge count

0 to 5. Except the score of 0, rest all were considered as asymmetrical.

Asymmetry of TRC, a-b ridge count, and ATD angles between right and left hands are determined by subtracting the values of the right hand from the left hand in each group.

Pattern types, TRC, a-b ridge count, and ATD angles were measured on both hands of all groups and comparisons of each entity were done between Groups A_M and B_M, A_F and B_F, and A_M and A_F.

The obtained values are formulated on Excel sheet and are sent for statistical analysis using IBM® SPSS 20 (software package for statistical analysis).

As the pattern type represents the qualitative data chi-square test was performed to test the significance between the groups. Whereas TRC, a-b ridge count and ATD angle asymmetry score being quantitative data, independent t-test was performed.

Results

Table 1 depicted the mean values of TRC, a-b ridge count, and ATD angle. There was a significant increase in TRC, a-b ridge count of both the hands in the study group when compared to their respective controls, whereas ATD angle

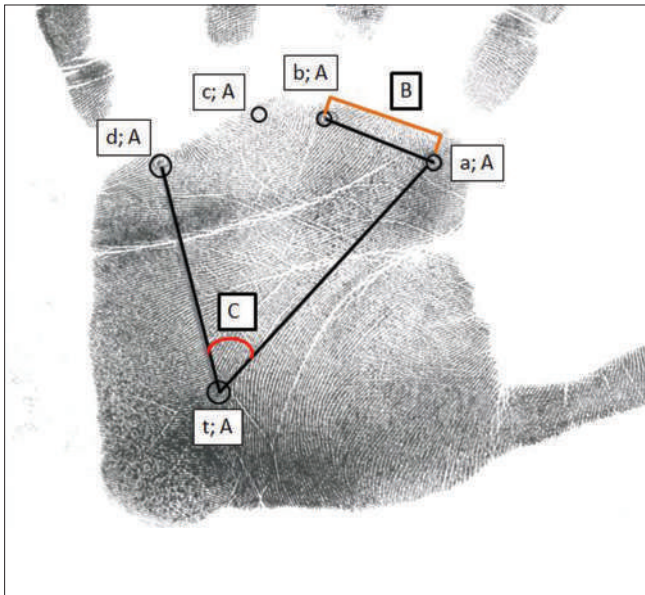


Figure 4: (A) Triradii – a, b, c, d, t. (B) A-b ridge count. (C) ATD angle

showed significant increase with respect to the values of the right hand. Table 2 illustrated the presence/absence of *t* point. A total of 28 cases in Group A_M exhibited the absence of *t* point. Out of these, 22 exhibited the absence of *t* point either in the right or left hand and 6 cases showed the absence of *t* point in both the hands and the difference obtained was statistically highly significant. Significant increase in asymmetry of pattern types in both male and female samples of the study group was represented in Table 3. Table 4 showed asymmetry values of TRC, a-b ridge count, and ATD angle. Significant asymmetry was detected in TRC of A_F when compared to B_F . A_M showed significant asymmetry in a-b ridge count and ATD angle.

Discussion

Clefts can be broadly classified into syndromic and NSCL/P.^[13] About 70% of CL/P and 50% of isolated cleft palate cases lack additional features and are categorized as “Nonsyndromic.”^[14] It was found that the prevalence of

Table 1: Comparison of TRC, a-b ridge count, atd angle between groups A_M , B_M ; A_F , B_F & A_F , A_M

Parameters	GROUPS		<i>t</i>	<i>P</i>	GROUPS		<i>t</i>	<i>P</i>	GROUPS		<i>t</i>	<i>P</i>
	A_M	B_M			A_F	B_F			A_M	A_F		
TRC R												
Min	14.0	32.0	11.21	<0.001	16.0	32.0	11.89	0.0001	14.0	16.0	0.07	0.94
Max	104.0	65.0			102.0	65.0			104.0	102.0		
Mean	75.8	44.6			76.0	44.2			75.8	76.0		
SD	8.5	9.5			17.7	9.3			8.5	17.7		
TRC L												
Min	16.0	27.0	10.39	<0.001	26.0	27.0	12.89	0.0001	16.0	26.0	1.5	0.25
Max	113.5	72.0			112.0	72.0			113.5	112.0		
Mean	77.6	44.4			80.7	44.3			77.6	80.7		
SD	20.7	12.3			17.8	12.8			20.7	17.8		
S a-b ridge count R												
Min	31.0	10.0	16.53	<0.001	30.0	10.0	14.91	<0.0001	31.0	30.0	0.34	0.94
Max	48.0	34.0			48.0	34.0			48.0	48.0		
Mean	40.6	30.2			40.8	29.9			40.6	40.8		
SD	3.8	3.3			4.1	4.4			3.8	4.1		
a-b ridge count L												
Min	30.0	10.0	11.09	<0.001	30.0	10.0	12.23	<0.0001	30.0	30.0	1.79	0.25
Max	52.0	40.0			48.0	40.0			52.0	48.0		
Mean	40.2	31.9			41.3	31.1			40.2	41.3		
SD	3.9	5.0			4.1	5.6			3.9	4.1		
atd angle R												
Min	30	28	2.135	0.035	38	28	3.46	0.001	30	38	0.35	0.73
Max	52	65			53	65			52	53		
Mean	44.63	42.44			44.84	41.66			44.63	44.84		
SD	4.26	7.59			3.759	7.284			4.26	3.759		
atd angle L												
Min	36	30	4.231	<0.001	30	30	1.94	0.05	36	30	4.9	<0.001
Max	53	50			50	50			53	50		
Mean	45.13	41.38			41.96	40.45			45.13	41.96		
SD	4.89	5.25			3.87	5.26			4.89	3.87		

TRC R – sum of total ridge counts of all fingers on right hand; TRC L – sum of total ridge counts of all fingers on left hand. $P < 0.05$ statistically significant (S), $P > 0.05$ not significant (NS); SD – Standard deviation

Table 2: Comparison of presence/absence of t point between groups A_M, B_M; A_F, B_F & A_F, A_M

Presence of t point in different groups	t point R				t point L			
	YES	NO	Chi square	P	YES	NO	Chi square	P
A _M	83	17	9.59	0.002	89	11	5.94	0.02
B _M	100	0			100	0		
A _F	97	3	0.78	0.38	95	5	0.066	0.797
B _F	97	3			97	3		
A _F	97	3	10.89	<0.001	95	5	2.45	0.12
A _M	83	17			89	11		

R – Right hand; L – Left hand. P<0.05 statistically significant (S), P>0.05 not significant (NS)

Table 3: Comparison of Asymmetry detected in pattern types between A_M, B_M & A_F, B_F

Asymmetry detected	Count%		Chi-square	P
	Yes	No		
A _M	54%	46%	22.104	<0.0001
B _M	14%	86%		
A _F	70%	30%	30.92	0.0001
B _F	22%	78%		

P<0.05 statistically significant (S), P>0.05 not significant (NS)

NSCL/P varies with ancestry and most commonly affected ones were Asian/Amerindian descendants.^[15,16] Hence, this particular craniofacial anomaly has been selected for the study.

All the physical traits are genetically controlled and are passed from one generation to the other which is governed by laws of inheritance propounded of Mendel and the features of dermatoglyphics and cleft deformities are not an exception.^[17] Threshold theory which was put forth by Carter and Wilkinson stated that abnormalities are the result of both genetic and environmental factors, but expressed only after exceeding threshold limit.^[18] According to this statement, before they could be physically expressed, there is an inevitable chance for few variations to occur in dermatoglyphics due to their correlated formation at intrauterine life. So as an attempt to derive the proportion and intensity of these genetic variations with reference to NSCL/P in the previous generations, the present study was planned with unaffected parents of cleft progeny.

The pattern types can easily be detected on the fingertips and any variations can be easily determined.^[19] Hence, pattern types were included in the study. Most frequently obtained pattern was loop in the present study. In similar studies conducted by Jahanbin *et al.*^[12] and Saxena *et al.*^[20] showed that the loop pattern was frequently seen in the normal individuals. Verbov (1970) stated that loops are the most common pattern types and represent about 70% of all finger patterns in Britain.^[21] In the present study, there was statistically significant difference seen between individual pattern types, where loops being more frequently seen, next being whorls, and finally the arches in all the four groups. This indicates the overall genetic predilection for loop pattern. In contrary to the present study, studies conducted

by Woolf and Gianas^[22,23] and Jahanbin *et al.*^[12] found that fathers of cleft patients had an increased frequency of arches. But in the present study, such difference has not been established.

Literature has shown that in the instances of genetic abnormality, there will be variation in the TRC. Hence, this particular entity has been included in the present study. There was significant increase in the mean TRC in both the groups of study population with reference to their respective controls. In contrast to these observations, Saxena *et al.*^[20] reported decreased mean TRC of parents with cleft progeny when compared to their controls. Furthermore, in a study conducted by Ma *et al.*,^[24] it was shown that there was no significant difference witnessed between the parents with CL/P children and control parents. The probable reason for reported dissimilarity might be that they reported increased occurrence of arch patterns in the parents with cleft children. Along with the increase in TRC a wide range between the minimum and maximum values of TRC in study group was expressively observed [Table 1]. This typically signifies developmental alteration during the formation of ridges in the parents of affected children.

The interdigital area between forefinger and middle finger said to be exposed to intrauterine environmental pressures for a greater period than other parts of palm.^[25] Hence, there are many chances for variations to occur in this area, so a-b ridge count was selected for the study. The mean a-b ridge count of controls was in the normal range of 10 and 40. It was found that there was a significant increase in the mean a-b ridge in cases where the minimum values were at the upper limit of the normal range and the maximum values were beyond the normal range (31–52), similar findings have been reported by Ma *et al.*^[24] and concluded that this could be a good index for predicting the future risk of CL/P which is also an inheritable palmar trait.

The reliability of ATD angle was proved by Brunson *et al.*^[26] saying that the ATD angle can be measured reliably whether the readings are made by one individual or multiple readers. Hence, the ATD angle was selected for the study. The normal ATD angle ranged between 30° and 65°.^[27] With regard to this, all selected individuals showed minimum and maximum values in the stated normal range,

Table 4: Comparison of Asymmetry in TRC, a-b ridge count, atd angle between A_M, B_M & A_F, B_F

Parameters	GROUPS		t	P	GROUPS		t	P	GROUPS		t	P
	A _M	B _M			A _F	B _F			A _M	A _F		
TRC R												
Min	14.0	32.0	11.21	<0.001	16.0	32.0	11.89	0.0001	14.0	16.0	0.07	0.94
Max	104.0	65.0			102.0	65.0			104.0	102.0		
Mean	75.8	44.6			76.0	44.2			75.8	76.0		
SD	8.5	9.5			17.7	9.3			8.5	17.7		
TRC L												
Min	16.0	27.0	10.39	<0.001	26.0	27.0	12.89	0.0001	16.0	26.0	1.5	0.25
Max	113.5	72.0			112.0	72.0			113.5	112.0		
Mean	77.6	44.4			80.7	44.3			77.6	80.7		
SD	20.7	12.3			17.8	12.8			20.7	17.8		
a-b ridge count R												
Min	31.0	10.0	16.53	<0.001	30.0	10.0	14.91	<0.0001	31.0	30.0	0.34	0.94
Max	48.0	34.0			48.0	34.0			48.0	48.0		
Mean	40.6	30.2			40.8	29.9			40.6	40.8		
SD	3.8	3.3			4.1	4.4			3.8	4.1		
a-b ridge count L												
Min	30.0	10.0	11.09	<0.001	30.0	10.0	12.23	<0.0001	30.0	30.0	1.79	0.25
Max	52.0	40.0			48.0	40.0			52.0	48.0		
Mean	40.2	31.9			41.3	31.1			40.2	41.3		
SD	3.9	5.0			4.1	5.6			3.9	4.1		
atd angle R												
Min	30	28	2.135	0.035	38	28	3.46	0.001	30	38	0.35	0.73
Max	52	65			53	65			52	53		
Mean	44.63	42.44			44.84	41.66			44.63	44.84		
SD	4.26	7.59			3.759	7.284			4.26	3.759		
atd angle L												
Min	36	30	4.231	<0.001	30	30	1.94	0.05	36	30	4.9	<0.001
Max	53	50			50	50			53	50		
Mean	45.13	41.38			41.96	40.45			45.13	41.96		
SD	4.89	5.25			3.87	5.26			4.89	3.87		

TRC – Total Ridge Count. $P < 0.05$ statistically significant (S), $P > 0.05$ not significant (NS); SD – Standard deviation

but there were increased mean scores in both the hands of study group when compared to those of controls and this difference was statistically significant. The overall increase in the ATD angle can be attributed to the fact that t triradius point [Figure 4A] was more distally placed which led to the increase in angle in parents with cleft children. In contrary, decrease in mean ATD angle was reported by Jahanbin *et al.*^[12] and Saxena *et al.*,^[20] but significant difference was reported only with the right hand by Saxena *et al.*^[20] Ma *et al.*^[24] did not notice any significant difference.

Another remarkable finding seen in the present study was the absence of t point in unaffected mothers of cleft progeny. Among the total sample of 100, 28 exhibited the absence of t point. Out of these, 22 exhibited the absence of t point either in the right or left hand and 6 cases showed the absence of t point in both the hands and the difference obtained was highly significant (p 0.002).

Genes in their optimal state are nearly symmetrical. Asymmetry will be illustrated in various human bilateral structures such as orofacial structures and hands depending

on alteration in genetic expression. Genetic damage takes place in phylogenic horizon in cleft which can be reflected in hands through dermatoglyphic asymmetry.^[5] It was evidently observed in the present study that there was increased asymmetry in the pattern types of both the parents with cleft progeny when compared to their respective controls [Table 3]. In a similar study conducted by Jahanbin *et al.*^[12] reported that only female cases differed significantly from controls and there was no significant difference between male cases and controls. The increased asymmetry in pattern types on 2nd, 3rd, and 4th fingers when compared to their respective controls was reported in a study piloted by Ma *et al.*^[24]

When the asymmetry of TRC, a-b ridge count, and ATD angle were compared between parents of cleft children and their controls, fathers in Group A showed increased asymmetry in TRC and mothers in Group A showed increased asymmetry in a-b ridge count and ATD angle [Table 4]. Jahanbin *et al.*^[12] found that the asymmetry of ATD angle of cases differed significantly from the scores

of controls which were in accordance with the present study. In a study conducted by Ma *et al.*,^[24] there was no significant difference seen in the asymmetry of a-b ridge count and ATD angle between cases and control groups which was in contrary to the present study.

Presence of few minor variations in dermatoglyphic patterns may not always be associated with the risk of having genetic abnormalities in the future generations, but the presence of marked disparities in these patterns could be a predictable indicator for determining the genetic aberration risk in the future generations. Further studies should be planned by considering these variations as standard predictable risk indicators and by calculating the risk probability. Determining the cross inheritance by studying the children dermatoglyphic patterns and relating them to the asymmetry of parents might aid in the better analysis.

Prospective studies would be valuable for the establishment of dermatoglyphic markers of the cleft. Dermatoglyphics, in turn, can be immensely helpful for the easy, accessible, noninvasive, and economical identification of groups at high risk of developing cleft and for timely prevention, especially in developing countries with enormous populations and limited health budgets.

Conclusion

Irrespective of the groups, most commonly found pattern was loop. The mean TRC, a-b ridge count, and values of ATD angle were significantly increased in the study group. Absence of *t* point and presence of asymmetry in the studied parameters were typically associated with study population, none of the controls exhibited these features.

Thus, it may be concluded that dermatoglyphics can be used as tool to study the DI of cleft anomalies and provide data to assess the individual variations, familial correlations, as well to know the genetic etiology of clefting.

At this juncture, from the findings of this study (even though the observations may not be authenticative), it proves that the particular type of dermatoglyphic variation has always been associated with specific genetic alteration. However, this study has definitely proved the usefulness of dermatoglyphics in analyzing altered genetic patterns.

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Conflicts of interest

There are no conflicts of interest.

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Antimicrobial Activity of Pomegranate (*Punica Granatum*) Pericarp Extract against *Streptococcus Mutans*- A Source For Natural Mouth Rinse: An *In-vitro* and *In-vivo* Study

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Plant materials were known as source of new antimicrobial agents. Many efforts have been made to develop alternative mouth rinses from natural products which are safe, easily available and substitute the standard pharmaceutical remedies. Thus, considering the therapeutic value of pomegranate fruit, the present study was designed to compare the antimicrobial efficacy of aqueous extract of Pomegranate pericarp and commercially available Chlorhexidine mouth wash against caries causative microorganisms in both *in-vitro* and *in-vivo*. To evaluate the antimicrobial efficacy of pomegranate pericarp extract (PPE) against *Streptococcus mutans* (*S.mutans*) and to determine its usefulness as anti caries mouth rinse. *In-vitro* antimicrobial efficacy was evaluated by disc inhibition zone method and broth dilution assay considering minimum inhibitory concentration of PPE. *In-vivo* evaluation was done as a randomised controlled trial which included thirty children aged between 6-12 years. They were divided randomly into 3 groups of ten each and subjected to different mouth rinses - Group I: PPE mouth rinse, Group II: 0.2% Chlorhexidine mouth rinse and Group III: distilled water (control). The salivary samples which were collected before and after (5 minutes) mouth rinsing were inoculated on Mutans Sanguis agar and the bacterial count was calculated. Statistically significant decrease in salivary *S.mutans* count was observed in group I and II compared to group III after mouth rinsing. However, there was no statistically significant difference between groups I and II. PPE mouthwash was effective in reducing the salivary *S.mutans* count and was comparable to chlorhexidine mouth rinse. Hence PPE mouth rinse may be considered as a potential anti caries mouth rinse.

Keywords: Chlorhexidine, Minimum Inhibitory Concentration, Mouthwash, Pomegranate pericarp extract, *Streptococcus mutans*.

Dental caries is one of the most common chronic infectious diseases in the world which is influenced by multiple factors such as diet, host

characteristics and cariogenic microorganisms, of which *Streptococcus mutans* (*S.mutans*) is a significant contributor to tooth decay.¹



The prime mode of preventing the development and progression of dental caries is through mechanical plaque control (regular tooth brushing). Mouth rinses are medicated solutions which are recommended as an antimicrobial, topical anti-inflammatory solutions so as to decrease halitosis and deliver fluoride for caries prevention in general. They are beneficial especially to mentally and physically challenged patients who lack manual dexterity. A multitude of products have arisen, during the past few decades which contain different active chemical ingredients like chlorhexidine, triclosan, fluoride mouth rinses etc.²

Chlorhexidine is considered as a gold standard anti-plaque agent because of its broad spectrum antimicrobial activity.³ However, its long term usage can cause tooth staining, unpleasant taste, increased calculus formation and mucosal erosion at higher concentrations.⁴ These shortcomings have led to the need for further research and introduction of new antibacterial agents which are derived naturally with minimal / no side effects on the oral tissues especially in children.

Nature has enormous plant sources which have good medicinal value and work against pathogenic microorganisms. Pomegranate (*Punica granatum*) is one such natural source that is currently finding important applications in the field of dental health.⁵ The healing property of pomegranate was discussed in one of the oldest medical texts, the Eber's Papyrus from ancient Egypt (1500 BC).³ In Ayurvedic medicine, pomegranate is considered "a pharmacy unto itself" and as a remedy for diabetes in Unani medicine. Various components of this plant such as the leaves, flowers, roots, bark and fruit extracts have been used for a variety of ailments.⁶

Even though there is ample evidence regarding the antimicrobial efficacy of PPE in various *In Vitro* studies, its clinical evidence is very minimal. Considering this fact, the present study is an attempt to evaluate the clinical applicability of naturally available PPE as mouth rinse in children.

MATERIALS AND METHODS

Following the approval from the institutional ethical committee, the present microbiological study was conducted in the department of Pedodontics and Preventive dentistry

in collaboration with department of Microbiology and Pharmacology.

Fresh ripen pomegranate fruits were procured from local market and the pericarps were separated manually, shade dried for 7 days, powdered and stored under freezing condition until its use [Figure I]. This powder was mixed in different concentrations (250, 500, 750 and 1000 mg) with 10 ml of distilled water in Jiffy's centrifuge tubes. These four concentrations of extracts were immersed in thermostatic water bath at a temperature of 60°C for 20 minutes following which they were left to cool and subjected to centrifugation at 2500 rpm for 10 minutes and the resultant supernatants were used to analyse the antimicrobial efficacy.⁷

The antimicrobial activity of PPE was assessed using disc inhibition zone method. *S. mutans* was first isolated from saliva by inoculation on Mitis Salivarius Bacitracin agar and PPE was loaded on the sterile filter paper discs at a concentration of 25, 50, 75 and 100 mg/ml, respectively. Filter paper disc dipped in 0.2% chlorhexidine was taken as positive control and distilled water as negative control. *S. mutans* streaked agar plates impregnated with discs were incubated in an anaerobic jar at 37°C for about 24 hrs. The zone of inhibition was assessed by measuring the diameter of inhibited growth [Figure II]. Broth dilution method was adopted to determine minimum inhibitory concentration (MIC) of the active extract. The lowest concentration of extract resulting in bacterial density lower than 300 colonies per plate was determined as MIC. As there was no bacterial colony growth at all the three concentrations (50, 75, 100 mg/ml) of PPE mouthwash, the lowest concentration i.e., 50 mg/ml was taken as MIC and was used to prepare the mouth rinse without adding any sweeteners.

A total of two hundred children between the age group of 6-12 years who were following routine oral hygiene practice with DMFT score ≤ 4 were screened without sex predilection. Subjects with draining abscess, sinus, cellulitis or any other conditions that require emergency dental treatment and patients with history of recent antibiotic usage (at least for past 1 month) were excluded from the study. After explaining the test procedure for the forty two children who have fulfilled the inclusion criteria, only thirty parents have given



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their consent to participate in the study voluntarily. Further these children were randomly divided into three groups depending upon the mouth rinse used - group I: PPE mouth rinse (experimental); group II: 0.2% chlorhexidine mouth rinse (positive control) and group III: Distilled water (negative control) with ten subjects in each group.

Salivary samples were collected in the morning in order to eliminate any bias in the concentration of saliva due to circadian rhythm. Following an initial swallow, about 1 ml of unstimulated saliva was collected in a sterile vial by instructing the children to drool for 2 minutes. Each child in their respective group was given 5ml of mouth rinse and asked to squish for about one minute. The same procedure was followed for salivary sample collection after 5 minutes following mouth rinsing. The collected samples were transported in an icebox within 2 hours to maintain the viability of microorganisms.

