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Addresses
Editorial Office
Dr. Avinash P Tamgadge
Vice Dean, Professor,
Department of Oral Pathology and Microbiology,
D.Y. Patil University, School of Dentistry,
Nerul, Navi Mumbai-400 706, India.
Ph: (+91) 9819619494
Email: avinash.pt@gmail.com
Website: www.ijohr.org

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INTRODUCTION

Bite mark may be defined as mark made by teeth either alone or in combination with other mouthparts.[1] Bite marks are a form of pattern injury, which means that the configuration is caused by a particular object. Occasionally, bite marks are obtained on various types of food substances, chocolate, chewing gum, fruits, vegetables.[2-4] Like fingerprints, the marks made by human teeth can be a tool for identification as this is unique in every individual. Bite marks disclose individual tooth imprints. In some cases, bite mark evidence is the only evidence on which conviction has been achieved, particularly alleged rape and child abuse cases. The current protocol for collection, management, preservation analysis, and interpretation of evidences should be employed if information is to be obtained for the court. The aim of this article is to give brief overview of bite mark analysis, its clinical applications, and limitations.

Humble (1933) used the transparencies for bite marks’ comparison.[8]

Webster in 1982 in an article of bite marks stated that bite marks have been reported in flesh, foodstuffs, and inanimate objects. The terminology used to describe food bite marks is very varied and thus gave classification of food bites in an effort to bring a degree of uniformity to the analysis of such marks.

Bernstein[9] has described in detail about the application of photography in forensic dentistry.

Gleen M. Wagner in 1986 in an article of bite marks identification stated that using tool-mark technology, comparisons are possible even in limited material. Computer enhancement of bite mark photographs increases a favorable comparison by further delineating unique characteristics of the arch and individual teeth.

Whittaker and McDonald emphasize that bite mark analysis starts with the examination of the wound.[10]

Aboshi et al. (1994) reported the identification of suspect arsonist by means of bite marks in cakes which were found at

Corresponding Author: Dr. Rakhee Modak,
Department of Oral Medicine and Radiology, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune, Maharashtra, India.
E-mail: drakheemodak@gmail.com

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the scene of the crime. A missing upper right central incisor was proved to be in patterned injury.

Sweet (1995) is of the view that no two human bite marks can be identical.[11]

Berlitz et al. (2000) reported a case of murder with a bite mark in a piece of cheese which was recorded. The pattern associated with comparison between the impression and a study model of the suspect was able to identify the perpetrator.

Franklin and Curtis have described in detail the method of bite mark OVERLAY Technique.[12]

Sheasby and MacDonald (2001) have described in detail about the primary and secondary distortions in the bite marks.[13]

Richard (2001) has written that unique characteristics of biter’s teeth are compared with that of the bite mark on the skin and which will help in identification.[14]

CLASSIFICATION OF BITE MARKS

In general, bite mark consists of superficial abrasions, or subsurface hemorrhage, or bruising of the skin because of bite.[2] The pattern of the injury is affected by the force and length in time of the bite, in combination with other mechanical and physiologic factors. Human bites may be classified in different ways, for example, defensive or offensive.[15]

MacDonald’s classification is most cited. MacDonald suggested an etiologic classification. It is pertinent to human bite marks but equally applicable to marks on other materials.

1. Tooth pressure marks: These are caused by incisal edges of the anterior teeth. They are stable and subjected to minimal distortion
2. Tongue pressure marks: Because of tongue pressure, impressions of the palatal surfaces of the teeth, cingulum, or palatal rugae may be produced. This causes distortion of marks
3. Tooth scrape marks: These are produced because of irregularities in the teeth due to fractures, restorations, etc.
4. Complex marks: These are a combination of the above types of marks. The shape depends on amount of tissue taken into a mouth.

COLLECTION OF BITE MARK EVIDENCE FROM THE BITE MARK VICTIM

Following information should be recorded both in living and deceased victim.

1. Demographics: Patients name, age, and gender along with case number, date of examination, and name of examiners should be recorded
2. Location of the bite mark: Anatomic location, contour of the surface (flat, curved, or irregular) underlying tissue such as bone, cartilage, muscle, or fat should be recorded
3. Shape of the bite mark: Shape of the bite mark such as round, ovoid, crescent, or irregular should be noted
4. Color and size of the mark: Both vertical and horizontal dimensions should be recorded
5. Type of injury: Petechiae, contusion, abrasions, and laceration caused by bite mark should be noted
6. Nature of the human bite mark: Human bite marks are usually semicircular or crescentic, with gap on either side. The teeth may cause clear, separate marks, or form a continuous or intermittently broken line. Bite marks may be abrasions, contusions or lacerations, or a combination of above any.

GUIDELINES FOR THE ANALYSIS OF BITE MARKS

To standardize the analysis of bite marks, the American Board of Forensic Odontostomatology (ABFO) established guidelines in 1986.[16]

The collection of evidence from the bite suspect commences only after proper consent has been acquired. The consent has to be signed by the suspect as well as the witness.

A detailed history of the individual including history of dental treatments (after and just before the bite marks) has to be noted.

The basic steps and tools used in the recording of bite marks are as follows.[6]

Photography

The most important evidence from bite mark victim is photography. It is performed by the forensic dentist or under the odontologist’s direction to ensure accurate and complete documentation. Extraoral photographs including full face and profile views, intraoral should include frontal views, two lateral views, and occlusal view of each arch, photograph of maximum mouth opening. All photographs should be taken with the camera perpendicular to injury. The bite marks are photographed at regular 24 h intervals on both deceased and living victim. Photographs of injury should be taken immediately.

1. In color and black and white
2. With and without the ABFO number 2 scale [Figure 1]
3. On and off camera flash
4. Close-ups that can easily be scaled 1:1
5. Ultraviolet (UV) photography if injury is fading
6. An overall body shot showing the location of injury
7. If the bite is on a movable anatomic location, then several body positions should be adopted to assess the effect of movement.

Color or speciality filters may be used to record the bite site in addition to unfiltered photographs. Alternative methods
of illumination may be used. A ring flash, natural light, and overhead lighting can be utilized to off angle lighting. Video/digital imaging may be used in addition to conventional photography.

Collection of swabs
Swabbing of bite mark injury is important to recover trace evidence. Stains of saliva or human cells for a DNA analysis should be collected whenever possible.[6,17]

The human beings secrete “ABO” antigens