The collected saliva was inoculated on Mutans Sanguis agar and the plates were incubated in an anaerobic jar for 48 hours followed by bacterial count using conventional plate count method [Figure III].

The whole procedure was conducted by a single investigator and the scores were recorded. To avoid bias in the results, a second investigator who was unaware of the prior results randomly evaluated the agar plates. As the inter examiner variability was not significant (P value < 0.5), the scores given by the first investigator were only considered. The values thus obtained were tabulated and subjected to statistical analysis using Wilcoxon signed rank test and Mann-Whitney U test.

Table 1. Intragroup comparison of salivary *S. mutans* count before and after mouth rinsing (in 10^3 CFU/ml)

Group	Before IQR (in 10^3 CFU/ml)	After IQR (in 10^3 CFU/ml)	P-value
I	383	218	0.005*
II	350	203	0.005*
III	350	375	1

Wilcoxon signed rank test

* Statistically highly significant if $P < 0.01$, IQR: Interquartile range

RESULTS

Intra group comparison in *S. mutans* count before and after mouth rinsing revealed significant decrease in number of *S. mutans* colony count in groups I and II ($p=0.001$). Nevertheless, this reduction was not statistically significant in group III ($p=1$) [Table I].

Intergroup comparison of salivary *S. mutans* count showed significant decrease in colony count between groups I and III; II and III ($p=0.001$). However, no statistical significant difference was observed when groups I and II were compared ($p=0.48$) [Table II].

DISCUSSION

Epidemiological studies showed that the prevention of dental caries was done by inhibiting plaque biofilm formation or removing plaque from the teeth that enhances oral hygiene. Common preventive strategies of dental caries are mechanical cleansing techniques such as regular brushing and flossing; use of systemic and topical fluorides; dietary modifications include altering frequency of sugar intake, use of sugar substitutes, fissure sealants, antimicrobial agents in mouth washes and probiotics.⁸ Among these, use of topical antimicrobial agents such as mouth rinses minimize caries risk by reducing the number of *S. mutans* in the mouth there by altering the oral environment.⁹

A variety of synthetic mouth washes containing Chlorhexidine, Triclosan and Cetylpyridinium chloride are available in the market. Over a period of last 40 years, chlorhexidine has been thoroughly investigated and successfully

Table 2. Intergroup comparison of difference in salivary *S. mutans* count before and after mouth rinsing (in 10^3 CFU/ml)

Inter group comparison of difference (after – before count) in IQR	P-value
I(285) Vs II(308)	0.48
I(285) Vs III(105)	0.001*
II(308) Vs III(105)	0.001*

Mann-Whitney U test

*Statistically highly significant if $P < 0.01$, IQR: Interquartile range



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used as antiplaque agent in dental practice.¹⁰ It is a synthetic bisbiguanide which is positively charged showing high affinity for negative ions found in the cell membrane of the microorganisms. It indirectly affects the enzymatic function of dehydrogenase and adenosine triphosphatase present in the cell wall of bacteria resulting in disruption of cell membrane leading to cell death. Proposed mechanism of caries inhibition is by interfering with the metabolic activity of *S. mutans*, particularly inhibition of phosphonyl pyruvate enzyme.¹¹

Chlorhexidine has high substantivity of 12 hours which is attributed to its controlled release system regulated by beta cyclodextrine. Greater the amount of beta cyclodextrine, the more progressive release of chlorhexidine.⁴ In the present study chlorhexidine was taken as positive control as it was considered to be gold standard anti plaque mouth rinse due to its prolonged broad spectrum antimicrobial activity. However certain local side effects were reported with its long term usage.¹²

To overcome these side effects, researchers are shifting their attention to herbal remedies to fight against microbial infections. Since plant extracts were known to be a good source of new antimicrobial agents, efforts have been made for development of alternate mouth wash from natural

products which were anticipated to be safer, easily available and substitute standard pharmaceutical remedies.

Pomegranate fruit is currently finding important applications in the field of dental health due to its consumption in ancient cultures for its medicinal purposes without adverse effects or toxicity. There are several *In Vitro* studies determining the antimicrobial activity of Pomegranate extract against *S. mutans* but very few *In Vivo* studies were conducted to prove its efficacy against dental caries. Thus PPE was selected in the present study to determine its clinical usefulness as anti caries mouth rinse.

Pomegranate pericarp contains different bioactive compounds like phenolics, flavonoids, proanthocyanidine compounds, minerals such as potassium, nitrogen, sodium and complex polysaccharides. Consuming pomegranate pericarp was considered beneficial for treatment of colic, colitis, menorrhagia, oxyuriasis, headache, diuretic, acne, piles, allergic dermatitis and treatment of oral diseases.²

Pomegranate fruit has many properties which include antimicrobial, anti-oxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic and inhibitory effect on invasion/motility, cell cycle arrest and apoptosis.¹³

The main compounds responsible for most of the purposeful properties of Pomegranate pericarp are phenolic compounds like ellagitannins and flavonoids. Chemically phenolic acids are defined as substances that possess an aromatic ring bound to one or more hydrogenated substituent.

Eating Pomegranate as a food could place antibacterial and antioxidant agents into the mouth and gum areas. On the other hand, better oral exposure to these agents could come from more



Fig. 1a. Dried pomegranate pericarp;b: Pomegranate pericarp powder.”

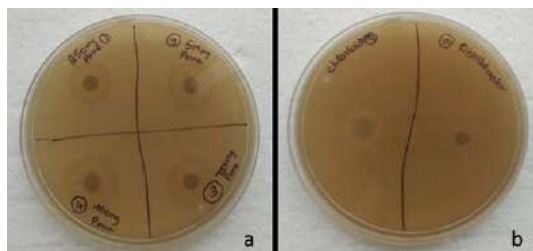


Fig. 2a. Zone of inhibition for PPE at different concentrations;b:Zone of inhibition for chlorhexidine and distilled water.”



Fig. 3. Bacterial colonies before and after PPE mouth rinsing.”

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direct and chronic exposure with active agents, such as mouth rinses. This is thought to occur due to the fact that the oral tissue would directly be exposed to polyphenols, which would subsequently get activated by enzymes, thereby destroying pathogenic bacteria.² Hence an approach has been tried out in the present study to use PPE as mouth rinse.

In the current study *S. mutans* was isolated using Mitis Salivarius Bacitracin (MSB) agar as suggested by Gold *et al.* (1973)¹⁴ who stated that MSB agar can be used as selective medium for isolating *S. mutans* from saliva.

Broth dilution assay, Agar dilution method, Disc diffusion method, Cup plate method and Ditch plate method are used to assess the antimicrobial activity of any natural or synthetic agent. In the present study disc diffusion method and broth dilution assay was followed as it was considered as standard and reliable.¹⁵ Furthermore this method involves direct contact of the tested substances with the microbial cultures, which is important for the evaluation of mouth rinses.

In a study conducted by Aldhafer *et al.* (2015)¹ the MIC of PPE was 15mg/ml. However, in this study it was 50mg/ml. This difference in the results may be attributed to the difference in the type of extract used and method of extract preparation. In the current study, aqueous extract was prepared rather than alcoholic extracts because of its easy availability and highest extraction capacity with water followed by methanol and ethanol. This is due to the relative polar nature of polyphenols in pomegranate and they are strongly soluble in polar solvents like water rather than non-polar solvents such as alcohol.¹⁶

The aqueous extract used in this contemporary study was prepared at 60°C temperature for 20 minutes. This procedure was followed according to the findings by Wissam *et al.* (2012)¹⁷ who stated that, there is effective extraction of polyphenols and PA at 60°C temperature using water as a solvent; however temperature above 70°C and time longer than 30 minutes may lead to possible polymerization of flavonoids leading to loss of phenolic compounds.

Results of the present study showed significant reduction in salivary *S. mutans* count with PPE compared to distilled water, whereas no significant difference was noticed

with chlorhexidine group. This implies that pomegranate mouth rinse is equally efficacious with chlorhexidine mouth rinse. The possible reason for this is due to the presence of tannins, which crosses bacterial cell wall and precipitate proteins through complex formation, increase bacterial lysis and impede bacterial adhesion by suppression of enzymes like glucosyl transferase which plays an important role in adhesion of *S. mutans* to tooth surface.⁶ According to Machado *et al.* (2002)¹⁸ ellagitannin-punicalgin is thought to be the primary constituent involved in the antimicrobial effect of pomegranate pericarp.

Similar results were noted by Smruti *et al.* (2011)¹⁹ who compared antiplaque efficacy of pomegranate mouth rinse against chlorhexidine. This study also concluded that pomegranate mouth rinse could be explored as a long-term anti-plaque rinse with prophylactic benefits.

The data obtained with the mouth wash of *Punica granatum* on *S. mutans* are consistent with the results shown in a clinical study conducted by Umar *et al.* (2016)²⁰ who stated that pomegranate mouth rinse may be used as an adjunct to prevent dental caries and maintain good oral hygiene.

There are certain limitations in the use of plant extracts as mouth rinse when compared to synthetic mouth rinses as they are time consuming, need of elaborate apparatus to isolate and characterise active molecules and shelf life. The isolation of active components faces many other challenges like inconsistency of source material, obscurity in isolating active components and cost of extraction.

However to consider the clinical applicability of this study certain issues have to be addressed, which include - appropriate concentration of mouth wash to be used, cost effectiveness, addition of preservatives for better shelf life, addition of colouring agents to improve acceptability by children, addition of flavouring agents for better palatability and its long term effectiveness as anti caries mouth rinse on large sample group.

CONCLUSION

The results of this study gives an inference that both PPE and chlorhexidine mouth rinse possess remarkable antimicrobial activity against



S.mutans. Hence PPE mouth rinse may be used as an alternative to chlorhexidine and also as an adjunct to conventional tooth brushing for prevention of dental caries and maintenance of oral hygiene in children.

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Original Article

Surface and Mechanical Properties of Different Coated Orthodontic Archwires

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INTRODUCTION

There is an increasing demand for esthetics in patients not only after treatment but during treatment as well. To meet their demands, research in material science has progressed in dentistry particularly in orthodontics. Ceramic brackets were introduced long ago, but archwires remained unchanged which has become an esthetic concern.

Coated archwires were thought to be one of the acceptable solutions for this problem. Metallic archwires are coated with tooth-colored polymers such as Teflon or epoxy resin by different manufacturers to meet the demand of esthetics from patients.^[1] But to be successful, best possible esthetics should be coupled with efficient performance. There is a possibility that original thickness of the archwire would be decreased to accommodate the

surface coating which, in turn, may alter some of the properties of the wire, primarily load deflection.^[2,3]

Surface topography plays an important role to determine the efficiency of the archwire. Coating of archwires with different materials and their loss during clinical use may alter the surface roughness which will affect the frictional properties.^[4] This study was undertaken as only limited number of studies^[5,6] was reported comparing the mechanical properties and surface roughness of as-received and retrieved coated archwires with similar coating. In this article,

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ABSTRACT

Objectives: The objective of this study was to evaluate the mechanical properties and surface characteristics of coated superelastic Ni–Ti archwires of three companies with different coatings before and after clinical use.

Materials and Methods: Coated 0.016 inch mandibular archwires of three different companies with different coatings (G & H – epoxy coated on all sides, Rocky Mountain Orthodontics (RMO) – Teflon coated on labial surface, and American Orthodontics (AO) – Teflon coated on all sides) were used in this study. Twenty wires from each company, 10 as received and 10 retrieved from patients after 4 weeks of clinical use, were tested for load deflection and surface roughness. An independent sample *t*-test was done to compare surface roughness and load-deflection characteristics and one-way ANOVA analysis to compare between groups. **Results:** Both retrieved and as-received G & H wires showed less force levels during loading and unloading compared to RMO and AO wires ($P < 0.05$). In both test and control groups, G & H wires produced slightly higher surface roughness values compared to AO and RMO. In all the three company wires, roughness values increased significantly before and after clinical use. **Conclusion:** Retrieved coated archwires of all the three companies produced lower loading and unloading force values compared to as-received coated archwires. Surface roughness of coated archwires increased after use.

KEYWORDS: Archwires, coated wires, epoxy coating, load deflection, Ni–Ti wires, retrieved coated archwires, surface roughness

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archwires with different coatings from different manufacturers were compared. The aims of the study were as follows:

1. To investigate three different orthodontic coated archwires, as received and retrieved from patients for surface roughness
2. To investigate three different orthodontic coated archwires, as received and retrieved from patients for load deflection.

MATERIALS AND METHODS

Thirty patients who were to undergo orthodontic treatment with fixed appliances were randomly selected with following inclusion criterion:

1. No congenitally missing or extraction in the anterior region
2. Pretreatment little's irregularity index >5.0 mm
3. No congenital clefts or craniofacial syndromes
4. No blocked out incisors in the mandibular arch.

Ethical approval was obtained from the Institutional Ethical Committee (Ref no: 17/IEC/CIR/14). Coated mandibular archwires of three different companies [Figures 1-3] were divided into six groups (I–III were test group wires retrieved from patient and IV to VI were control group wires as received from the manufacturer) [Table 1]. Randomization was done by submitting three groups of wires to three different operators who were unaware about details of the wire. The selected patients undergoing fixed orthodontic therapy were randomly allocated to each operator, and the archwires were ligated using Teflon-coated ligature wires (Classic Orthodontics, San Diego, CA, USA) [Figures 4 and 5]. Details of the patient, type of wire, date of insertion, and date of removal of the wires were noted by an observer.

After 4 weeks of clinical use, the archwires were retrieved and cleaned with distilled water as described by Eliades *et al.*^[5] and were sent to laboratory within 48 h for testing the load deflection and surface roughness using universal testing machine and contact stylus profilometer, respectively. As-received wires from the same companies were also tested for surface roughness and load deflection, and the results were recorded and statistically analyzed.

Table 1: Coated mandibular archwires of three different companies divided into six groups

Company	G & H (Franklin, Indiana)	RMO; Denver, Colorado	AO; Sheboygan, Wisconsin
Cross section	0.016 Ni–Ti	0.016 Ni–Ti	0.016 Ni–Ti
Coating	Epoxy	Teflon	Teflon
Coated surfaces	All surfaces	Labial	All surfaces

RMO: Rocky Mountain Orthodontics, AO: American Orthodontics

Load deflection testing procedure

For three-point bending, a custom-made jig was fabricated, and two MBT brackets with a slot size of 0.022 × 0.028 inches were attached on the top of the parallel rods of the jig as proposed by



Figure 1: G & H, 0.016 inch ultraesthetic coated archwires

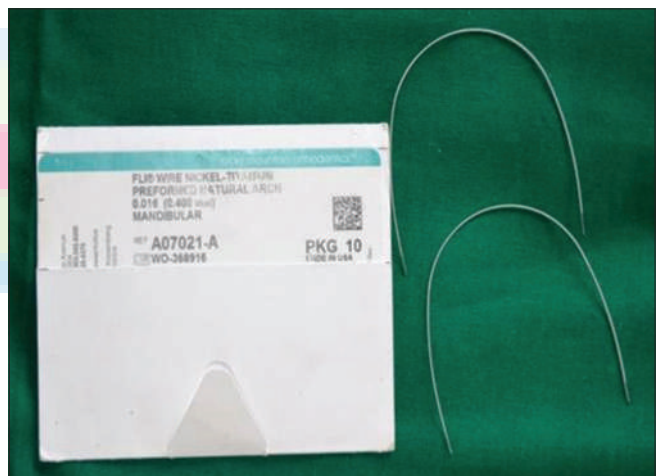


Figure 2: Rocky Mountain Orthodontics, 0.016 inch coated archwires

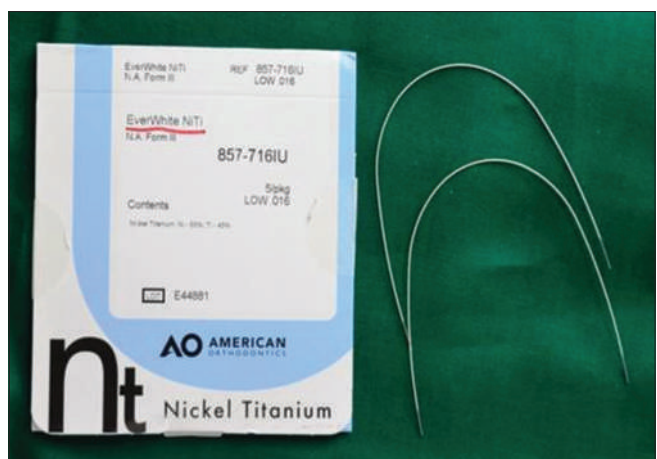


Figure 3: American Orthodontics, 0.016 inch EverWhite coated archwires

Elayyan *et al.*^[6] The jig was fixed to the base of universal testing machine [Figure 6]. The wire sample was attached to the brackets using ligature wire. The force was applied vertically with a rod attached to the moving part of the universal testing machine at a crosshead speed of 1 mm/min. The wire was deflected first by 1, 2, 3 mm, and loading values were noted at each of these deflections. Unloading was done in the order of 2 and 1 mm, and values were recorded.

Surface roughness testing procedure

A contact stylus profilometer [Figure 7] with a stylus radius of 2.5 μm and tip angle of 90° traversing at a speed of 1 mm/s was used to evaluate the surface roughness of the wire samples. Cutoff length was 0.08 mm and measuring length was 1 mm. The following three readings were recorded Ra (arithmetic mean deviation of the roughness profile), Rq (root-mean-square deviation of the roughness profile) and Rz (10-spot average roughness) using contact stylus profilometer.

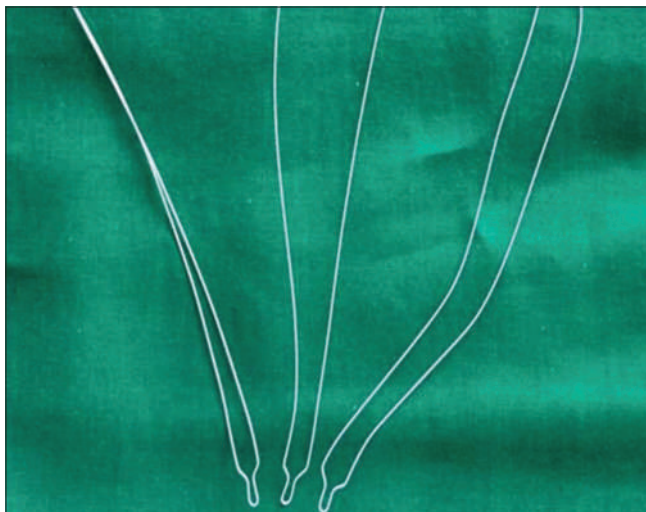


Figure 4: Teflon-coated ligature wires

Statistical analysis

All the statistical analysis was done in the Statistical Package for the Social Sciences software (version 15, SPSS Inc., Chicago, USA). Descriptive statistics, means, and standard deviations were calculated and the associated *P* values were listed in tables [Tables 2 and 3]. An independent sample *t*-test was conducted to compare the surface roughness and load-deflection characteristics of as-received and retrieved coated archwires. One-way ANOVA analysis was computed to compare surface roughness and load-deflection characteristics between groups.

RESULTS

In the retrieved wires, Group I (G & H) wires showed less force levels to deflect the wire at 1, 2, and 3 mm during loading and unloading compared to Group II (Rocky Mountain Orthodontics [RMO] and Group III (American Orthodontics [AO]) wires ($P < 0.05$) [Table 2]. Group II and III wires required almost similar forces. There was a statistically



Figure 5: Coated archwire ligated using coated ligature wire



Figure 6: Jig with wire under universal testing machine


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Figure 7: Contact stylus profilometer

significant difference between the three groups with $P < 0.05$.

In the as-received wires, Group IV (G & H) wires required less force levels to deflect the wire at 1, 2, and 3 mm during loading and unloading as compared to Group V (RMO) and Group VI (AO) wires. Group V wires produced slightly higher forces compared to other groups. However, there was no statistically significant difference between the groups with $P > 0.05$. Intragroup comparison was done between control and test sample [Table 2].

In both test and control groups, epoxy-coated (G & H) wires produced slightly higher surface roughness values compared to Teflon-coated wires (AO and RMO) but with no statistically significant difference between them ($P > 0.05$). In all the three wires, roughness values increased significantly after clinical use [Table 3].

DISCUSSION

The purpose of this study was to evaluate and compare the surface roughness and load-deflection properties of coated Ni–Ti wires of 0.016” from three different manufacturers under the same testing conditions using a contact stylus profilometer to measure surface roughness and three-point bending test to measure load-deflection properties.

In both retrieved wires and as-received wires, epoxy-coated (G & H) wires showed less force levels to deflect the wire, during loading and unloading compared to Teflon-coated (RMO and AO) wires. This could be due to the fact that the Teflon (RMO and AO) coating adds a minimal thickness (0.0008–0.001 inch) to the archwire, while the epoxy (G & H) coating adds considerable thickness (0.002 inch) to the archwire, so the coating of epoxy is thicker than that of the Teflon layer and may result in a smaller Ni–Ti inner core.^[7,8]

Table 2: Mean comparison among loading and unloading deflections of materials in test group and control group

Loading/unloading	Test	Mean	SD	P	Control	Mean	SD	P
1 mm (loading)	G & H	0.78	0.27	0.000 (S)	G & H	1.86	0.24	0.074 (NS)
	RMO	1.29	0.31		RMO	2.06	0.30	
	AO	1.31	0.23		AO	1.95	0.26	
2 mm (loading)	G & H	0.90	0.26	0.000 (S)	G & H	2.50	0.16	0.137 (NS)
	RMO	1.43	0.45		RMO	2.74	0.36	
	AO	1.47	0.42		AO	2.72	0.30	
3 mm (loading)	G & H	1.91	0.44	0.004 (S)	G & H	3.37	0.22	0.165 (NS)
	RMO	3.04	0.70		RMO	3.47	0.39	
	AO	2.39	0.55		AO	3.42	0.29	
1 mm (unloading)	G & H	0.42	0.29	0.001 (S)	G & H	1.34	0.16	0.047 (S)
	RMO	0.99	0.30		RMO	1.64	0.36	
	AO	1.02	0.32		AO	1.46	0.21	
2 mm (unloading)	G & H	0.90	0.26	0.004 (S)	G & H	2.30	0.24	0.349 (NS)
	RMO	1.43	0.45		RMO	2.49	0.40	
	AO	1.47	0.42		AO	2.34	0.25	

Statistical analysis: Intergroup: Independent sample *t*-test, Intragroup: ANOVA one-way test. Statistically significant if $P < 0.05$. SD: Standard deviation, NS: Not significant, S: Significant, RMO: Rocky Mountain Orthodontics, AO: American Orthodontics

Table 3: Mean comparison among G and H, Rocky Mountain Orthodontics, and American Orthodontics surface roughness (µm) of RA, RQ, and RZ in test and control groups

Surface roughness	Test	Mean	SD	P	Control	Mean	SD	P
RA	G & H	1.04	0.74	0.053 (NS)	G & H	0.33	0.19	0.213 (NS)
	RMO	0.71	0.41		RMO	0.24	0.07	
	AO	0.45	0.30		AO	0.20	0.19	
RQ	G & H	1.27	0.88	0.053 (NS)	G & H	0.45	0.19	0.094 (NS)
	RMO	0.89	0.54		RMO	0.33	0.07	
	AO	0.54	0.35		AO	0.27	0.23	
RZ	G & H	4.73	3.19	0.030 (S)	G & H	0.92	0.25	0.653 (NS)
	RMO	2.85	1.52		RMO	0.78	0.22	
	AO	2.04	1.29		AO	0.87	0.78	

Statistical analysis: Intergroup: Independent sample *t*-test, Intragroup: ANOVA one-way test. Statistically significant if $P < 0.05$. SD: Standard deviation, NS: Not significant, S: Significant, RMO: Rocky Mountain Orthodontics, AO: American Orthodontics

Even though statistically nonsignificant, RMO as-received wires required slightly higher forces during loading and produced slightly higher forces during unloading. This could be due to two reasons. This may be due to the reduced thickness of Teflon coating, allows an increased wire dimension underneath the coating in RMO archwires. The other reason may be RMO wires were coated only on labial side which further decreases the coating and increases the wire dimensions which further decreases the coating thickness and increases the wire dimension which was also in agreement with the study done by da Silva *et al.*^[9]

Results showed that loading and unloading forces were more in as-received wires compared to retrieved wires which are also in agreement with the study conducted by Elayyan *et al.*^[6] This could be due to increased frictional forces between the irregular surface of the coating and brackets, and the forces of mastication may also lead to permanent wire deformation. The increased irregular surface and friction might result in binding of the archwire during testing. Results of this study also suggest that superelastic property of coated Ni–Ti wires was lost significantly after 1 month. Hence, these wires cannot be used for more than 1 month.

In all the three company wires, roughness values increased significantly after clinical use. Abrasive effect of tooth brushing, food consistency, and interaction between the arch wire coating and bracket edges may be the reason for this increase in roughness.^[10,11]

CONCLUSION

- In the as received wires there were no significant differences in surface roughness.
- In all the three companies surface roughness increased in retrieved wires compared to as received wires.
- In the retrieved wires the epoxy coated (G&H) showed more roughness compared to Teflon coated (AO and RMO).
- In the as received wires epoxy coated (G&H) wires showed lowest forces compared to Teflon coated

(AO and RMO). but with no statistically significant difference.

- In the retrieved wires epoxy coated (G&H) wires showed lowest forces compared to Teflon coated (AO and RMO).
- In all the three companies forces decreased in retrieved wires compared to as received wires.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Implant-Supported Maxillary Incisor Intrusion

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Abstract

Deep bite management and retention are cumbersome as the stability is questionable, especially when the correction is achieved by posterior extrusion in nongrowing patients. Hence, it is advisable to intrude the anteriors; however, as conventional methods of anterior intrusion tax the anchorage, choosing biomechanics which ensure stable anchorage is our goal. The use of mini-implants has revolutionized biomechanics in orthodontics with better results as far as anchorage is concerned. This case report supports the literature regarding the implant-supported anchorage for true intrusion.

Keywords: Deep bite, maxillary incisor intrusion, mini-implants, nongrowing patient

INTRODUCTION

Anterior deep bite is a common problem which makes orthodontists focus on biomechanics to eliminate the problem by extrusion of posterior teeth or intrusion of the anteriors. Etiological factors for deep bite must be thoroughly evaluated to establish a correct diagnosis which will aid treatment planning.^[1] Some etiological factors for excessive gingival display include vertical maxillary excess, supraeruption of the maxillary incisors, and shortness or hypermobility of the upper lip.^[2,3]

The maxillary incisors can be predictably intruded about 2 mm with orthodontic appliances.^[4] Any further correction may generate esthetic problems, such as reverse smile architecture due to the discrepancy between the posterior occlusal planes and the anterior incisal plane.

Anchorage control is fundamental to successful orthodontic treatment. Although extraoral anchorage supplements tooth-borne anchorage, it requires excellent patient cooperation.^[5] Nowadays, skeletal anchorage systems such as miniplates, palatal plates, and mini-implants have revolutionized in providing a much more stable anchorage. Studies have shown that mini-implants are one of the best options for this purpose due to the multiple advantages they offer mainly easy management and placement in various anatomical areas as well as their low cost.^[6,7]

In this case report, we have discussed the treatment of deep bite with mini-implant-aided intrusion.

DIAGNOSIS AND TREATMENT PLANNING

A 19-year-old female patient came with a chief complaint of forwardly placed upper front teeth. On extraoral examination, the patient had convex profile, incompetent lips, interlabial gap of 8 mm at rest, and gingival exposure of 4 mm during smile. Intraoral examination revealed Angle's class I malocclusion, proclined upper anteriors with generalized spacing between upper anteriors, and severe deep bite (8 mm) [Figure 1].

Cephalometric examination revealed skeletal class II due to mild prognathic maxilla with proclined upper and lower anteriors and normodivergent growth pattern [Figure 2]. After evaluation of clinical aspects and cephalometric values [Table 1], as the patient has nearly 8 mm of spaces between upper anteriors, nonextraction line of treatment was planned. Initial alignment of the upper arch was followed by intrusion of upper anteriors with orthodontic mini-implants for the correction of deep bite and proclination.

TREATMENT PROGRESS

Banding and bonding of both the arches were done with 0.022" × 0.028" MBT prescription (Gemini series, 3M Unitek,

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Monrovia, CA, USA). Initial alignment and leveling was done with wire sequence of 0.016" NiTi, 0.018" NiTi, 0.016" × 0.022" NiTi, and 0.019" × 0.025" NiTi archwires. By the time initial alignment and leveling was completed, 6.5 mm deep bite was present.

Mini-implants of 1.2 mm × 6 mm dimension were placed in interradicular space between lateral incisor and canine bilaterally in the upper arch. Sectional 0.019" × 0.025" stainless steel (SS) stabilizing wires were placed in both posterior and anterior segments [Figure 3]. A prestretched elastic chain which exerted a force of 50 g to the anterior segment from mini-implants was placed bilaterally for intruding maxillary incisors. After 4 months of active intrusion, 3.5 mm of overbite was corrected [Figures 4 and 5].

Space closure was done with 0.019" × 0.025" SS wire with step-up bend given distal to lateral incisor to maintain the intrusion achieved. Finishing and detailing was performed. After 20 months of total treatment time, debonding was done and lingual bonded retainer was given in the upper and lower anterior region.

TREATMENT RESULTS

Posttreatment records [Figures 6 and 7] revealed that Angle's class I molar and canine relationship was maintained. Ideal overjet and overbite were achieved. There was an improvement in profile, lip competency, interlabial gap at rest, and gingival exposure during smile. Superimposition of pretreatment and posttreatment lateral cephalogram [Figure 8] also confirmed the clinical findings along with improved esthetics.

DISCUSSION

Correction of deep bite by extrusion of posterior teeth is less stable, especially in nongrowing patients when compared to growing patients.^[8,9] Hence, it was decided to correct the deep bite and gummy smile by maxillary incisor intrusion. Various intrusion arches such as utility arch and Burstone intrusion arch are frequently used for incisor intrusion. They create a force to elongate the molars which compromises the posterior anchorage in turn reducing the ability to intrude incisors.

Mini-implants are routinely used for intrusion and retraction of anteriors for the correction of deep bite and correction of anterior open bite by intrusion of the posterior teeth. When sufficient interradicular space is not available for implant

Table 1: Cephalometric data – pre- and post-treatment

Measurement	Pretreatment	Posttreatment
SNA	85°	83°
SNB	81°	80°
ANB	4°	3°
Pn-A	2 mm	1 mm
SN-Go-Gn	33°	32°
FMA	28°	27°
Max I-SN	115°	109°
UI - NA	30°	25°
	8 mm	4 mm
IMPA	99°	98°
LI-NB	34°	33°
	6 mm	5 mm
Iu-NF	31°	27°
Nasolabial angle	106°	111°



Figure 1: Pretreatment photographs



Figure 3: Preintrusion photographs



Figure 2: Pretreatment radiographs



Figure 4: Postintrusion photographs



Figure 5: Postintrusion radiographs



Figure 7: Posttreatment radiographs

placement, additional space can be created by intentional separation of the dental roots during the initial stages of orthodontic treatment.^[1] Sufficient interdental bone and a larger anterior segment which requires greater control provoked us to use mini-implants in the present case.

True intrusion without axial inclination change can only be obtained by directing the intrusive force through the center of resistance of the anterior teeth.^[1] Hence, mini-implants were placed between the roots of the canine and lateral incisors, bilaterally in the present case. A light intrusive force was delivered by a prestretched elastic chain to the anterior segment so that true intrusion of the anterior teeth could be achieved along their long axes. The present case revealed that maxillary intrusion by implant anchorage does not have an effect on vertical molar position and requires minimal patient cooperation.

External apical root resorption (EARR) occurs during treatment when forces at the apex exceed the resistance and reparative ability of the periapical tissues.^[10,11] Therefore, extremely light forces (15–25 g) should be used to produce appropriate pressure within the periodontal ligament.^[11] In the present case, a prestretched elastic chain introduced this optimal light force from mini-implant anchorage. As a result, optimal intrusion was obtained without EARR during the active treatment period.

CONCLUSION

Significant maxillary incisor intrusion with mini-implants was obtained without relying on patient cooperation and also with good control over the direction and amount of force. Cent percent anchorage was maintained during intrusion with no extrusion of the posterior teeth. This demonstrated that the mini-implant anchorage method was useful for achieving an excellent improvement of a dental deep bite and gummy smile in this patient.



Figure 6: Posttreatment photographs

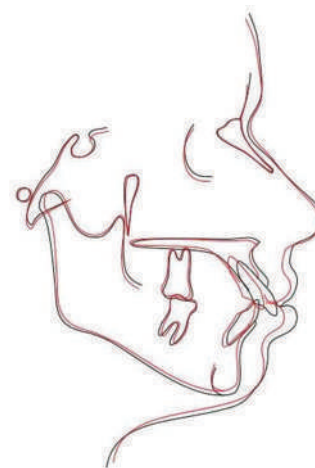


Figure 8: Pretreatment and posttreatment superimposition

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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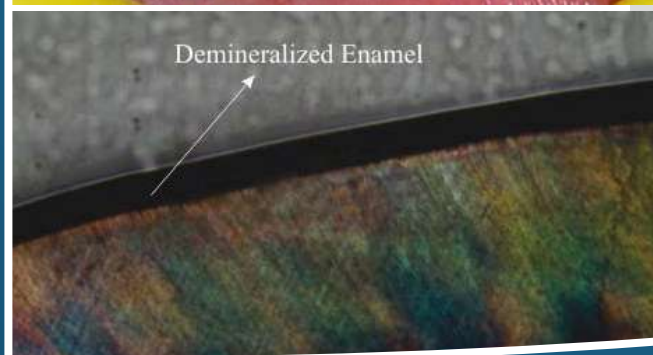


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Case Report

Bilateral Sagittal Split Osteotomy: Surgery First Approach for Correction of Skeletal Class II

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ABSTRACT

Most of the skeletal malocclusions which require orthognathic surgeries are treated by traditional approach which requires time-consuming and unesthetic presurgical orthodontic phase. Surgery first approach (SFA) avoids these disadvantages of the traditional approach. A 24-year-old female patient with skeletal and dental class II malocclusion due to retrognathic mandible was treated with SFA. Bilateral sagittal split osteotomy with mandibular advancement was done immediately after initial alignment and closure of the existing spaces in the maxillary arch. Angle's class I molar and canine relation was achieved after surgery. Bonding of the mandibular arch was done after 1 month of orthognathic surgery and treatment was completed within 13 months. A wrap-around retainer was placed in upper arch, and bonded lingual retainer was given in the lower arch.

KEYWORDS: *Bilateral sagittal split osteotomy, orthognathic surgery, regional acceleratory phenomenon, skeletal class II, surgery first approach*

INTRODUCTION

Patients with severe skeletal problem need to undergo orthognathic surgical procedures. The traditional orthognathic surgical procedure consists of three steps, i.e., presurgical orthodontic preparation, surgery and postsurgical orthodontic treatment for finishing and detailing.^[1] This traditional procedure takes long duration and decompensation during presurgical orthodontic procedure results in worsening of the profile which might not address the chief complaint of the patient. These shortcomings of traditional orthognathic surgical procedure shifted the trend toward "surgery first approach" (SFA) where the time taking presurgical orthodontic procedure is eliminated, jaws are repositioned in desired relation and then orthodontic treatment for correction of dental problems follows. Patients appreciate the immediate improvement in facial appearance while the orthodontist can utilize the increased bone turnover to achieve accelerated tooth movement.^[2-4]

This article presents the treatment of a skeletal class II patient with SFA method.

DIAGNOSIS AND TREATMENT PLANNING

A 24-year-old female patient came with a chief complaint of forwardly placed upper front teeth. Examination of pretreatment records [Figures 1-3a, 4a] revealed skeletal class II due to retrognathic mandible, hypodivergent growth pattern, Angles class II div 1 malocclusion, deep bite and lower midline shift to the left by 3 mm. After confirming with the cephalometric values [Table 1], bilateral sagittal split osteotomy (BSSO) with the advancement of mandible was planned. Based on patient's concern about esthetics, surgery first option was explained to her.

After evaluation of clinical aspects and cephalometric values, a nonextraction approach was preferred. The treatment plan was to do the initial alignment of the upper arch followed by BSSO and advancing the mandible by 6 mm.

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Figure 1: Pretreatment intraoral photographs



Figure 2: Pretreatment extraoral photographs

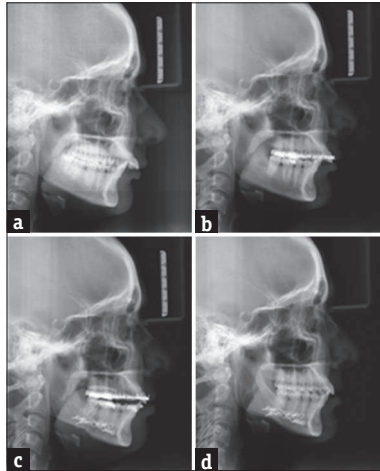


Figure 3: (a) Pretreatment lateral cephalogram, (b) presurgical lateral cephalogram, (c) postsurgical lateral cephalogram, (d) posttreatment lateral cephalogram

Table 1: Cephalometric data pretreatment and posttreatment

Measurement	Normal	Pretreatment	Posttreatment
SNA	82±2°	78°	78°
SNB	80±2°	72°	75°
ANB	2°	6°	3°
WITS	0-1 mm	7 mm	2 mm
B [⊥] -N [⊥]	-2 mm	-6 mm	-3 mm
N'Sn Pog'	161°	148°	150°
Pn -A	0±2	-6 mm	-3 mm
SN-Go-Gn	32°	27°	31°
FMA	25°	22°	24°
Max1-SN	102°	117°	109°
U1-NA	22°	39°	30°
	4 mm	9 mm	6 mm
IMPA	95	104o	96o
LI-NB	25°	25°	27°
	4 mm	5 mm	7 mm

TREATMENT PROGRESS

Before surgery, banding and bonding of the upper arch was done with 0.022" MBT prescription (Gemini series, 3M Unitek, Monrovia, California). After 4 months of initial alignment and leveling of

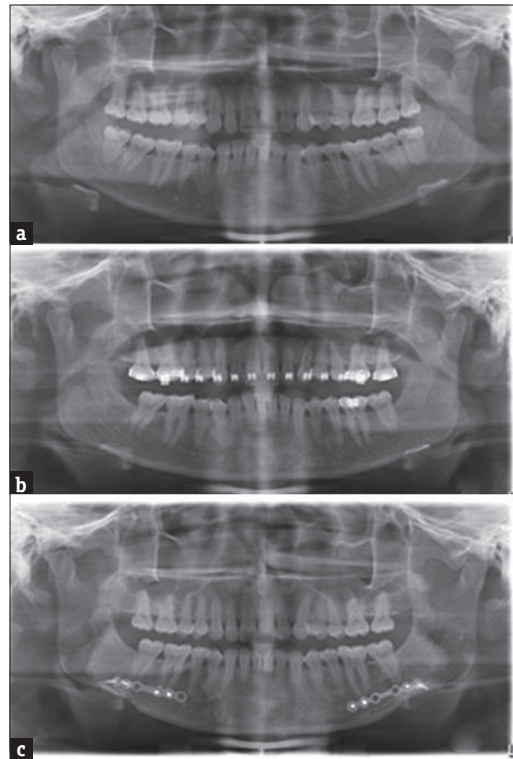


Figure 4: (a) Pretreatment orthopantomogram, (b) presurgical orthopantomogram, (c) posttreatment orthopantomogram

the upper arch with nickel-titanium (NiTi) wires, a 0.019" × 0.025" stainless steel (SS) stabilizing wire was placed, and presurgical records were taken [Figures 3b, 4b, 5 and 6]. Model surgery was done based on cephalometric prediction and surgical splint fabricated. BSSO was performed; mandible was advanced by 6 mm and stabilized with titanium plates. Postsurgical records [Figures 3c, 7 and 8] showed class I molar and canine relationship with improved facial profile. One month postorthognathic surgery, lower arch strap up was done, aligned and leveled with NiTi wires, space closure was done with SS wires followed by finishing and detailing.

After 13 months of total treatment time, brackets were removed and wrap around retainer was placed in the



Figure 5: Presurgical intraoral photographs



Figure 7: Postsurgical intraoral photographs



Figure 9: Posttreatment intraoral photographs

maxillary arch, and bonded lingual retainer was given in the lower anterior region.

TREATMENT RESULTS

Posttreatment records [Figures 3d, 4c, 9 and 10] showed resolution of skeletal and dental problems with good facial esthetics. Angle's class I molar and canine relationship was achieved with ideal overjet and overbite. Superimposition of pre- and post-treatment lateral cephalograms [Figure 11] showed that the treatment goals have been achieved. The patient was pleased with the treatment outcome.

DISCUSSION

Skeletal class II malocclusion in most of the cases presents with dentoalveolar compensation, proclination of mandibular incisors and upright or mild proclination of maxillary incisors. Traditional orthognathic surgical procedures consume much time for decompensation of skeletal class II before surgery.



Figure 6: Presurgical extraoral photographs



Figure 8: Postsurgical extraoral photographs



Figure 10: Posttreatment extraoral photographs

SFA was chosen owing to advantages like initial correction of complex skeletal problems, will normalize surrounding soft tissues lips, cheeks, and tongue which facilitate postoperative tooth movement and reduce the length of orthodontic treatment.^[5-7] Tooth movement would be accelerated by regional acceleratory phenomenon. Alveolar bone adjacent to the osteotomies performed during orthognathic surgery results in increased bone turnover. This could account for the more efficient orthodontic movements immediately after surgery which will ultimately decrease the total treatment time and patient trauma. Improvement in the patient's profile and esthetics during early stages of treatment will have the psychological advantage and acceptance of further treatment with much interest and cooperation.^[8-11]

Several protocols are being followed for SFA. Most of the orthodontists prefer to bond brackets and place

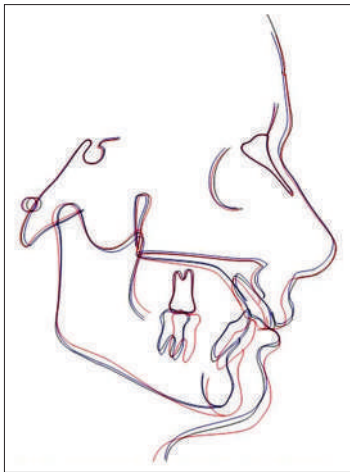


Figure 11: Pretreatment, presurgical, and posttreatment superimposition

passive SS archwires just before surgery, but some prefer to directly bond the wire or other orthodontic attachments to the tooth surface. Surgery first orthodontic approach may be favorable for a class II patient with a retrusive mandible. Immediately after surgery, the class II malocclusion becomes a super class I or class III relationship following mandibular advancement, with an edge-to-edge incisor relationship or bimaxillary dentoalveolar protrusion. This can be corrected by the use of class III orthodontic mechanics or by extracting all first premolars followed by retraction as in class I bimaxillary protrusion cases.^[11]

However in this case, as upper arch has spacings, we preferred to complete the upper arch alignment and leveling, and close the spaces before surgery, to have a better idea about overjet and to plan mandibular advancement accordingly. Furthermore, the upper arch alignment done is helpful for proper surgical splint fabrication and to guide the mandible into proper occlusion during surgery without any occlusal interference.^[3] Only mandibular first molars were banded before surgery so that immediately after surgery, training elastics could be given.

CONCLUSION

The SFA provides significant benefits compared with traditional orthognathic surgical treatment. Among its advantages is immediate profile improvement, reduced treatment time and may be more efficient and effective decompensation. With ideal case selection, correct diagnosis and treatment planning, proper model setup, and splint fabrication these advantages of SFA can be achieved.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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A study to assess expression of human papillomavirus types 16 and 18 in oral squamous cell carcinoma using polymerase chain reaction

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Abstract

Objective: The diverse subset of oral squamous cell carcinoma (OSCC) with different clinical appearance and outcome, independent of traditional risk factors has led to increasing attention in human papillomavirus (HPV) infection.

Materials and Methods: The investigation followed a case-control design. Information pertaining to the subjects was retrieved from hospital records. Twenty cases of OSCC and twenty age-matched controls were analyzed to ascertain the prevalence of HPV types 16 and 18. DNA was extracted from the blocks of formalin-fixed paraffin embedded tissues, and HPV-DNA was amplified using HPV type-specific primers by polymerase chain reaction (PCR) method. Data analysis was carried out using Chi-square test.

Results: HPV-DNA was detected in 55% of cases (11/20; HPV 16 = 6, HPV 18 = 3 and HPV 16 and 18 = 2) and 30% of controls (6/20; HPV 16 = 3, HPV 18 = 1 and HPV 16 and 18 = 2) indicating higher percentage of HPV presence among OSCC cases. No significant association was found between the presence of HPV and gender, age, site and grade of differentiation of OSCC.

Conclusion: Although the presence of HPV was higher in cases compared to controls, none of these differences were statistically significant. HPV 16 and 18 are commonly found in normal oral mucosa mandating the need for distinguishing clinical, subclinical and latent HPV infections.

Keywords: Human papillomavirus types 16 and 18, oral squamous cell carcinoma, polymerase chain reaction

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INTRODUCTION

Head and neck cancer is one of the ten most common types of cancer worldwide distressing more than 500,000 individuals every year.^[1]

Oral squamous cell carcinoma (OSCC) is most frequent accounting for over 90% of oral cancers. It represents the sixth most frequent malignant tumor worldwide.^[2]

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Tobacco, smokeless tobacco products such as gutka, pan masala, betel quid and alcohol are the risk factors for the development of OSCC.^[3]

Viruses such as human papillomavirus (HPV), Epstein–Barr virus and herpes simplex virus-1 (HSV-1) are also implicated to play a role in the development of OSCC.^[4]

HPVs are small DNA viruses infecting various human epithelial tissues. More than 130 HPV types have been identified and are classified into low- or high-risk groups based on their oncogenic potential.^[5]

HPV gives rise to a distinct clinical entity of oropharyngeal squamous-cell carcinomas (OPSCCs) with a considerably better prognosis than HPV-negative OPSCC, often related to tobacco and alcohol consumption.^[6]

There is a growing evidence of causal association of high-risk HPV types mainly HPV 16/18 and OSCC. A number of studies have shown that HPV is associated with increased risk of oral cancer, independent of exposure to tobacco and alcohol. This association is valid for HPV 16 and 18 because of its detection in oral dysplastic lesions and oral cancers.^[7]

The nature of the relationship between HPVs and OSCC remains unclear owing to difficulties with interpreting studies that have demonstrated prevalence rates ranging from 0% to 100%. The diverse populations and assays with varying degrees of sensitivity for detecting viral DNA also make interpretation difficult.^[8]

HPV is a sexually transmitted infection, and findings suggest that the number of lifetime sexual partners is an important risk factor for the development of HPV-associated head and neck SCC. In case–control studies, the odds of HPV-positive malignant disease increased 2-fold in individuals who reported between one and five-lifetime oral sexual partners and 5-fold in those with six or more, compared with those recalling no oral sex.^[9]

A wide range of variation has been noticed in HPV positivity rates in cancers at different sites in head and neck region. Highest rates being reported in tonsillar region followed by cancers of tongue and buccal mucosa.^[10]

A meta-analysis including 94 studies on HPV presence in oral mucosa showed that oral dysplasia and OSCC are more commonly associated with HPV infection particularly subtypes 16 and 18 compared to that of normal oral mucosa (NOM).^[11]

The field of human cancer research has been advanced with the application of highly sensitive molecular biology tools such as polymerase chain reaction (PCR) which permits virus detection soon after infection and even before the onset of disease. The purpose of the current study is to assess the prevalence of HPV in OSCC.

MATERIALS AND METHODS

Source of data

In the present study, tissues were collected from clinically suspected patients of OSCC who attended the Department of Oral Pathology and Microbiology. Tissues from retromolar area were collected from patients who underwent surgery for impactions and used as controls after approval from the Institutional Ethics Committee. The study consisted of 40 samples categorized into two groups; twenty cases of OSCC and twenty age-matched controls.

Methodology

Part of the tissue was processed and sections were stained and examined for routine hematoxylin and eosin to confirm the diagnosis. From remaining part of the histologically proven tissues, DNA extraction was done and subjected to PCR for the evaluation of HPV-positive samples.

Collection of sample

Specimens were collected from both OSCC patients and controls. After obtaining, tissues were kept in a small ziplock bag for immersing into liquid nitrogen then stored at -20° until use.

DNA extraction procedure from fresh tissue samples

Tissues collected were subjected to dehydration by the addition of 1 ml of alcohol for 30 min then the mixture is centrifuged, and the supernatant is discarded. Later, the pellet was suspended in 500 μ l TE buffer and Vortexed. Then centrifuged at 10,000 rpm for 5 min and supernatant was discarded and washed with fresh TE buffer for 2–3 times. Supernatant was discarded and 50 μ l lysis buffer I was added, Vortexed and kept for 5 min. Later, 50 μ l Lysis buffer II was added along with 10 μ l Proteinase–K (10 mg/ml), vortexed vigorously. Kept in water bath at 60°C for 2 h. Then, kept in boiling water bath for 10 min for enzyme deactivation. The supernatant containing DNA was taken to fresh tube and stored at -20°C . Amplification was done by conventional PCR using HPV 16 and 18 primers [Table 1].

Polymerase chain reaction procedure

The detection of HPV 16 and HPV 18 was carried out in two separate reactions for each sample. The reaction mixture preparation steps for PCR are as follows:

- Gently vortexed and briefly centrifuge PCR master mix after thawing
- A thin-walled PCR tube is placed on ice, and the following components are added for each 50 µl reaction
- A premixture was prepared and aliquoted into each tube. The premix contains following components in a final volume of 20 µl/aliquot
- The samples are gently vortexed and spinned down
- Then tubes are placed in conventional thermal cycler (Applied Biosystems, USA).

The polymerase chain reaction conditions were as follows

Initial denaturation was carried out at 95°C for 5 min. Denaturation, annealing and extension were carried out at temperatures of 95°C, 53°C and 72°C, respectively, for 1 min and extension over a period of 2 min. Final extension was done at 72°C for 5 min.

The amplified products were run on 2% agarose gel electrophoresis for detection of HPV 16 and HPV 18 specific bands. The gel for HPV 16 and HPV 18 reactions was run separately. Then, the photo of gel under ultraviolet light transilluminator was taken, and the bands were recorded using Gel documentation system (Major Science, USA).

Amplicon size of 120 base pair corresponds to HPV 16. Rest other bands were considered as nonspecific [Figure 1]. Amplicon size of 100 base pair corresponds to HPV 18. Rest other bands were considered as nonspecific [Figure 2].

Statistical analysis

The collected data were entered into the excel sheet, and statistical analysis was done using software, Statistical

Package for Social Sciences (SPSS) version 20.0. Comparison of two groups with respect to HPV 16, 18 and 16 and 18 positivity was done by Chi-square test.

RESULTS

A total of 20 OSCC cases and 20 controls were included in the study. Distribution of age among study and control groups was done at an intervals of ten from 20 to 60 years. Pertaining to gender, controls consisted of 10 males and 10 females, and cases consisted of 12 males and 8 females.

HPV-DNA was detected in 11 out of 20 cases and 6 out of 20 controls indicating a higher percentage of HPV presence among OSCC cases. Statistical analysis was performed using Chi-square test, and the difference was not statistically significant ($P = 0.110$). HPV 16 positive status among cases and controls was 6/20 and 3/20, respectively. HPV 18 positive status was 3/20 among cases and 1/20 among controls. HPV 16 and 18 positivity was noticed in 2 cases and 2 controls. However, this difference was not statistically significant ($P = 0.378$).

No significant association was found between the presence of HPV and gender and age [Table 2], site and grade of differentiation of OSCC [Table 3].

Table 1: Primer sequences for human papilloma virus 16 and 18

HPV type	Primer sequence
HPV 16	Forward primer: 5'- TCA AAA GCC ACT GTG TCC TG-3' Reverse primer: 5' - CGT GTT CTT GAT GAT CTG CA-3'
HPV 18	Forward primer: 5'- ACC TTA ATG AAA AAC GAC GA-3' Reverse primer: 5' - CGT CGT TGG AGT CGT TCC TG-3'

HPV: Human papilloma virus

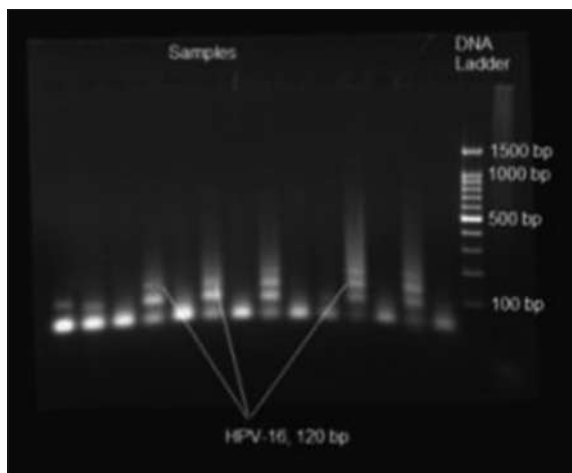


Figure 1: Human papilloma virus 16-specific primer mediated polymerase chain reaction of DNA extracted from oral squamous cell carcinomas. Polymerase chain reaction products shown after gel electrophoresis

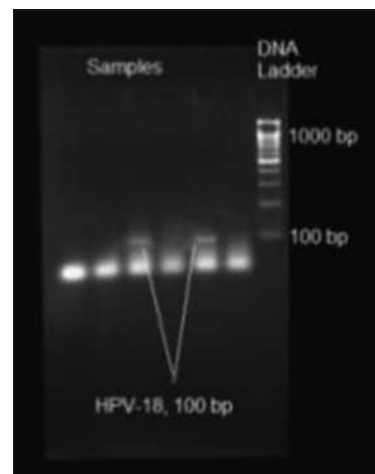


Figure 2: Human papilloma virus 18 -specific primer mediated polymerase chain reaction of DNA extracted from Oral squamous cell carcinomas. Polymerase chain reaction products shown after gel electrophoresis

Table 2: Comparison of human papilloma virus prevalence among cases in relation to gender and age

Parameters	Total	HPV 16	HPV 18	Both	None	χ^2	df	P
Gender								
Males	12	5	1	1	5	2.407	3	0.492**
Females	8	1	2	1	4			
Age (years)								
20-30	3	1	1	0	1	8.519	9	0.483**
31-40	2	1	0	0	1			
41-50	5	1	0	2	2			
51-60	10	3	2	0	5			

Chi-square test, * $P < 0.05$ (S), ** $P > 0.05$ (NS). HPV: Human papilloma virus, S: Significant, NS: Not significant

Table 3: Comparison of human papilloma virus prevalence among cases in relation to site and grade of differentiation

Parameters	Total	HPV 16	HPV 18	Both	None	χ^2	df	P
Site								
Posterior most area	9	1	1	2	5	6.617	9	0.677**
Buccal mucosa	5	2	1	0	2			
Tongue	5	2	1	0	2			
Lower anterior area	1	1	0	0	0			
Grade of differentiation								
Well differentiated	17	6	2	2	7	8.497	6	0.204**
Moderately	2	0	0	0	2			
Basaloid	1	0	1	0	0			

Chi-square test, * $P < 0.05$ (S), ** $P > 0.05$ (NS). HPV: Human papilloma virus, S: Significant, NS: Not significant

DISCUSSION

Squamous cell carcinoma (SCC) is the most frequent oral cavity malignancy accounting for over 90% of oral cancers, representing the sixth most frequent malignant tumor worldwide.^[12]

Tobacco and alcohol consumption are implicated in 75% of OSCC, and smoking accounts for 42% of deaths from cancers of oral cavity and heavy alcohol consumption for 16% of the deaths. The rest 25% of OSCC are attributed to HPV infection. Even though at least 15 HPV types are thought to have oncogenic potential, the most prevalent type causing HPV-associated oral squamous cell cancers is HPV 16, which is also implicated in HPV-associated anogenital cancers.^[13]

The ethnicity and geographic origin of patients are responsible for differences in HPV prevalence in head and neck SCC (HNSCC). Asiatic countries, in particular, Japan, have the highest worldwide frequency. This high prevalence of HPV in Asiatic patients with oral cancers indicate that viral infection may be an important etiological agent and along with dietary habits and a probable genetic predisposition can cause additional mutations leading to malignancy. The lowest prevalence of HPV-positive HNSCC was noticed in Africa.^[14]

In the present study, when gender and viral prevalence are considered, out of 12 male subjects 7 of them exhibited HPV positivity and 4 females were HPV positive out of 8 cases. This is in accordance with the studies conducted by Brandwein *et al.* and Benson *et al.* where they stated that OSCC is most commonly diagnosed among men compared to women.^[15,16]

In the current study, HPV was detected in 11 out of 20 cases and 6 out of 20 controls. Results of the present study indicated a higher percentage of HPV prevalence among cases compared to controls, which is in accordance to studies performed by Gan *et al.* where they mentioned that HPV prevalence was higher among cases compared to controls.^[13]

Zhu *et al.* did meta-analysis to evaluate the relationship of OSCC with HPV infection in Chinese population and stated that high incidences of HPV infection particularly HPV 16 was found in the samples of Chinese OSCC that elevates the risk of OSCC tumorigenesis. This is in accordance with our current study where a relatively higher percentage of HPV 16 presence was observed.^[17]

D'Costa J *et al.* conducted a study to detect HPV 16 and 18 DNA in tissues from patients with oral cancer, potentially malignant lesions (PMLs) and subjects having normal mucosa using PCR. They found HPV 16 positivity in 15% of OSCC, 34% of PMLs and 15% of subjects with NOM indicating that HPV infections are important but may not be sufficient for the progression to malignancies and that synergistic actions with other carcinogenic agents may be required.^[18]

Giovannelli *et al.* in 2002 studied the presence of HPV-DNA in various oral mucosal lesions (13 SCCs, 59 PMLs, 49 benign erosive ulcerative lesions) through nested PCR and found in 80% of the HPV-positive controls.^[19]

In the present study, HPV positivity (6/20) was also noticed among controls. HPV is commonly found in NOM mandating the need for distinguishing clinical, subclinical and latent HPV infections.

Paz *et al.* have done a study to assess the association of HPV 16 with SCC and found HPV sequences in 25 out of 167 tumors (15%), but HPV was detected most frequently in tumors in Waldeyer's tonsillar ring.^[20]

In this study, when site and HPV prevalence was observed, 5 cases out of 9 taken from posterior-most areas of the oral

cavity were positive for HPV. With regard to tissue taken from buccal mucosa, three subjects were HPV positive out of five. While considering lower anterior region, one subject included in the study expressed positivity. Out of five cases selected from tongue region, three of them exhibited positivity indicating HPV predilection for certain sites in head and neck region particularly tonsil and base of the tongue.

Westra WH mentioned that HPV-related oropharyngeal cancers are highly differentiated and not poorly differentiated. A subtype of HNSCC, basaloid SCC presents with aggressive clinical behavior. Within the basaloid subtype, detection of HPV is a highly favorable prognostic factor that helps in identifying a subset of cancers that departs from the highly aggressive behavior associated with this variant.^[21]

Benson *et al.* in 2014 mentioned that histologically, HPV-HNSCCs are non-keratinizing with basaloid features. Initially, they were described as poorly differentiated, but on further analysis, they are similar in morphology to the reticulated epithelium of the tonsillar crypts from which they are thought to arise and therefore are more appropriately now described as well differentiated.^[16]

In this study on the comparison of grade of differentiation of OSCC with HPV-positive status, 9 well-differentiated OSCC cases were HPV positive out of 17. One basaloid variant of OSCC exhibited HPV 18 positivity while 2 cases of moderately differentiated OSCC were HPV negative. The result of our present study correlated with above-mentioned statement pertaining to grades of OSCC differentiation.

CONCLUSION

In the present study, expression of HPV was higher among OSCC cases when compared to controls with a relatively higher percentage of HPV 16 positivity. However, the difference was not statistically significant. Most of the cases exhibiting HPV positivity belonged to well-differentiated pattern of OSCC. Although the presence of HPV was higher in cases compared to controls, none of these differences were statistically significant. HPV 16 and 18 are commonly found in NOM mandating the need for distinguishing clinical, subclinical and latent HPV infections. Hence, further studies on larger samples using sensitive detecting techniques such as real-time-PCR provide conclusive results.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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A Study on the Expression of CD44 Adhesion Molecule in Oral Squamous Cell Carcinoma and its Correlation with Tumor Histological Grading

Abstract

Background: The increasing incidence of oral squamous cell carcinoma (OSCC) combined with its poor and unchanged prognosis motivates a need for research into the aspects of tumor biology such as the expression of adhesion molecules. Alterations in the properties of adhesion molecules could play a pivotal role in the development and progression of invasive cancer and distant metastasis. **Aim:** This study aims to quantify the expression of cluster of differentiation 44 (CD44) in the different grades of OSCC. **Materials and Methods:** Immunohistochemical staining for CD44 was performed in 10 tissue sections each of normal mucosa, well-differentiated OSCC, moderately differentiated OSCC, and poorly differentiated OSCC. CD44 positivity was analyzed quantitatively in 10 representative fields of each section under 20× magnification. **Statistical Analysis Used:** Statistical analysis was performed using Statistical Package for Social Sciences software, version 20.0 (SPSS Inc., Chicago, IL, United States). **Results:** The mean value of the number of positive cells in controls was 745 ± 68.17 , in well-differentiated OSCC was 694.2 ± 145.47 , in moderately differentiated OSCC was 349 ± 78.40 , and in poorly differentiated OSCC was 108.8 ± 34.39 . **Conclusion:** The results of this study suggest an altered expression of CD44 in OSCC with weak immunostaining in poorly differentiated squamous cell carcinoma. Thus, it can be inferred that the loss of cell adhesion, correlated to the decrease of CD44 expression, might be of value in determining the progression of OSCC.

Keywords: CD44, immunohistochemistry, oral squamous cell carcinoma

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Introduction

Head and neck cancer is a major burden in Asia particularly in India, where more than 200,000 new cases are diagnosed each year. Moreover, 7.8% of the global cancer burden and 8.33% of global cancer deaths are contributed by the Indian subcontinent.^[1] Oral cancer accounts for 30% of all the cancers in the country.^[2] Squamous cell carcinoma accounts for 95% of the malignant tumors of the oral cavity. More than 60% of the patients present with an advanced stage of the disease.^[3] It is the sixth most common cancer in the world, eighth most common cancer in men, and fifth most common cancer in women.^[4] As per the global cancer statistics published in 2012, the incidence of oral squamous cell carcinoma (OSCC) is estimated at 300,400 new cases and 145,400 deaths annually.^[5] The overall 5-year survival rate is <50% and has not improved significantly over the

last three decades.^[6] Despite the accessibility of oral cavity for visual examination along with well-defined clinical diagnostic features for oral cancers, they are being detected in their advanced stages. In the Indian scenario, 60–80% of patients present with an advanced stage of disease as compared to 40% in developed countries.^[7]

Carcinogenesis is a multistep process, which alters the genetic events within signal transduction pathways that govern normal cellular physiology such as cell division, differentiation, senescence, and adhesion. As the alterations or mutations in these pathways get accumulated, the cells become functionally independent from the surrounding normal epithelial cells with enhanced ability to proliferate and invade or metastasize to distant sites. The accumulation of such changes will correlate to the histologic grade of the differentiation of cancer and also reflects on the prognosis.^[8]

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The increasing incidence of OSCC, combined with its poor and unchanged prognosis, motivates a need for research into the aspects of tumor biology such as the expression of adhesion molecules. Alterations in the properties of adhesion molecules could play a pivotal role in the development and progression of invasive cancer and distant metastasis. The loss of cell–cell adhesion allows malignant cells to escape from their site of origin, degrade the extracellular matrix, and acquire a motile and invasion phenotype that can metastasize.^[9]

The cluster of differentiation 44 (CD44) is a transmembrane cell adhesion molecule involved in cell-to-cell and cell-to-matrix interactions by binding with hyaluronan, extracellular matrix proteins, and growth factors.^[10] Dalchau *et al.* in 1980 first described CD44 as brain-granulocyte-T lymphocyte antigen.^[11] It was first identified on the lymphocytes having functions essential for cell homing and is encoded by a gene located on the short arm of chromosome 11.^[12] CD44 consists of a cytoplasmic domain and a transmembrane domain. It is constituted by 20 exons, in which the first five and the last five exons are constant, and the remaining 10 exons result in the generation of a variable region.^[13] CD44 has various isoforms that exist due to the splicing of variable exons, which encode the proximal portions of the extracellular cytoplasmic domain.^[14]

The invasion and dissemination of OSCC requires active cell migration through the extracellular matrix with a remodeling of intercellular adhesions.^[15] CD44 is thought to undergo structural and functional alterations during malignant transformation, which lends the cancer cells to detach from the site of tumor growth and invade the surrounding tissues. Hence, CD44 deserves considerable attention with respect to its adhesive, locomotive, and growth-transducing functions among the cancer cells.^[16] CD44 is also a cancer stem cell marker with two distinct phenotypes such as: (i) The epithelial–mesenchymal transition phenotype cancer stem cells that express proteolysis-resistant CD44s isoform and (ii) The cancer stem cells with epithelial phenotype that expresses trypsin-sensitive CD44v isoforms. This results in the loss of CD44 expression in epithelial phenotype cancer stem cells and a higher expression in the epithelial–mesenchymal transition phenotype during isolation.^[17]

The field of human cancer research is rapidly advancing, and the application of molecular biology tools such as immunohistochemistry made the diagnosis much easier. This study aimed to assess the immunohistochemical expression of CD44 in the different grades of OSCC and to evaluate its role as an indicator of cancer progression.

Materials and Methods

This study was performed using 10 formalin-fixed, paraffin-embedded tissue blocks of each category, which were diagnosed histologically as well-differentiated, moderately differentiated, and poorly differentiated OSCC, and were retrieved from the archives of the Department of Oral Pathology and Microbiology, SIBAR Institute of Dental

sciences, Guntur. As controls, 10 formalin-fixed, paraffin-embedded tissue blocks of the normal oral mucosa over the impacted third molars was obtained from the patients undergoing surgery for impactions after informed consent. Ethical approval for this study was obtained from the Ethical Committee of SIBAR Institute of Dental Sciences, Guntur, on 24th December 2013 (Ref.no.20/IEC-SIBAR/2013). The grading of OSCC was performed based on the criteria proposed by the World Health Organization in 2005.

Samples were categorized into four groups as follows:

- (1) Group I—Controls (10).
- (2) Group II—Well-differentiated OSCC (10).
- (3) Group III—Moderately differentiated OSCC (10).
- (4) Group IV—Poorly differentiated OSCC (10).

Control group comprised apparently normal healthy oral mucosa, which was obtained from patients without any tobacco smoking or chewing habits, alcohol consumption, and any clinically obvious lesions.

Serial sections having a thickness of 4 µm were prepared. The sections of Groups I–IV were first subjected to routine hematoxylin and eosin examination to reconfirm the diagnosis. Later, other sections of all the four groups were subjected to immunohistochemical analysis using anti-CD44 antibody. For immunohistochemical staining, sections were carefully fixed on microslides coated with ploy-l-lysine. The tissue was deparaffinized by giving two dips of 10 min each in fresh xylene. The rehydration of the tissue was performed by giving three dips for 5 min each in 90, 80, and 70% alcohol. Thereafter, the tissue was placed in a distilled water bath and was not allowed to dry. Tissue sections were dipped in buffer solution prepared by mixing 1.21 g of Tris Ethylene diamine tetra acetic acid (EDTA) buffer with 0.37 g of EDTA and incubated at 850 W for 5 min, 600 W for 10 min, and 400 W for 5 min in a microwave oven. The slides were allowed to cool and then placed in a wash buffer prepared by adding 950 ml of distilled water with 50 ml of buffer. Excess buffer from the slides was removed using tissue paper. Then the tissue specimen was covered with 50 µl of hydrogen peroxide, incubated for 5 min, and gently washed twice with phosphate buffered saline. The tissue specimen was then covered with primary antibody—a monoclonal anti-CD44 antibody—incubated for 1 h at room temperature and gently washed twice with phosphate buffered saline. The secondary link antibody was then added. Specimens were incubated for 30 min at room temperature and given three gentle rinses with phosphate buffered saline. The substrate chromogen solution prepared by mixing 1 ml of substrate buffer and a drop of diaminobenzidine was incubated for 5 min at room temperature and later gently rinsed with phosphate buffered saline. Then the slides were counterstained in a bath of hematoxylin for 2–5 min and washed under tap water for 5 min.

Positive CD44 expression was seen as a light brown stain in the cell membrane. All stained areas demonstrating positivity

for CD44 were identified at a magnification of 20 \times , and the number of positively stained cells was counted on 10 representative areas of the section, in a minimum of 100 cells per field [Figures 1–8]. The CD44 positivity was estimated according to the criteria of Simionescu *et al.* as follows:^[18]

- (1) Score 0—<10% positive cells.
- (2) Score 1—Between 10 and 25% positive cells.
- (3) Score 2—Between 25 and 50% positive cells.
- (4) Score 3—Between 50 and 75% positive cells.
- (5) Score 4—Over 75% positive cells.

Statistical analysis

The collected data were entered in the Excel spreadsheet, and statistical analysis was performed using the Statistical Package for Social Sciences software, version 20.0 (SPSS Inc., Chicago, IL, United States). The normal distribution of the number of positive cells was determined by “Kolmogorov–Smirnov test.” A comparison of the four groups with respect to the percentage of positive cells, as

well as the scores, was performed using “Kruskal–Wallis analysis of variance (ANOVA) test.” A pairwise comparison of the three grades of OSCC and the controls was performed using “Mann–Whitney *U* test.”

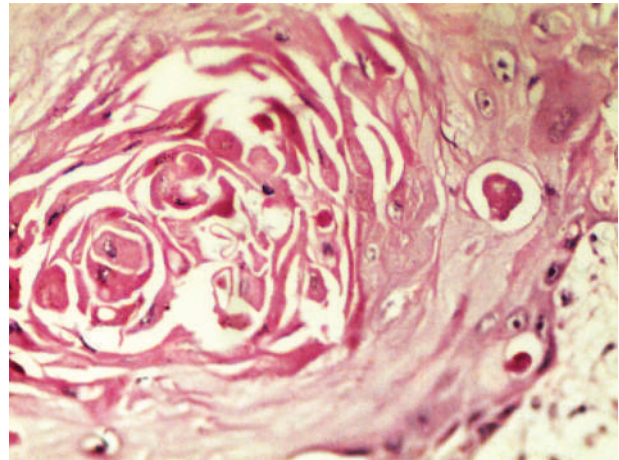


Figure 3: 20 \times view of hematoxylin and eosin stained photomicrograph of well-differentiated squamous cell carcinoma

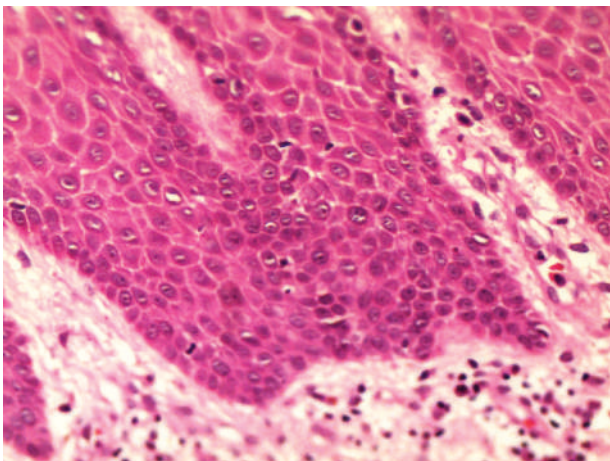


Figure 1: 20 \times view of hematoxylin and eosin stained photomicrograph of normal oral mucosa

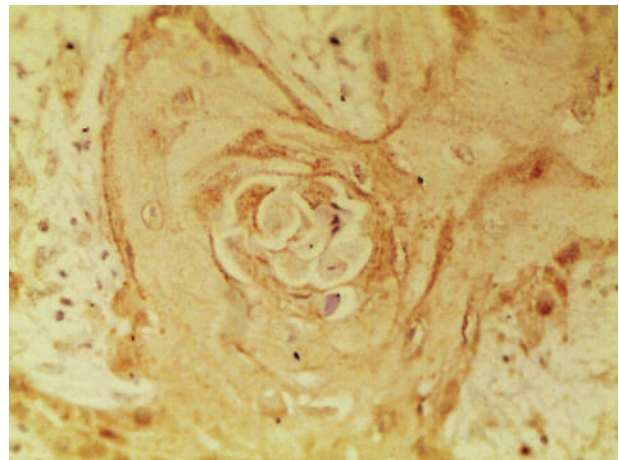


Figure 4: 20 \times view of photomicrograph of well-differentiated squamous cell carcinoma showing CD44 immunopositivity

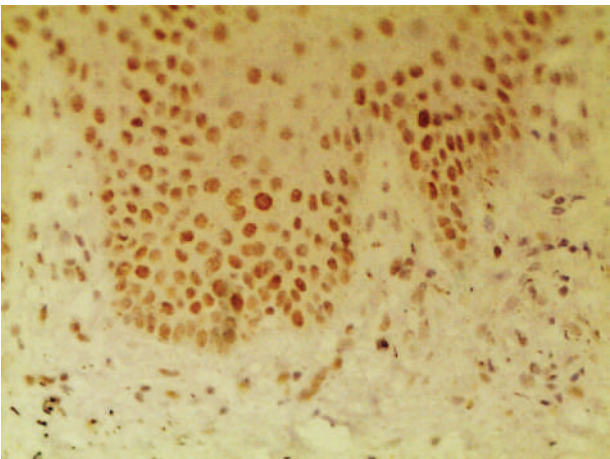


Figure 2: 20 \times view of photomicrograph of normal oral mucosa showing CD44 immunopositivity

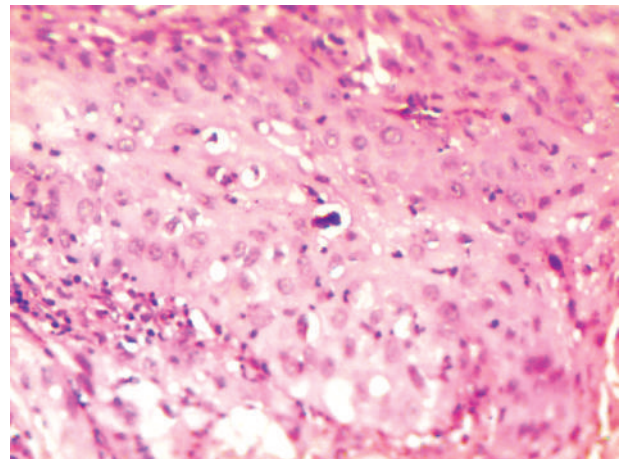


Figure 5: 20 \times view of hematoxylin and eosin stained photomicrograph of moderately differentiated squamous cell carcinoma

Results

A total of 30 cases of OSCC, divided into three groups with 10 samples each of well-differentiated, moderately

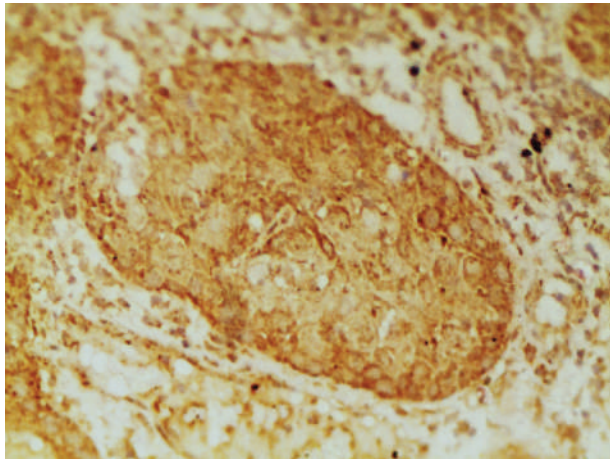


Figure 6: 20x view of photomicrograph of moderately differentiated squamous cell carcinoma showing CD44 immunopositivity

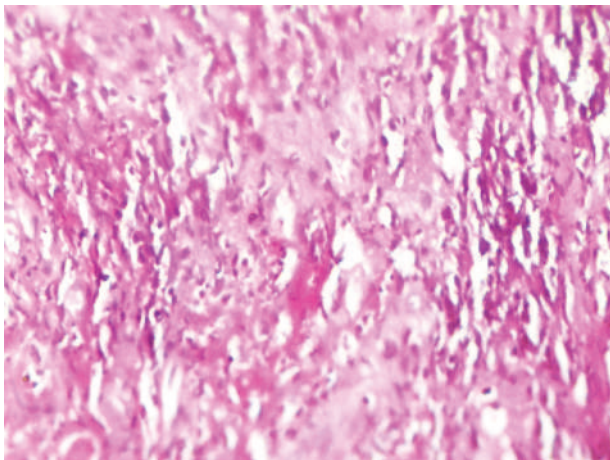


Figure 7: 20x view of hematoxylin and eosin stained photomicrograph of poorly differentiated squamous cell carcinoma

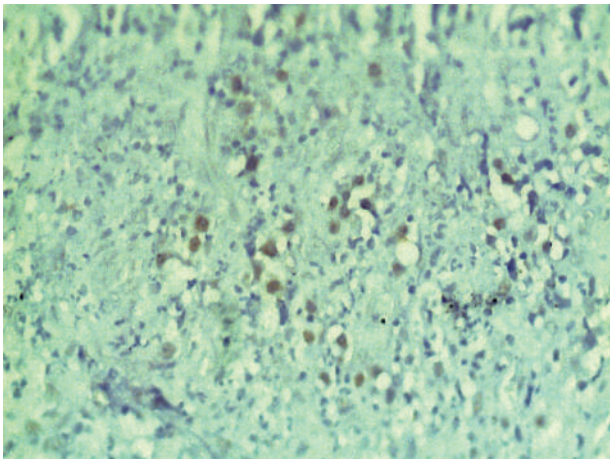


Figure 8: 20x view of photomicrograph of poorly differentiated squamous cell carcinoma showing CD44 immunopositivity

differentiated, and poorly differentiated squamous cell carcinomas and 10 samples of controls were taken for the study. All the groups were subjected to immunohistochemical analysis using anti-CD44 antibody. The number of immunopositive cells was evaluated in each case, and a score was given accordingly.

The mean value with respect to the percentage of positive cells was 74.5 ± 6.82 in Group I (controls), 69.42 ± 14.55 in Group II (well-differentiated OSCC), 34.9 ± 7.84 in Group III (moderately differentiated OSCC), and 10.88 ± 3.44 in Group IV (poorly differentiated OSCC). Statistical analysis was performed using “Kruskal–Wallis ANOVA test,” and a high statistically significant difference of mean was obtained ($P = 0.00001$) [Table 1].

A pairwise comparison of the four groups with respect to the percentage of positive cells was performed using “Mann–Whitney *U* test.” A high statistical significance was noticed on comparing Group I and Group III, Group I and Group IV, Group II and Group III, Group II and Group IV, and Group III and Group IV ($P = 0.0002$). However, a comparison of Group I and Group II did not show any statistical significance ($P = 0.3258$) [Table 2].

A comparison of the four groups with respect to the score given based on the number of immunopositive cells was performed using “Kruskal–Wallis ANOVA test.” Of the 10 cases in Group I (controls), five (50%) had Score 3, and the other five (50%) had Score 4. One case (10%) of Group II (well-differentiated OSCC) had Score 2, five cases (50%) had Score 3, and four cases (40%) had Score 4. In Group III (moderately differentiated OSCC), nine cases (90%) had Score 2, and one case (10%) had Score 3. Five cases (50%) of Group IV had Score 0, and the other five cases (50%) had Score 1. Of the total 40 samples, Score 0 and Score 1 were seen in five cases each, with all being poorly differentiated squamous cell carcinoma. Score 2 was seen in 10 cases, with nine being moderately differentiated OSCC and one being well-differentiated OSCC. Score 3 was seen in 11 cases, with five each in controls and well-differentiated OSCC and one in moderately differentiated OSCC. Score 4 was observed in nine cases, with five and four each in

Table 1: Comparison of four groups with respect to the percentage of positive cells by Kruskal–Wallis ANOVA test

Groups	Mean	SD	SE	Sum of ranks
Group I	74.55	6.82	2.16	318
Group II	69.42	14.55	4.60	291
Group III	34.90	7.84	2.48	156
Group IV	10.88	3.44	1.09	55
<i>H</i> -value			32.9751	
<i>P</i> -value			0.00001*	

SD, standard deviation; SE, standard error. * $P < 0.05$.

controls and well-differentiated OSCC, respectively. A high statistical significance ($P = 0.00001$) was obtained [Table 3].

A pairwise comparison of the four groups with respect to score was performed using “Mann–Whitney U test.” A high statistical significance was noticed on comparing Group I and Group III ($P = 0.0003$), Group I and Group IV ($P = 0.0002$), Group II and Group III ($P = 0.0015$), Group II and Group IV ($P = 0.0002$), and Group III and Group IV ($P = 0.0002$). However, there was no statistical significance between Group I and Group II ($P = 0.5708$) [Table 4].

Discussion

Oral cancer being the sixth most common cancer and with OSCC being the most common among the general population, they represent a major health problem in many parts of the globe.^[19] Carcinogenesis is a multistep process resulting from the accumulation of genetic alterations that occur even before the development of a malignant phenotype. As the tumor progresses, cells undergo further mutations, leading to an increased heterogeneity of the tumor cell population. The cells detach from the tumor mass due to the loss of adherence property and invade the neighboring

tissues. The detached cells enter the blood and lymph, through which they are transported to distant sites (metastasis) and lead to the development of secondary tumors.^[20] In this study, the immunoexpression of CD44 was assessed among the various grades of OSCC.

In this study, a decline in the expression of CD44 was observed with the increasing grades of OSCC when compared with the normal oral mucosa, which was in accordance with studies conducted by Mende *et al.*, Kuo *et al.*, Satoa *et al.*, Carinci *et al.*, Gonzalez-Moles *et al.*, Mostaana *et al.*, and Krump and Ehrmann.^[21-27] A strong expression of CD44 in the normal epithelium when compared with the OSCC indicates that CD44 is required for the maintenance of epithelial structure as a whole by the homing of the epithelial cells. The loss of CD44 expression favors the invasiveness of tumor cells by emancipating the cells from their attachment with the neighboring cells. It plays a crucial role in the behavior of malignant tumors.^[28]

The mean value of CD44 immunopositive cells in this study is higher in well-differentiated squamous cell carcinoma when compared to moderately and poorly differentiated squamous cell carcinomas. Poorly differentiated squamous

Table 2: Pairwise comparison of four groups with respect to the percentage of positive cells by Mann–Whitney U test

Groups	Mean	SD	Sum of ranks	U -value	Z -value	P -value
Group I	74.55	6.82	118.00			
Group II	69.42	14.55	154.00	37.00	-0.9827	0.3258
Group I	74.55	6.82	118.00			
Group III	34.90	7.84	56.00	0.00	-3.7796	0.0002*
Group I	74.55	6.82	118.00			
Group IV	10.88	3.44	55.00	0.00	-3.7796	0.0002*
Group II	69.42	14.55	154.00			
Group III	34.90	7.84	56.00	1.00	-3.7041	0.0002*
Group II	69.42	14.55	154.00			
Group IV	10.88	3.44	55.00	0.00	-3.7796	0.0002*
Group III	34.90	7.84	56.00			
Group IV	10.88	3.44	55.00	0.00	-3.7796	0.0002*

SD, standard deviation. * $P < 0.05$.

Table 3: Comparison of four groups with respect to score

Score	Group I	%	Group II	%	Group III	%	Group IV	%	Total
Score 0	0	0.00	0	0.00	0	0.00	5	50	5
Score 1	0	0.00	0	0.00	0	0.00	5	50	5
Score 2	0	0.00	1	10	9	90	0	0.00	10
Score 3	5	50	5	50	1	10	0	0.00	11
Score 4	5	50	4	40	0	0.00	0	0.00	9
Total	10	100	10	100	10	100	10	100	40
H -value	32.5614								
P -value	0.00001*								

* $P < 0.05$.

Table 4: Pairwise comparisons of four groups with respect to score by Mann–Whitney *U* test

Group I vs. Group II	<i>P</i> = 0.5708
Group I vs. Group III	<i>P</i> = 0.0003*
Group I vs. Group IV	<i>P</i> = 0.0002*
Group II vs. Group III	<i>P</i> = 0.0015*
Group II vs. Group IV	<i>P</i> = 0.0002*
Group III vs. Group IV	<i>P</i> = 0.0002*

**P* < 0.05.

cell carcinoma cases showed diminished CD44 expression. A correlation is made with the immunostaining degree, as given by the total number of positive cells in each case. The immunostaining degree is proportional to the grade of the differentiation of OSCC. All cases of well-differentiated squamous cell carcinoma expressed the highest immunostaining score of 3 and 4 with 50–75% and over 75% positive cells, respectively. Moderately differentiated squamous cell carcinoma cases presented a lower immunostaining score of 2 with 25–50% of immunopositive cells. When poorly differentiated squamous cell carcinoma was evaluated, half the cases presented a weak immunostaining score of 1 with 10–25% positive cells, and the other half of the cases showed zero degree with <10% of CD44-positive cells. We can assess that a total absence of score or Score 1 on immunostaining was associated with undifferentiated OSCC having an unpredictable prognosis. These findings are in accordance with the studies of Salmi *et al.*, Bahar *et al.*, Kunishi *et al.*, Soukka *et al.*, Ue *et al.*, Stoll *et al.*, Kanke *et al.*, Fonseca *et al.*, Simionescu *et al.*, Hedesiú *et al.*, da Cruz *et al.*, and Hema *et al.*^[29–40]

The restricted expression of CD44 in various grades of OSCC was related to the degree of cellular proliferation and differentiation. The decrease in the immunostaining expression of CD44 with increase in the grade of tumor may suggest a loss of cell–cell adhesion, thereby contributing to the easy detachment of cells from a rigid configuration. The varying pattern of expression in the different grades of OSCC was related to the presence of pleomorphic cells. Highly pleomorphic cells with a weak resemblance to the parent tissue were present in poorly differentiated squamous cell carcinoma, and hence, a weak CD44 immunostaining is associated with poorly differentiated squamous cell carcinoma. CD44 also signals for the orientation of epithelial cells that differentiate and migrate upward. These events are switched off as the cells reach their end differentiation and are disclosed from their intercellular junctions. CD44 downregulation might reflect an early cellular change from normal cell–cell and cell–matrix interactions to a bizarre heterotypic cell surface adhesion property that predisposes the cells to attain invasiveness.^[41] The normal oral epithelium showed an extensive expression of CD44 in the basal and parabasal

cells with negative expression in the corneal and subcorneal layers. In OSCC, CD44 is localized to the basal and parabasal layers and around the peripheral cells of the tumor nests. Similar findings were observed in the study by Andratschke *et al.*^[42] The strong expression of CD44 observed in the generative cells of the epithelium implicate that these cells could be the targets for malignant transformation.^[43]

In this study, the expression of CD44 in the normal oral epithelium was detected as membranous staining localized on to the surface of the epithelial cells. On evaluation of the OSCC cases, apart from the membranous staining, cytoplasmic labeling was also evident. This could be due to the interaction of the cytoplasmic domain of CD44 with cytoskeletal linker proteins such as ezrin and ankyrin through which it can mediate cell migration on hyaluronan.^[44,45]

The disturbances in the expression of CD44 adhesion molecule during the process of oral carcinogenesis can be associated with unfavorable prognosis and an increased probability of developing metastasis. Therefore, poorly differentiated carcinomas with a weak immunostaining of CD44 can present with unpredictable prognosis.

Conclusion

The results of the present study suggest an altered expression of CD44 in OSCC with weak immunostaining in poorly differentiated squamous cell carcinoma. It implies that the adhesive functions of CD44 in cell–cell and cell–matrix interactions are needed in the maintenance of the normal architecture of the epithelium. The downregulation of CD44 could pave way for the cells to detach and invade. The expression of CD44 could also elucidate the difference in the biological behavior of the different grades of OSCC. Thus, it can be inferred that the loss of cell adhesion correlated to the decrease of CD44 expression might be of prognostic value in the evolution of OSCC.

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Conflicts of interest

There are no conflicts of interest.

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Quantitative analysis of dental age estimation by incremental line of cementum

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Abstract

Aim: This study aims to examine the correlation between age and number of incremental lines in human dental cementum among single-rooted teeth (incisors and canines) and to assess the best tooth to estimate age group by studying cementum under phase-contrast microscope and to assess the use of cementum in age estimation.

Materials and Methods: The present study was carried out in the Department of Oral Pathology and Microbiology. A sample of eighty single-rooted undecalcified longitudinal ground sections is prepared from freshly collected teeth with age noted separately and observed under phase-contrast microscope, photographed and counted. Counting the number of alternating dark and light lines of the cementum and adding them to the average age at which the analyzed tooth erupts provided an estimate of the chronological age of the individual.

Results: The results obtained in the present study were statistically significant and positive correlation was observed between the actual age and the estimated age. The number of incremental lines was found to be gradually increased with increase in age of the individual. On an average, ± 2 years of age difference were observed in our study on comparing the actual age with estimated age.

Conclusion: Hence, the incremental lines of the cementum were found to be gradually increased with increase in age and hence can be used as one of the adjuvant tools in dental age estimation.

Keywords: Actual age, cementum, estimated age, incremental lines, phase-contrast microscopy

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INTRODUCTION

Age is one of the indispensable factors in forming the uniqueness of the individual. Age estimation is a technique implemented by many archaeologists, anthropologists and many forensic scientists. There are numerous methods such as odontometric, histological, radiographies and biochemical methods for dental age

estimation, but none of the methods are precise for dental age estimation; each method will have some limitations and they have not given satisfactory results.^[1] The concept of age estimation changes era in forensic odontology. In the identification of dead bodies, age estimation becomes obligatory if there are no antemortem details available and if there is a need for renovation of particular profile.

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Moreover, estimation of age can be done invaluable archaeological skeletal material cutting back to hundreds of centuries.^[2] Tooth development begins in intrauterine life, and the individual age will start at the age of 21 to 60 years. Young individuals will show strong correlation of chronological age with estimated age.^[3]

In a developing country like India, a huge numeral of persons is uneducated and do not have awareness or archives of their birth date which is obligatory in cases of illegal acts, documentation, jurisdictional punishment, agreement, rape, unlawful abortion, service, accomplishment of majority, abduction and in cases of prostitution. In all of these circumstances, estimation can be used as an adjuvant tool in approximating the age of the particular individual.^[4] Estimation of age is also compulsory for entrance in schools, joining services, at the time of retirement and during the improvement of pensions to the old. Hence, the technical determination of age plays an important role.^[4]

Further age can also be designed from the advent of ossification centers and their unions during skeletal advance.^[5] Even though forensic odontology is a quite trivial sphere, it has been exploited for countless years particularly in forming individuality.^[6] The chief reported crime in the antiquity was cracked when bite marks were revealed in the remnants of the prohibited fruit in the Orchard of Eden and recognized as those belonging to Adam and Eve.^[7] Teeth are one of the structure will with stand with external factors such as temperature, decomposition.^[8] Dental cementum is the mineralized, a vascular dental tissue covers the anatomical roots of human teeth. The mineralized substance of the cementum continuously deposited on the root surface. Dental cementum is one of the tooth structures we can use for dental age estimation.^[9] Continue apposition of cementum will cause deposition of cementum and resting of cementum will appear as incremental deposition of cementum. These incremental lines can be used for dental age estimation.^[10] Counting the alternative dark and white bands of incremental lines plus average age at which tooth erupts provides an estimation of an individual. Estimation of dental age using incremental lines of cementum first described by Scheffer in 1950.^[11] Stott *et al.*, in 1982 done a study to correlation between incremental lines of cementum with dental age, he found that estimated age is close to actual age.^[12]

There are different techniques used for assessment of incremental lines of cementum, but a decreased accuracy of the technique in more advanced age.^[13] The purpose of this study was to evaluate the correlation between the number of incremental cemental annulation with knowing

age group using phase-contrast microscope.

MATERIALS AND METHODS

Eighty freshly extracted single-rooted teeth were collected from eighty individuals with known age. Teeth were collected from the Department of Oral and Maxillofacial surgery, SIBAR institute of Dental Sciences, Guntur, South India. All teeth were extracted for discretionary dental treatment. The inclusion criteria were extracted due to periodontitis and trauma and exclusion criteria were teeth extracted due to caries, grossly decayed teeth, deciduous teeth and teeth with cementum associate pathologies are excluded from the study. After collection of teeth kept in formalin and teeth were grouped according to age grouped into I–VIII, each tooth is cut into thin sections using a diamond tipped disc and sectioning was done long axis of the tooth. The teeth were then ground on Arkansas stone with water to 80 μ m thicknesses. The sections were cleared with xylene and mounted using dibutyl phthalate in xylene mounting media on glass slides. In each section, the area at the junction of the cervical and middle third of the root where a cellular cementum is present is considered for counting the incremental lines of the cementum. Digital images for the incremental lines were taken for every section using phase-contrast microscope under $\times 20$ magnification. Photomicrographs were visualized on the computer and the cemental lines were counted with the help of image analysis software image express pro version 6.0 attached to the Olympus phase-contrast microscope (BX 51M) with Jenoptik digital CCD camera. Alternate dark and white bands are observed in photomicrographs in the present study [Figure 1]. Estimated age of the teeth was calculated by using the formula given by Stott in 1982.^[12]

Incremental lines are calculated with formula: Estimated age = Number of incremental lines + eruption age of that particular tooth.

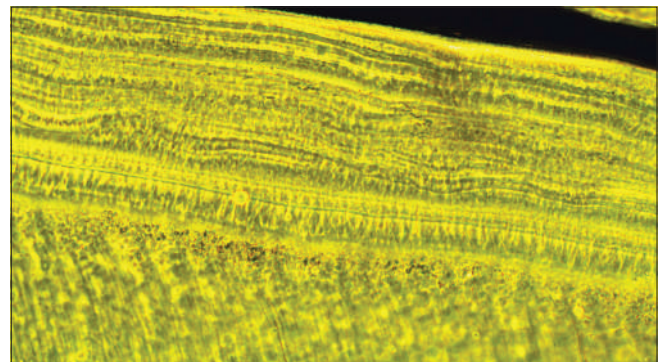


Figure 1: Incremental lines of cementum using phase-contrast microscope ($\times 200$)

Statistical analysis

Number of incremental lines were counted, and number was entered in excel sheet and data, statistical analysis were performed using Statistical Software Package for the Social Sciences (SPSS) 20.0 version. Comparison of actual age and estimated age was done by paired *t*-test. Intragroup correlations were carried out for each group by applying Karl Pearson's correlation coefficient.

RESULTS

The study sample consists of eighty single rooted teeth, which were divided into eight groups according to the age with ten teeth in each group [Table 1]. Association between age and number of incremental lines was calculated using descriptive statistical analysis and the results revealed that at the age of the individual increased, the number of incremental lines was found to be increased significantly [Table 2]. The actual age was minimum of 22 years and maximum of 60 years. Comparison of mean, among actual age with an estimated age by using paired *t*-test. The mean and standard deviation of actual age is 40.63 ± 11.55 . The mean and standard deviation of estimated age is 40.19 ± 11.67 . Hence, a positive correlation was observed between actual age and estimated age [Table 3]. When compared with mean of actual age and estimated age the observations showed statistically not significant with $P \geq 0.05$, whereas correlation between actual age with number of incremental lines are statistically significant ($P \leq 0.05$) [Table 4]. Comparison between estimated age with number of incremental lines by using Karl Pearson's correlation coefficient observation shown statistically significant ($P \leq 0.05$), but when correlated between estimated age and eruption age, it is not statistically significant ($P \geq 0.05$) [Table 4].

Multiple regression analysis of actual age was done with number of incremental lines and the eruption age with independent variables. Intercept value was statistically significant ($P \leq 0.05$). Independent variables using a number of incremental lines were found to be statistically significant ($P \leq 0.05$) [Table 5].

DISCUSSION

Dental age estimation is very important aspect in forensic investigations and application ranges from comparison of antemortem with postmortem identification to estimating age in childrens and adults.^[13] Jones *et al.* in 1974 mentioned cementum as a hard avascular connective tissue that covers the roots of the teeth. The primary function of the cementum is to anchor the tooth root to the gum

maintaining the crown in position for effective occlusion.^[9] As the position of cementum is intermediary, it forms the interface between root dentin and periodontal ligament. One of the main functions of cementum is to anchor the principal collagen fibers of the periodontal ligament to the root surface, but it also has adaptive and reparative functions, playing a crucial role in maintaining occlusal relationships and in protecting the integrity of the root surface.^[14] The unique property of cementum is that it does not undergo continuous remodeling like bone but

Table 1: Groupwise distribution of the study participants according to age

Serial number	Groups	Age group
1	Group I	21-25
2	Group II	26-30
3	Group III	31-35
4	Group IV	36-40
5	Group V	41-45
6	Group VI	46-50
7	Group VII	51-55
8	Group VIII	56-60

Table 2: Descriptive statistics on groups with mean of incremental lines

Serial number	Groups	Mean number of incremental lines
1	Group I	11.9
2	Group II	18.3
3	Group III	21.5
4	Group IV	24.7
5	Group V	30.6
6	Group VI	35.7
7	Group VII	40.1
8	Group VIII	46.2

Table 3: Comparison of mean, among actual age with an estimated age by using a paired *t*-test

Variable	Mean±SD	<i>t</i>	<i>P</i>	Inference
Actual age	40.63±11.55	1.18	0.24	NS
Estimated age	40.19±11.67			

SD: Standard deviation, NS: Not significant

Table 4: Correlation between actual age and estimated age with number of incremental lines and eruption age by Karl Pearson's correlation coefficient

Variables	Correlation between actual age with			Correlation between estimated age with		
	<i>r</i>	<i>t</i>	<i>P</i>	<i>r</i>	<i>t</i>	<i>P</i>
Number of lines	0.9570	29.1268	0.0001*	0.9889	58.8279	0.0001*
Eruption age	0.1416	1.2633	0.2102	0.2034	1.8351	0.0703

* $P < 0.05$

Table 5: Multiple regression analysis of actual age by number of incremental lines and eruption age

Indpt variables	Estimate	SE of estimate	<i>t</i>	<i>P</i>
Intercept	6.1790	2.6326	2.3471	0.0215*
Number of lines	0.9636	0.0320	30.1209	0.0001*
Eruption age	0.5943	0.2160	2.7515	0.0074*

* $P < 0.05$. SE: Standard error

continues to grow in thickness throughout life. Unlike dentin and enamel, where there are clear differences in the proteins present in these tissues and the factors regulating their functions when compared with bone, cementum has not demonstrated to express specific proteins and factors in common with bone and to be developmentally controlled by similar factors.^[15] The importance of incremental lines of cementum was highlighted by explaining the properties that make archeologists and wildlife biologists to be helpful in many ways and also enlightened the seasonal variations of incremental lines of the cementum.^[16] When sectioned and analyzed under microscope, they appear as alternating translucent and opaque bands.

Zander and Hurzeler in 1958 done a study to correlate between cementum annulations with dental age, in their study they observed that straight line relationship between actual age with incremental lines of cementum and also said that when the dental age increases the number of incremental lines also increases.^[17] Selukar *et al.* in 2002 done a study on cemental apposition with dental age. They found that significant positive correlation between age of individual and cementum apposition. They concluded that when age advances the cemental apposition and number of incremental lines are increased.^[18] We have observed in our study that incremental lines of cementum with dental age estimation, we observed that positive correlation between incremental lines of cementum with estimated age, the mean difference was $\pm 2-3$ years. When the age advances the number of incremental lines of cementum were increased, these observations were similar to Selukar *et al.*, in 2002. Gupta *et al.* in 2014 done a study to investigate the role of cementum as an aid for age estimation and to get the correlation between secondary cementum and age of the individuals. A strong positive correlation was found between the estimated age, which was calculated by using cemental lines and actual age. They found that the thickness of the cementum was increased with increase in age. So they concluded that countable cemental annulations are present in human teeth and quantification of cementum annuli is a moderately reliable means which can be used for age estimation in humans.^[19] in our study when correlated between estimated age and actual age we observed that positive correlation in both the genders and estimated age and actual age.

CONCLUSION

Age estimation from human teeth is well recognized. Dissimilar methods and abundant procedures have been established for age estimation, every single one signifying specific accuracy, exactness and reliability. In

all circumstances, reproducible and consistent estimation effects are possible when the suitable methods are appropriately applied and used. Miscalculation is present in every single case. Hence, the forensic odontologist should follow diverse techniques and accomplish repetitive depths and calculations in order to come to a consistent conclusion. When compared with other methods used in forensic odontology for dental age estimation, counting cemental annulations is easy to perform, not technique sensitive, less time taking and economically feasible. Moreover, cementum does not undergo much pathological changes when compared with other structures of the teeth. Hence, in conclusion, incremental lines of the cementum can be used as an adjuvant tool in dental age estimation.

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Conflicts of interest

There are no conflicts of interest.

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Is the Intention Being Realized in Execution: Evaluation of Oral Health Promotion Program at a South Indian University

Abstract

Introduction: There are accessibility problems for rural Indian population with respect to oral health care. Dental Council of India envisioned that establishment of satellite clinics would be a beneficial strategy for rural population to have better access to oral health care, curtailing the accessibility problems and financial barriers for oral health care utilization to some extent. **Aim and Objective:** The aim of this study was to procure data on the functioning of these satellite clinics and evaluate an oral health promotion program in a satellite clinic located in a South Indian university. **Materials and Methods:** A total of 301 participants were selected through two-stage random sampling to evaluate the functioning of a dental satellite clinic located in a South Indian university. The data collected include demographics, utilization of services at the satellite clinics, and self-reported oral and general health status. **Results:** The utilization of services was only 9.3%, and a majority of the participants were not aware of the existence of the clinic. Promotion of available services and provision of oral health education were the major suggestions received from the study participants. **Conclusion:** Evaluation of satellite clinics both from the students' perspective and with regard to oral health status of concerned populations must be seriously undertaken.

Keywords: Evaluation, oral health promotion, perceived unmet dental needs

Introduction

It is beyond doubt that there has been improvement in the people's awareness of oral health and utilization of oral health care services over the past few years. However, oral health disparities continue to exist with poor oral health being a routine observation among the marginalized sections and those who are geographically disadvantaged.^[1-3] Literature suggests that rural areas are dentally underserved as the existent public health system in India does not integrate dental surgeons as much as it needs to^[4-6]; there is urban preference among dental graduates in the establishment of clinical practices.^[7-9] In light of these findings, it is important that prospective strategies for promotion of oral health in rural areas and for the marginalized sections of people be seriously considered both by the public administrators and the dental fraternity.^[10] Teaching dental institutions play a significant role in the promotion of oral health by reaching out to people through

community outreach programs. The Dental Council of India made it imperative for all the teaching dental institutions in India to establish satellite clinics in rural areas, which benefit the people residing in these areas.^[11] These clinics address the accessibility issues to a great extent and also offer services at nominal costs. Satellite clinics possess an invaluable potential to implement common risk factor approach, which serves to integrate oral health with the general health of people.

Community outreach programs differ from individual care in that they primarily focus on population, including those persons who do not and cannot access care. Evaluation of such initiatives is an intricate and complex task that has been customarily neglected and often underfunded.^[12] Evaluation of outreach programs is important as a source of development of good practice, as a guide for making best use of the available resources, and to inform policy development.^[13] Thus, evaluation is beneficial not only in curtailing the execution-related issues but also in

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mitigating the problems pertaining to the formulation of the future programs. In this context, the aim of this study is to evaluate the effectiveness of oral health promotion in one such satellite clinic located in a South Indian university.

Materials and Methods

This cross-sectional study was conducted in a South Indian university located on the Kolkata–Chennai national highway,^[7] that spreads over 293 acres. The university has six colleges within the campus, and the student strength of the campus is over 5000. It is a destination university for diverse students from Iran, Iraq, Nepal, Bhutan, Tanzania, Ethiopia, and Libya. The satellite clinic in the campus established by a teaching dental institution provides free oral health services that include screening, prophylaxis, restorations, extractions, and referral services.

Ethical approval for the study (133/IEC/SIBAR/2018) was obtained from the Institutional Ethical Committee. The study was conducted between June and August 2018. A two-stage random sampling was performed in which three groups were selected from the seven academic groups at the university (students from each of the six colleges and staff) in the first stage by simple random sampling. In the second stage, a random sample of participants was selected from each chosen group, proportional to the population size of that group. The sampling details of the study are presented in Figure 1.

The effectiveness of oral health promotion was evaluated based on the perceived unmet dental needs in spite of the free services available. In view of the provision of free oral health

services and setup of satellite clinic within the university, it is apparent that accessibility and cost of treatment, the two most common barriers for utilization of dental services, were taken out of the equation in our study.^[14] In light of these relatively favorable conditions, beneficiaries are expected to convert their needs to demand, at least the perceived needs if not the normative. A self-administered questionnaire was developed seeking information on the perceived dental needs in the past 12 months, which remained unmet in spite of the above-mentioned favorable circumstances. The expression “perceived unmet needs” refers to these needs, which remained unmet despite perception. The questionnaire also included the self-rated of oral health status and self-rated general health status on a five-point Likert scale. Information was also sought on the utilization of dental services at the satellite clinic. Demographic data were collected on age, gender, and academic group. The questionnaire was administered to all the participants during the working hours of the university. The principal investigator (VCC) made himself available, at the time of administration, for the participants to clarify doubts, should there be any.

The questionnaire was readministered to 40 randomly selected participants, after a period of 10 days, with a purpose of correlating the scores on the two occasions. The observed agreement between the responses was found to be considerably greater than the expected agreement by chance, as was assessed by Cohen’s Kappa (0.89). Statistical analysis was performed using IBM SPSS statistics for Windows, Version 20.0 (IBM Corp, Armonk, New York, USA). The data on self-rated oral and general health status

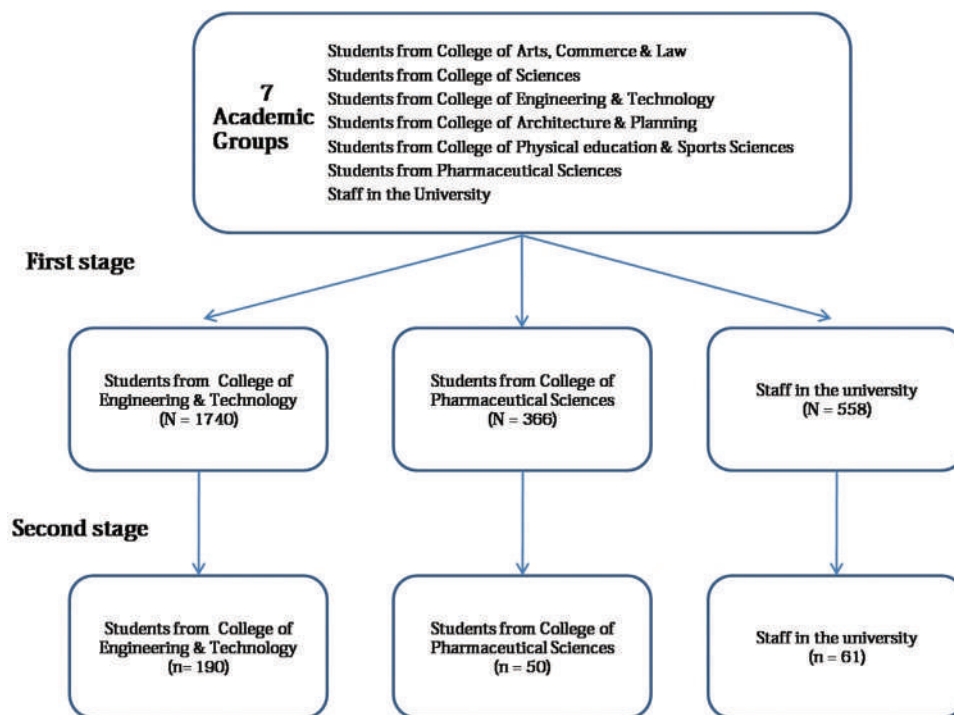


Figure 1: Sampling details of the study

Table 1: Sample distribution according to gender, age, self-reported oral health and general health, perceived unmet dental needs in the past year, and utilization of services at the satellite clinic

Variable	Academic group			P value	
	Engineering students (n = 190)	Pharmacy students (n = 50)	Staff (n = 61)		
Gender	Male	92 (48.4%)	13 (26%)	38 (62.3%)	<0.01 ^a
	Female	98 (51.6%)	37 (74%)	23 (37.7%)	
Age, years; mean (95% CI)	19.31 (19.13–19.49)	19.74 (19.18–20.3)	38.11 (35.69–40.54)	<0.001 ^b	
Self-reported oral health mean (95% CI)	3.38 (3.22–3.55) ^m	2.88 (2.51–3.25)	3.30 (2.99–3.6) ^m	<0.05 ^c	
Self-reported general health mean (95% CI)	3.57 (3.41–3.73)	3.24 (2.87–3.61)	3.49 (3.22–3.76)	0.237 ^c	
Perceived unmet dental needs in past 12 months	56 (29.5%)	22 (44%)	22 (36.1%)	0.381 ^a	
Utilization of services at the satellite clinic	10 (5.26%)	6 (12%)	12 (19.67%)	0.012 ^a	

^aChi-square test; ^b One-way ANOVA; ^c Kruskal–Wallis *H*-test.^mSuperscripts with the same letter within the row indicate that there is no statistically significant difference ($*P > 0.05$) between the academic groups as assessed by Mann–Whitney test with Bonferroni correction. $P \leq 0.05$ considered statistically significant.

were dichotomized as “good” (very good/good) and “poor” (fair/poor/very poor) to facilitate in identification of the possible discrepancies between the two measures.^[15] Parametric and nonparametric tests were performed as appropriate. The study has been conducted in full accordance with the World Medical Association Declaration of Helsinki (as amended in Edinburgh, 2000). Informed consent was obtained from the principals of the selected colleges and the participants.

Results

Sample distribution according to gender, age, self-reported oral and general health status, perceived unmet dental needs in the past year, and utilization of services at the satellite clinic was presented in Table 1. Around 80% of the participants belonged to the age group of 16 to 25 years, and the mean age of the study population was 23.19 ± 8.742 years, indicating a considerably wider spread of age among the study participants. Significant differences were observed between the academic groups with regard to self-rated oral health status. The details of the distribution of responses on self-rated oral and general health status on Likert scale are provided in Figures 2 and 3, respectively. The tendency of the participants to report oral health and general health status toward “good” and “very good” can be clearly observed in these diverging stacked bar diagrams. On dichotomization of Likert responses as aforementioned, it was identified that 40.9% of the respondents reported poor oral health status, whereas only 32.9% reported poor health status. A total of 30.69% of those participants, who reported their health status

to be good, rated their oral health status as poor. Spearman correlation test showed significant weak positive correlation ($r = 0.226$) between the self-rated oral and general health status of the participants ($P = 0.000$).

Nearly 33% of the participants reported having perceived unmet dental needs in the past 12 months. There was no significant difference between the three academic groups with respect to the perceived unmet dental needs in the past year. Among the 100 participants with perceived unmet dental needs, 73 were unaware of the existence of dental satellite center at the university. The common reasons for not utilizing the services at the satellite clinic were lack of knowledge about existence of satellite clinic (60.8%), absence of dental needs (25.61%), poor perception of importance of oral health (9.63%), and lack of confidence in the quality of services offered (1.8%). Only 9.3% of the study population utilized the services at the satellite center. Utilization of available services was significantly higher among staff compared to the students from the two selected colleges ($P = 0.012$) [Table 1]. The percentage of study participants unaware of existence of satellite center was 60.8% (183/301). Promotion of available services was the major suggestion received from the participants (79.4%) followed by provision of oral health education (13.95%).

Discussion

There could be two reasons for the low prevalence of unmet dental need in our study. The participants may have had a dental problem in the past 12 months for which he/she had sought dental care or the classic mismatch between normative

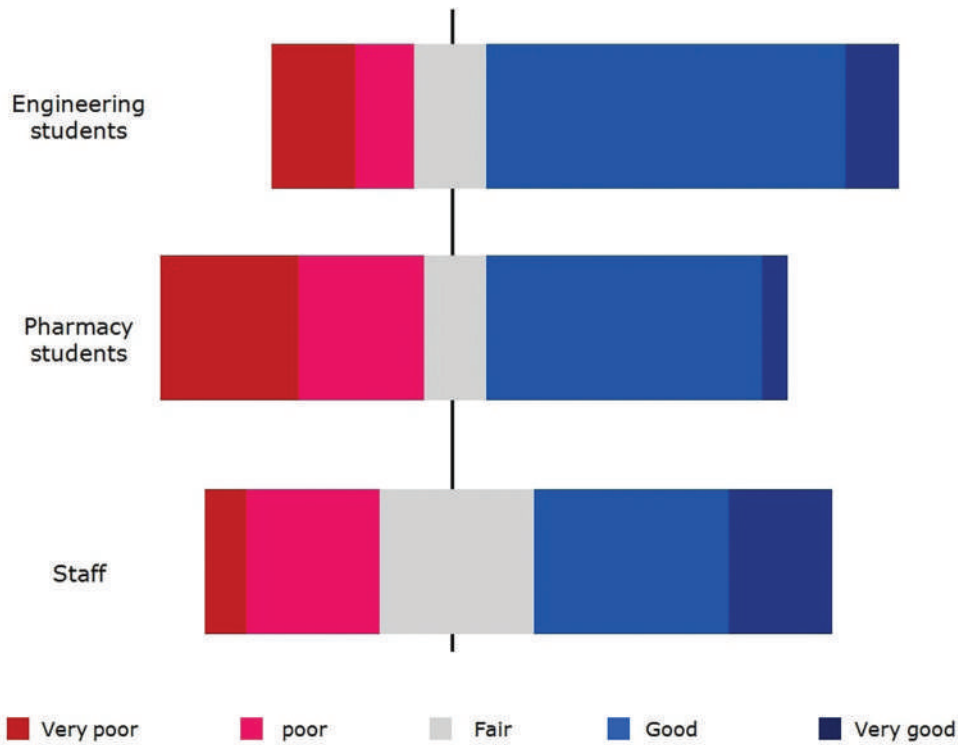


Figure 2: Diverging stacked bar diagram comparing self-rated oral health between academic groups

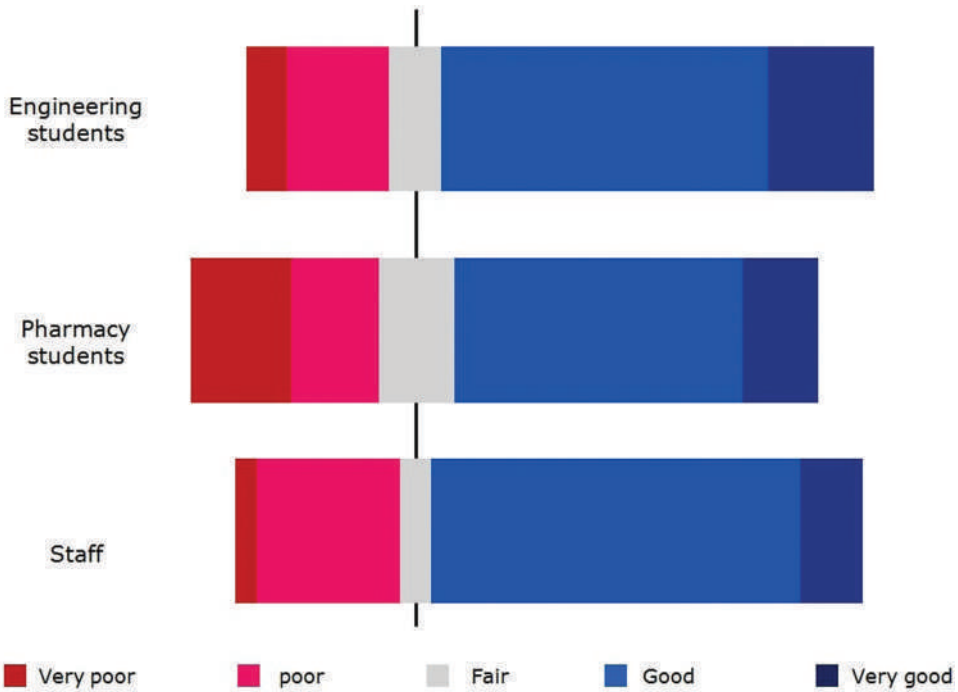


Figure 3: Diverging stacked bar diagram comparing self-rated general health between academic groups

need and perceived need may have played a role.^[16-18] An important finding in this study is the discrepancy between an individual's self-rated oral and self-rated general health. More than one-third of the participants with good self-rated general health reported poor oral health. This emphasizes that the campaign "oral health is an integral

part of general health" has not been well received by the general population.^[19-22] Care was taken in formatting the questionnaire in such a way that inquiry on self-reported oral health status was reserved to the end, virtually eliminating the scope for respondents to disreackon oral health while responding on perceived health status, which

could have happened if these questions were posed in a sequential manner. However, the authors admit that this is too complex a conclusion to draw from a questionnaire study. But the inference opens new avenues and lays foundation for future research in this direction.

The utilization of available oral health services was low (9.3%) in the present study. This is comparable with the utilization rates at satellite clinics in Nigeria^[23] and the utilization of oral health services in India, in general.^[24] A majority of participants were not aware of the existence of the satellite clinic, signifying the need for promotion of the available oral health services, which must be pursued by the dental institutions and the local authorities where the satellite clinics are located. The fact that the care provided by the dental students at this satellite clinic is currently procedure based, where no one student is solely responsible for the execution of the sequential treatment plan unlike comprehensive care, could be a reason for the low utilization observed as the patients may feel disenfranchised with different care providers providing care under the supervision of different faculty members each time they visit.^[25] However, this was not explicitly mentioned by any of the participants. Information through patients' surveys has proven to be a successful way of strategic evaluation and improving the quality of health services.^[26] Therefore, suggestions were received from the target population on what could be done to improve the services at the satellite clinic. In light of lack of knowledge about existence of satellite clinic, promotion of available services was the major suggestion received followed by oral health education. To ensure that the rationale and aims of outreach programs are met, health education is necessary.^[27]

It should also be noted here that satellite clinics possess an invaluable potential to implement common risk factor approach, which serves to integrate oral health with the general health of people.^[28] Evaluation of functioning of satellite clinics helps us better understand the outreach activities and thereby improve the quality of services provided. It is recommended to use established criteria for evaluation of these programs.^[29] Another important concern is the quality of community exposure to dental students. Qualitative interviews must be conducted on dental students and faculty members to tailor the outreach programs that best benefit the cause and also to make necessary modifications in the planning of future dental curriculum. Although this aspect of outreach programs was given due importance across many parts of the world, students' perspectives regarding outreach activities were not well documented in India.^[30-32]

Limitations of this study are seeking dental care through other oral health care resources was not considered, which could have been a reason for nonutilization of services at the satellite center. Clinical evaluation of oral health status of

the individuals could have been performed to evaluate the normative need and consequently to determine any mismatch between the perceived and the normative needs.

Policy Recommendations

- India is a vast geographic extending over 3287,263 km² and stands at 7th position globally in terms of the geographical area. The number of dental institutions in the country has grown phenomenally over the years, with 313 functioning dental institutions in the country at present. If a policy guideline can be formulated, which mandates each dental institution to establish four satellite clinics at a distance of 15 km covering the entire perimeter of the institution, each institution could cover an area of around 700 km², providing necessary oral health services for the population residing in that area. This translates to covering an area of around 215,330 km², when all the dental institutions in the nation are taken into account, which is over 15% of the geographical area of the country. However, this percentage must be interpreted with caution owing to the uneven distribution of dental institutions in the country across its states and clustering of few dental institutions in certain areas, the consequence of which is that such dental institutions does not cover mutually exclusive areas. Therefore, clear policy guidelines on the number of satellite clinics to be established by a dental institution and the distance between satellite clinics and corresponding dental institutions must be established.
- Guidelines should also include the range of oral health services that must be available in the satellite clinics in view of the fact that a great proportion of oral health care provided at satellite clinics constitute tooth extractions.
- Special emphasis must be placed on methods and strategies for oral cancer diagnosis while formulating policy guidelines, as the incidence of oral cancer in India has been on a constant rise. The role of satellite clinics in habit cessation counseling must also be established.
- Guidelines must ensure periodic evaluation of satellite clinics both in terms of improvement in oral health status of corresponding populations and the improvement in diagnostic and clinical competence of dental students.

Conclusion

Evaluation of satellite clinics both from the dental students' perspective and with regard to oral health status of concerned populations must be seriously undertaken, as periodic evaluation and critical appraisal are as important as the formulation and execution of oral health promotion programs.

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Nil.



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Conflicts of interest

There are no conflicts of interest.

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Association between Tobacco Usage and Dental Caries among 35–44-Year-Old Fishermen of North Coastal Region of South Indian State, Andhra Pradesh

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Abstract

Introduction: Tobacco usage is popular among rural and urban communities of India. Sporadic studies that were conducted to know the caries experience among tobacco users yielded conflicting conclusions. Fisherfolk communities form a special population in light of the variations in their dietary habits compared to general population. **Aim:** The aim of this study was to explore the dental caries (DC) experience among tobacco users and nonusers in fisherman communities residing in the north coastal region of a south Indian state. **Materials and Methods:** A comparative cross-sectional survey was done in fisherman community belonging to a north coastal region of a South Indian state. Stratified randomized sampling technique was employed to recruit 374 adult participants aged 35–44 years. The survey included interviewer-administered questionnaire and clinical examination for measuring DC experience. The questionnaire consisted of demographic data, brushing aids, 2 days sweet score, and a detailed record on tobacco use of subjects. Decayed missed and filled index (DMFT) and Simplified Oral Hygiene Index (OHI-S) were recorded. The study participants were dichotomized based on tobacco usage. The tests used were independent samples *t*-test, analysis of variance, and Chi-square test. **Results:** DC was present among 46.5% of tobacco users and 65.8% of nonusers. The mean DFT score for tobacco users (0.62 ± 0.23) was less when compared to that of nonusers (1.40 ± 0.52) after excluding missing component, which is statistically significant ($P < 0.001$). The mean OHI-S was higher among tobacco users than nonusers. There was no statistically significant difference in the mean sweet scores between these two groups ($P = 0.172$). **Conclusion:** Despite higher mean OHI-S scores among tobacco users and similar sweet scores among both the groups, DC experience among tobacco users was less when compared to nonusers. Longitudinal studies are recommended for further confirmative evidence.

Keywords: Dental caries, diet records, oral hygiene index, smoking, sweetening agents

INTRODUCTION

Tobacco usage is one of the greatest epidemic threats to public health globally. Approximately one-third of the adult population in the world use tobacco either in smoking, chewing, or any other forms and about half of them die prematurely. Due to severe addiction to nicotine, 4.9 million people died in the year 2000 worldwide as per the estimate by the World Health Organization (WHO).^[1] Approximately 7 million people are killed annually by tobacco use, which is assumed to increase to 10 million with 70% deaths occurring in low- and middle-income countries.^[2]

Tobacco use has a detrimental impact on both general and oral health. Major systemic adverse effects of tobacco consumption

include various form of cancer (mainly lung cancer) and cardiovascular diseases. Likewise, there is strong evidence that tobacco use has numerous negative effects on oral health, ranging from staining of teeth and dental restorations to reduction in the ability to taste, development of oral diseases such as smokers' palate, oral cancer, potentially malignant disorders, oral candidiasis, and periodontal disease.^[3,4]

Dental caries (DC) is the most prevalent pandemic chronic noncommunicable disease affecting any age group and

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is dependent on a number of factors such as lifestyle, socioeconomic and sociodemographic gradients, and the tobacco use. Although it is regarded currently as preventable disease with the regular oral hygiene habits, frequent fluoride usage, and less intake of sugars as major recommendations, it is still effecting the quality-of-life of many individuals of low- and high-income countries.^[5,6]

Despite its multifactorial etiology,^[7] there exists an evocative relationship between tobacco use and incidence of DC. However, the veracious relation between these two is unclear till now since some studies suggest a positive association,^[8-10] whereas some negative.^[4,11,12]

Andhra Pradesh has 974 km of coastal line covering nine coastal districts from Srikakulam (North) to Nellore (South). Fishing is the main occupation of these fisherfolk communities living along the shores of these coastal lines and they also possess different cultures, traditions, and dietary habits which consist mostly of seafoods when compared to the general population. Thus, with this background, the current study was conducted to evaluate the association between tobacco usage and DC among 35–44-year-old fisherfolk population of north coastal region of South Indian state, Andhra Pradesh.

MATERIALS AND METHODS

This study was carried out in 2015 among a fisherman community in North coastal region of a South Indian state, Andhra Pradesh. The study population comprised of 35–44-year-old individuals. A pilot study was conducted before the initiation of the main study among 50 individuals belonging to the same community who were not included in the main study through which a prevalence (p) of 37% was obtained. Accordingly, 187 tobacco users and 187 nonusers were recruited into the study with the total sample size being 374. Participants in the age group of 35–44 years without known systemic illnesses and drug usage that could influence DC experience were selected. For each tobacco user, a nonuser was selected randomly with gender and maximum permissible age variation of ± 2 years as the matching criteria. Clearance for this study was obtained from the Institutional Ethical Board (protocol No. Pr. 08/IEC/SIBAR/2015), following the ethical guidelines Declaration of Helsinki. All examinations were carried out after proper authorization by participants through an informed written consent form which includes details of the current study along with participant's signature. Training and calibration of the examiner for clinical scoring were conducted before the initiation of the study, which yielded a $\kappa = 0.85$.

The study was conducted in the months of August to October 2015. Data collection was done through structured questionnaires and clinical oral examinations. The study was carried out outside the dwellings in the fisherman communities. Optimum lighting and ventilation were ensured for clinical examination. An interviewer-administered questionnaire which included information on demographic details, oral hygiene

habits, and a comprehensive history on tobacco and alcohol consumption was used. A 48-h diet history including 1 weekday and 1 weekend was incorporated into the questionnaire. From this food sweetened with added sugars or natural sweets were extracted. Later, these sweetened foods were categorized and scored using Nizel and Papas method.^[13]

After completion of the questionnaire, a single examiner conducted clinical examination in natural illumination using WHO probe and mouth mirror following decay, missing, filled teeth (DMFT) index criteria.^[14] Oral hygiene status was also examined following Simplified Oral Hygiene Index (OHI-S).^[15]

Sweet and OHI-S scores were categorized for further analysis as follows: the median of sweet scores of total population was calculated and those scores above the median were categorized into high-sugar consumption (>5) and those scores below the median (<5) were categorized to low-sugar consumption. OHI-S scores were also categorized into good, fair, and poor based on the OHI-S index criteria.^[15]

All examinations were carried out after proper authorization by participants through an informed written consent form which includes details of the current study along with participant's signature.

Data were processed and analyzed using the Statistical Package for the Social Sciences software (IBM SPSS statistics for windows version 20, Armonk, NY, USA). Independent sample t -test and analysis of variance were used to compare the quantitative variables between the various groups. Chi-square test was used to test the association between tobacco usage and caries experience. $P \leq 0.05$ was considered statistically significant.

RESULTS

DC was present among 58.57% of tobacco nonusers, which was high when compared to tobacco users (41.43%) and the difference was statistically significant [Table 1]. The mean decayed filled teeth (DFT) of tobacco users and nonusers was 0.62 ± 0.23 and 1.40 ± 0.52 , respectively, and the difference was statistically significant ($P < 0.000$). There was a statistically significant difference ($P = 0.001$) in the mean OHI-S scores among tobacco users and nonusers with the tobacco users having a higher mean of 3.45 ± 0.56 compared to nonusers having a mean of 1.69 ± 0.80 . Tobacco users and nonusers were having a mean sweet scores 5.24 ± 1.00 and 5.17 ± 0.86 , respectively, and the difference was not statistically significant ($P = 0.172$) [Table 2]. The mean DFT was higher among individuals using both forms of tobacco (1.00 ± 0.52) followed by individuals using smokeless form (0.77 ± 0.23) and smoke forms (0.55 ± 0.24), and the difference was not statistically significant [Table 3].

The mean DFT was lower among the tobacco users with low- and high-sugar consumptions compared to their counterparts, and the difference was statistically significant ($P < 0.001$, $P = 0.008$) [Table 4]. The mean DFT of tobacco



users with poor, fair, and good OHI-S scores was lower when compared to that of tobacco nonusers, and the differences were statistically significant ($P = 0.005$ $P < 0.001$ $P < 0.001$) [Table 5].

DISCUSSION

Since its introduction by Portuguese in 600 AD, there has been a continuous raise in the usage of tobacco in various forms by the Indian population with a current data of 267 million adult tobacco users.^[16] Nicotine which is found in substantial amount in tobacco products makes addicts out of tobacco users. It is a stimulant with properties similar to those of cocaine and amphetamines and is 1000 times more potent than alcohol, 10–100 times more potent than barbiturates, and 5–10 times more potent than cocaine or morphine in its addictive potential. This addictive effect of nicotine is due to its capacity to trigger the release of dopamine which is a chemical in the brain that is associated with feelings of pleasure.^[17] This could be the induction of addiction to tobacco use among the people leading to an increased burden on their general and oral health.

The various ill effects tobacco has on the oral health have been supported in literature till decades.^[4-6,8,17] However, tobacco usage and its effects on DC is a subject of many opinions. Early literature supports a low DC among smokers.^[11,12] Schmidt, in 1951, supported this belief when he reported that increase in tobacco smoking was followed by a decrease in caries rate.^[11] Smoking increases thiocyanate level in saliva.^[4,12] Thiocyanate, which is a normal constituent of saliva, was found to have a possible caries-inhibiting effect. To date, quite a few investigators have discovered a correlation between elevated smoking level and DC. The higher number of lactobacilli and *Streptococcus mutans* group and the decreased buffering effect of smoker's saliva may indicate an increased susceptibility to caries.^[3,18] Studies have also shown that smoking is associated with lower salivary cystatin activity and lower output of cystatin C during gingival inflammation. These cystatins are thought to contribute to maintaining oral health by inhibiting certain proteolytic enzymes thereby increasing DC.^[3,18]

This is the first study investigating the association of tobacco and DC among the individuals selected from a specific community. Since the full effect of DC can be appraised at 35–44 years, this age group has been included in the current study.^[19] DC being a multifactorial disease, several other predictor variables such as age, gender, oral hygiene habits, oral hygiene status, and sugar consumption had also been included in the current study along with tobacco usage. An assumption was made that these individuals own the same socioeconomic class since they were selected from the same community. Since diet, especially sugar consumption, and oral hygiene could act as effect modifiers in the association between smoking and DC experience, sugar scores and OHI-S scores were recorded. However, while defining DC experience, the missing component had been excluded due to the difficulty in distinguishing the reason for tooth loss

Table 1: Dental caries distribution according to tobacco habit

DFT	Tobacco habit		P
	Present, n (%)	Absent, n (%)	
Present	87 (41.43)	123 (58.57)	<0.001*
Absent	100 (60.97)	64 (39.03)	

Chi-square test, *Statistically Significant. DFT – Decayed filled teeth

Table 2: Comparison of mean decayed filled teeth, Oral Hygiene Index, and sweet scores between tobacco users and nonusers

Variables	Tobacco habit (mean±SD)		P
	Present (n=187)	Absent (n=187)	
DFT	0.62±0.23	1.40±0.52	<0.001*
Decay	0.60±0.21	1.35±0.46	<0.001*
Filled	0.04±0.01	0.07±0.03	0.060
OHI	3.45±0.56	1.69±0.80	<0.001*
Sweet score	5.24±1.00	5.17±0.86	0.172

Unpaired t-test, *Statistically Significant. DFT – Decayed filled teeth, SD – Standard deviation, OHI – Oral Hygiene Index

Table 3: Comparison of mean DFT among the tobacco users according to the form of tobacco

Form of tobacco among tobacco users	n	Mean DFT	P
Smoke form	99	0.55±0.24	0.13
Smokeless form	57	0.77±0.23	
Both forms	31	1.00 ± 0.52	

One way ANOVA, *Statistically Significant. DFT – Decayed filled teeth

Table 4: Comparison of the mean decayed filled teeth between low- and high-sugar consumption groups according to tobacco usage

Sugar score	Tobacco usage	n	DFT (mean±SD)	P
Low-sugar score	Present	176	0.62±0.32	<0.001*
	Absent	179	1.39±0.80	
High-sugar score	Present	11	0.64±0.23	0.008*
	Absent	8	1.63±0.56	

Unpaired t-test, *Statistically Significant. SD – Standard deviation, DFT – Decayed filled teeth

Table 5: Comparison of the mean decayed filled teeth between good, fair and poor Simplified Oral Hygiene Index groups according to tobacco usage

OHI-S	Tobacco habit	n	DFT (mean±SD)	P
Poor	Present	130	0.56±0.24	0.005*
	Absent	10	1.80±0.62	
Fair	Present	47	0.83±0.34	<0.001*
	Absent	79	1.32±0.44	
Good	Present	10	0.40±0.23	<0.001*
	Absent	96	1.43±0.42	

Unpaired t-test, *Statistically Significant. OHI-S – Simplified Oral Hygiene Index, SD – Standard deviation, DFT – Decayed filled teeth

which could be due to periodontitis which is more prevalent among tobacco users.^[4]

There was no statistically significant difference in the mean age of tobacco users and nonusers with an equal distribution of these two groups in both the genders. These findings confirmed the precision of matching. Indigenous methods of brushing were followed by 7.4% of tobacco users and 3% of tobacco nonusers, which exposes the oral health negligence among them. Majority of the tobacco users were using smoke form of tobacco (68.4%) and most of them have been having the habit from 11 to 15 years, depicting the chronicity of tobacco habit in this region.

The DC experience was high among tobacco nonusers compared to users with a significant difference between them. These results were in accordance with studies done by Schmidt^[11] and Hugoson *et al.*^[20] However, contradicting findings were observed in the studies done by Aguilar-Zinser *et al.*^[10] and Tada and Hanada.^[21] The reason for lower caries experience among tobacco users could be due to the elevated levels of salivary constituent thiocyanate in tobacco users, which have been proved to have an inhibitory effect on DC.^[4,12] The inference behind the studies showing high caries experience among tobacco users could be due to the inclusion of missing component of the DMFT which has an unpredictable cause.^[10,21] To negotiate, this overestimation of caries experience due to missing component, the M component has been excluded in the current study. Individuals using smokeless form of tobacco were having high caries compared to individuals using smoke form which was in par with studies done by Weintraub and Burt^[22] and Tomar and Winn.^[23] This could be due to the addition of various sugar constituents to the smokeless tobacco products.^[23]

Diet, especially with high-sugar content, can cause an increase in DC.^[24] The findings of the current study also showed a low mean DFT among individuals consuming low-sugar diet compared to individuals consuming high-sugar diet. However, there was no statistically significant difference between tobacco users and nonusers with respect to the mean sweet score. Among individuals with low and high sweet scores, tobacco users were having less mean DFT compared to their counter parts. Furthermore, among the individuals with poor, fair, and good OHI-S, tobacco users were having low mean DFT compared to nonusers. These two findings confirm the fact of less caries experience among tobacco users in spite of similar sweet scores and oral hygiene status. These verdicts of the current study confirm the role of tobacco solely in less caries experience. The factor of fish consumption was found to be equally distributed between tobacco users and nonusers, eliminating the effect modification possibility of fish consumption and DC, since DC is found to be less among individuals consuming high quantity of fish.^[25]

In spite of high caries experience in the current study population, the mean number of filled teeth was found to be less, which shows lack of awareness or inability to access

proper dental care in this region since it is located in the rural coastal area. This alarming situation reflects the desideratum of health-care facilities to satiate the dental needs of this community.

It is the fact that people in minorities observe similar customs, traditions, practices, and holds similar beliefs. They are more homogenous compared to people who constitute a majority of population. Fisherman community being a minority section in India is expected not to be different from the above supposition. Keeping this in view, the authors opine that the results obtained in this study can be generalized to fisherman communities.

The limitations of this study include the shortcomings inherent to questionnaires like social desirability bias in responding to tobacco use. Employment of multilevel models would have yielded more confirmatory results. Elimination of missing component of DMFT, though beneficial in terms of not overestimating caries experience, however, carries the disadvantage of underestimating the same in case the reason for loss is caries.

The habit of tobacco use is high among the fisherman community compared to general population, regardless of gender. This study highlights the need for tobacco cessation counseling among these communities. Future studies attempting to find association between tobacco use and DC could consider the inclusion of both empirical and conceptually justified potential effect modifiers in this association and incorporate the interaction terms between these effect modifiers and exposure variables in the analysis of data.

CONCLUSION

The results obtained in this study highlighting less caries experience among tobacco users clearly do not promote the use of tobacco by any means. The study should be understood only in terms of the scientific quest of the authors to discern the association between tobacco and DC. The inherent shortcomings of cross-sectional studies apply here and it is recommended that well-designed longitudinal studies be conducted to find the exact nature of association between tobacco and DC.

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Conflicts of interest

There are no conflicts of interest.

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Evaluation of Serum Leptin and Adiponectin in Obese Individuals with Chronic Periodontitis

Abstract

Objectives: Studies have reported changes in leptin and adiponectin levels in the gingival crevicular fluid, saliva, and blood serum of obese patients with periodontal disease. The aim of the study is to evaluate serum leptin and adiponectin levels in obese and nonobese individuals with chronic periodontitis and to deduce a relationship between the clinical parameters and the inflammatory biochemical parameters. **Materials and Methods:** In this case-control study, a total of fifty individuals were selected based on the body mass index (BMI): Group I of obese individuals with chronic periodontitis and Group II of nonobese individuals with chronic periodontitis. Periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, and clinical attachment level. The effects of obesity and periodontal status on serum leptin and adiponectin levels of both groups were statistically analyzed using an independent *t*-test. **Results:** Statistical analysis showed that the effect of BMI on serum leptin and adiponectin levels was statistically significant ($P < 0.01$), and the effect of chronic periodontitis on the serum leptin and adiponectin levels was also statistically significant compared to nonobese individuals ($P < 0.0001$). However, there is no statistically significant correlation between serum leptin and adiponectin, which indicates that both are independent to each other. **Conclusions:** In obese individuals with chronic periodontitis, serum leptin levels were significantly high compared to nonobese individuals and serum adiponectin levels were significantly lower in obese individuals compared to nonobese individuals with periodontitis, though both the parameters were independent of each other.

Keywords: Adiponectin, chronic periodontitis, enzyme-linked immunosorbent assay, leptin, obesity

Introduction

Periodontal problems are part of health issues having implications on systemic health, rather than just general oral health problems. Obesity that is of late considered a health issue, primarily caused by the changed lifestyles, is related to periodontitis when studied in terms of the obese individual's body mass index (BMI) using parameters such as ratio of waist-to-hip circumference, fat content in the body, and consumption of oxygen. Based on these findings, certain conditions associated with periodontitis can aggravate obesity, as could be seen from "the metabolic syndrome," dyslipidemia clustering, and insulin resistance. Of these diseases linked to obesity, the potential danger of cardiovascular diseases (CVDs) is shown to have been influenced by periodontitis.^[1,2] Obesity is a condition similar to overall inflammation, occurring with metabolic and immune characteristics,

rendering one susceptible to periodontal disease.^[3] To establish these connections of chronic inflammation such as periodontitis and obesity, several hosts of mediators have been recognized. Functioning as endocrine organ, adipose tissue is a connective tissue, loose in nature, and its adipocytes not only function as a passive triglyceride reservoir but also generate cytokines and hormones which can result in erratic responses of immunity.^[4] Adipokines perform a number of different roles like the ones played by leptin and adiponectin, wherein they function as hormone like proteins.^[5] These adipocytokines play an important role in initiation of periodontal disease by activating monocytes, which increases the production of inflammatory cytokines. Release of inflammatory cytokines causes an alteration in the host immune responses that links to a higher susceptibility to bacterial infection.^[6]

Leptin enhances the host immune mechanism by activation of monocytes and

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macrophage and manages activities such as phagocytosis, cytokine production, chemotaxis, and regulation of oxidative species stimulated by neutrophils, development of natural killer cells, shift of T-cell responses toward Th1 cytokine type, and inhibition of Th2 cell type.^[7] Adiponectin is produced primarily by adipocytes, and its plasma levels decrease in obese individuals as compared to normal-weight individuals.^[8] It has certain constructive outcomes such as anti-inflammatory, vasoprotective, and antidiabetic effects. These defensive effects occur due to suppression of tumor necrosis factor- α and interleukin-6, along with induction of interleukin-1 receptor antagonist.^[9] Iwayama *et al.* demonstrated that adiponectin exerts anti-inflammatory effects on human gingival fibroblasts and mouse gingival fibroblasts and promotes osteoblastogenesis of human periodontal ligament cells and concluded that adiponectin has potent beneficial functions to maintain homeostasis of periodontal health which contributes to wound healing and tissue regeneration.^[10]

Materials and Methods

Ethical consideration and study design

This cross-sectional study was approved by the Ethical Committee of Mamata Dental College and Hospital, Khammam, Telangana, India. The individuals participating in the study were volunteers who received detailed information regarding the proposed research and provided signed informed consent.

Study population and inclusion criteria

Twenty-five systemically healthy individuals, 19 males and 6 females, 25–65 years old (mean age: 43.24 years), comprised the control group; 25 obese individuals, 18 males and 7 females with mean age of 54.92 years, comprising the test group participated in the study. These individuals were selected from an initial sample of 66 patients who were referred for periodontal treatment to the Outpatient Department of Periodontics, Mamata Dental College and Hospital, Khammam, from March 2017 to June 2017. The patients enrolled in the study met the following inclusion criteria: (1) age >25 years; (2) the presence of at least 15 teeth, excluding third molars, (3) nonsmokers, and (4) normal weight or obese Type I or II status.^[11] All consecutive patients who met these inclusion criteria were invited to participate in the study and 25 individuals were randomly allocated in the test group, i.e., obese patients with chronic periodontitis and 25 individuals in the control group, i.e., nonobese individuals with chronic periodontitis, respectively, using a coin toss method. The study design was double-blinded randomized study to avoid bias. Of the 66 individuals screened, 16 failed to meet the required inclusion criteria. Patients with a diagnosis of gingivitis or aggressive periodontitis or a known systemic disease such as acquired immunodeficiency syndrome or diabetes mellitus, pregnant/lactating, currently undergoing orthodontic

treatment or submitted to periodontal therapy during the previous 6 months, or having undergone antimicrobial, anti-inflammatory, and/or immunosuppressive therapies during the previous 6 months were excluded from the study.

Body mass index assessment

BMI was calculated based on the each individual's weight in kilograms divided by the square of his height in meters (kg/m^2). Weight and height measurements were performed by a single, trained examiner. Obesity was defined according to the World health organization (2012) as Type I (i.e., $\text{BMI} \geq 30 \text{ kg}/\text{m}^2$ but $< 35 \text{ kg}/\text{m}^2$) or Type II (i.e., $\text{BMI} \geq 35 \text{ kg}/\text{m}^2$ but $< 40 \text{ kg}/\text{m}^2$).

Demographic and clinical parameters

Patient's account of his/her medical history was taken systemic diseases was assessed. The clinical periodontal parameter measured were gingival index (GI) of Loe and Silness, plaque index (PI) of Silness and Loe, probing pocket depth (PD) measured from the gingival margin to the base of the periodontal pocket and clinical attachment level (CAL) measured from a fixed reference point of cemento-enamel junction to the base of pocket/gingival sulcus. PD and CAL were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual). A standard UNC-15 probe (University of North Carolina) was used for the measuring periodontal parameters. All recordings were performed by a single experienced clinician to avoid any interobservational bias at the single session, twice over a gap of 30 min.

Collection of serum

Two milliliters of blood was collected from the antecubital fossa by venipuncture using 20 gauge needle with a 5 ml syringe. Blood sample was allowed to clot at room temperature, and after 1 h, serum was separated from blood by centrifuging at 3000 rpm for 5 min and 0.5 ml of the extracted serum was immediately transferred to 1.5 ml aliquots. Each aliquot was designated a tracking number and stored at -80°C until further analysis.

Since adipokines form important biomarkers, knowing the normal values of the same would be significant in correlating it with specific metabolic states that could have the potential to impacting directly on the metabolic homeostasis of the system. Obesity and CVD are a few to mention to show dysregulated levels of adipokines among a vast number of other pathological changes.

Leptin analysis

Highly sensitive enzyme-linked immunosorbent assay (ELISA) kits were used to detect serum leptin levels as prescribed by the manufacturer. Each plate was checked before use, to ensure that the calibration curve measured leptin standards (0–1000 pg/ml) within the stated limits of

the assay. Absorbance of the substrate color reaction was read on ELISA reader. The total leptin was determined in picograms (pg), and calculation of concentration in each sample was performed by dividing the amount of leptin by the volume of sample (pg/ml).

Adiponectin analysis

Serum adiponectin Acrp30 concentration was measured by immunoassay method using a commercially available human adiponectin ELISA kit. According to the manufacture’s guidelines, the lowest detectable level of serum leptin was 0.2 ng/mL. Monitoring the levels of adiponectin and its receptors, namely Adipo R1 and Adipo R2, is promising target for prevention and treatment of obesity, insulin resistance, hyperlipidemia, and atherosclerosis.

Statistical analysis

Data entry and analysis were done using the SPSS version 22.0 IBM SPSS (IBM Inc. Chicago, USA). Descriptive statistics, such as mean and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables, were determined. The data were found normally distributed. Independent *t*-test was done for intergroup comparison.

Table 1 and Graph 1 shows the mean and SD of the age and BMI. There is statistically significant difference present in age and BMI in obese and nonobese individuals with periodontitis, with higher mean values seen in obese individuals. Table 2 and Graph 2 shows the comparison of mean values of various periodontal clinical parameters in both the groups. There is statistically significant difference present in the mean PI scores and mean GI scores in obese individuals with chronic periodontitis than in nonobese individuals with chronic periodontitis, showing higher scores in the former group. Table 3 and Graph 3 shows the mean and SD of various biochemical parameters in obese chronic periodontitis group and nonobese chronic periodontitis individuals, wherein it can be inferred that there is highly significant difference in the mean serum leptin levels in obese and nonobese patients, with higher values of leptin in obese patients and decreased adiponectin in obese individuals with chronic periodontitis. Correlation of serum leptin values with clinical parameters in obese chronic periodontitis group is shown in Table 4, which inferred that there is a statistically significant correlation between serum leptin values and the various clinical parameters. From Table 5, statistically significant correlation between serum adiponectin values and various clinical parameters such as GI and PI was seen. However, no statistical difference was seen between probing depth and clinical attachment loss. Graph 4 represents the relationship between serum leptin and serum adiponectin.

Results

The means ± SDs of serum leptin levels in obese individuals and nonobese individuals with chronic periodontitis were

Table 1: Distribution of individuals according to age and gender

Parameter	Obese patients (n=25)	Healthy patients (n=25)	P
Age (mean±SD)	54.92±6.82	43.24±8.96	<0.001**
BMI (mean±SD)	31.68±4.16	22.42±2.11	<0.001**
Male, n (%)	18 (72)	19 (76)	0.257 (NS)
Female, n (%)	7 (28)	6 (24)	

P<0.05 is considered statistically significant. **Highly significant (*P*<0.01), NS: Not significant (*P*>0.05). BMI: Body mass index; SD: Standard deviation

Table 2: Comparison of mean values of various clinical parameters in both the groups

Parameter	Mean±SD		P
	Obese patients (n=25)	Healthy patients (n=25)	
PI	2.24±0.59	1.89±0.4	0.020*
GI	2.66±0.4	2.55±0.17	0.018*
Probing depth	6.83±0.99	6.44±0.52	0.089 (NS)
Clinical attachment loss	3.92±1.09	3.95±0.75	0.905 (NS)

*Statistically significant (*P*<0.05), NS: Not significant (*P*>0.05). PI: Plaque index; GI: Gingival index; SD: Standard deviation

Table 3: Comparison of biochemical parameters in both groups

Parameter	Mean±SD		P
	Obese patients (n=25)	Healthy patients (n=25)	
Serum leptin	26.33±4.47	1.92±0.55	<0.001**
Serum adiponectin	11.82±5.13	16.96±4.69	<0.001**
Leptin/adiponectin	2.66±1.23	0.131±0.065	<0.001**

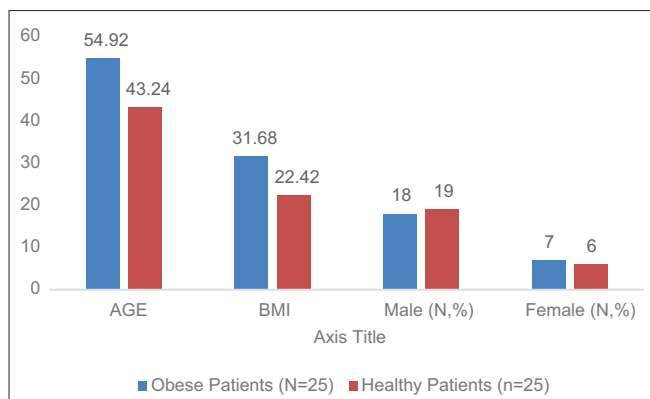
**Highly significant (*P*<0.01). SD: Standard deviation

Table 4: Correlation of serum leptin values with clinical parameters in obese chronic periodontitis group

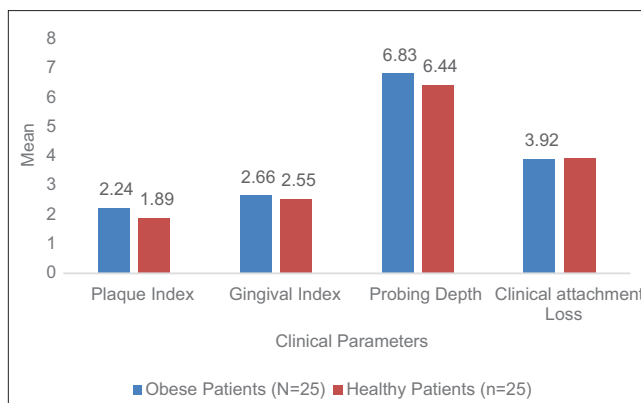
Parameter	Correlation value (r)	P
PI	0.001	0.009*
GI	0.275	0.008*
Probing depth	0.324	0.009*
Clinical attachment loss	0.28	0.005*

*Significant (*P*<0.01). PI: Plaque index; GI: Gingival index

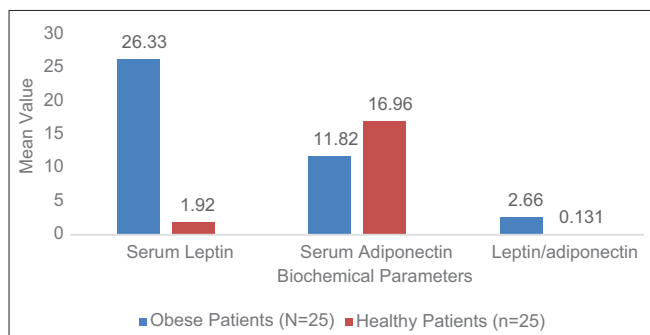
26.33 ± 4.47 pg/mL and 1.92 ± 0.55 pg/mL, respectively. The means ± SDs of serum adiponectin levels in obese individuals and nonobese individuals with chronic periodontitis were and 11.82 ± 5.13 pg/mL and 16.96 ± 4.69, respectively. Statistical analysis showed that the effects of BMI on serum leptin and adiponectin levels were statistically significant (*P* < 0.01), and the effects of chronic periodontitis on the serum leptin and adiponectin levels in obese individuals with chronic periodontitis were significant compared to nonobese individuals with chronic periodontitis (*P* < 0.0001). However, there is no



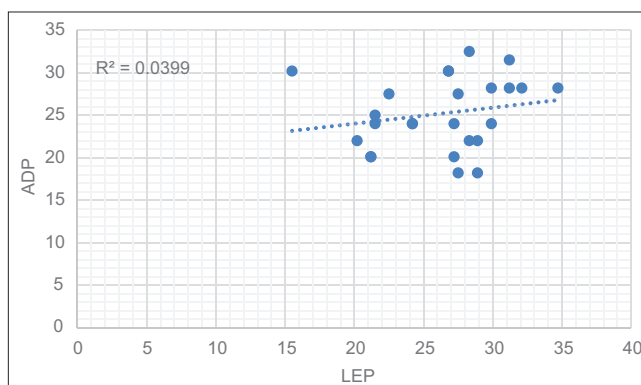
Graph 1: Distribution of individuals according to age and sex



Graph 2: Mean values of various clinical parameters



Graph 3: Mean values of various biochemical parameters



Graph 4: Relationship between serum leptin and serum adiponectin

statistically significant correlation between serum leptin and adiponectin, which indicates that both are independent to each other as assessed from Table 6.

Discussion

Obesity is considered a medical condition of accumulation of excess body fat which may have adverse effect on one’s health, leading to reduction in life expectancy and associated health problems, defined in terms of BMI and evaluated in terms of distribution of fat via waist-hip ratio and total cardiovascular risk factor. Obesity may also influence periodontal disease status by increasing lipid and glucose blood levels, which may, in turn, have deleterious consequences on the host response by altering T cells and monocyte/macrophage functions, as well as increasing cytokine production.^[1] A variety of potential mechanisms can explain an association between obesity and periodontitis.^[12] The biological mechanisms for association of obesity with periodontitis are not well established. However, adipose tissue-derived cytokines and hormones may play a role.^[3] Overweight in young individuals may have unhealthy dietary patterns such as insufficient micronutrients and excess sugar and fat content, which may increase the risk for periodontal disease and also changes in host immunity and/or increased stress levels, that are often associated with gain of excess fat in early part of life.^[13] Iwayama *et al.* established that adiponectin with its potent beneficial function can maintain homeostasis of periodontal health, improve periodontal

Table 5: Correlation of serum adiponectin values with clinical parameters

Parameter	Correlation value (r)	P
PI	0.218	0.001*
GI	0.230	0.001*
Probing depth	0.309	0.132 (NS)
Clinical attachment loss	0.330	0.107 (NS)

*Highly significant ($P < 0.01$), NS: Not significant ($P > 0.05$). PI: Plaque index; GI: Gingival index

Table 6: Correlation of serum leptin/adiponectin values with clinical parameters

Parameter	Correlation value (r)	P
PI	0.150	0.474 (NS)
GI	0.228	0.272 (NS)
Probing depth	0.380	0.061 (NS)
Clinical attachment loss	0.372	0.067 (NS)

Statistically significant ($P < 0.05$), NS: Not significant ($P > 0.05$). PI: Plaque index; GI: Gingival index

lesion, and has wound healing effects besides supporting periodontal regeneration.^[10] Singh *et al.* did a study to evaluate the relationship between obesity and periodontitis and showed that the prevalence of periodontitis was significantly more in obese than in nonobese individuals. It was concluded that strong correlation exists between obesity

and periodontitis and stated that obese with high serum triglycerides and low-density lipoproteins could be at higher risk of periodontitis.^[11] Palle *et al.* demonstrated a significant association between measures of overall and abdominal obesity (BMI and waist circumference) and periodontal disease showed significant association.^[14] In a review of seventy cross-sectional studies, systematically conducted by Chafee *et al.*, association between chronic periodontal disease and obesity, with strong association across people from diverse backgrounds, was seen. Obese patients are likely to have more incidence of periodontal disease, though no evidence is currently available to recommend differences in treatment planning.^[15] Keller *et al.* studied eight longitudinal and five interventional reviews, of which two of the longitudinal studies showed a direct association between obesity and the risk of developing periodontitis and three studies found to have a direct relationship between obesity and development of periodontitis among the adults.^[16] Leptin plays an important role in regulating energy intake and consumption, including appetite and metabolism. However, in relation to periodontitis associated with obesity, the same still needs to be examined. Karthikeyan and Pradeep reported that serum leptin levels increase in periodontitis.^[17] Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. There is an inverse association between adiponectin and markers of inflammation. Yamaguchi *et al.* showed that adiponectin might not function efficiently in periodontal disease sites owing to reduced number of receptors, and this might occur in worsening of periodontitis.^[18]

Conclusions

Obesity is a complex and multifactorial disease. Its relationship with periodontal disease and other chronic disease is well documented, but underlying mechanism is under investigation. Information at a chronic level is multidirectionally linked to periodontal diseases, obesity, and associated conditions, and a periodontist can share related information with the patients as to help improve oral and general health of the patient. This study highlights the significant roles played by serum levels of leptin and adiponectin in pathogenesis of periodontitis and the levels that appear to be indicative to disease occurrence.

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Conflicts of interest

There are no conflicts of interest.

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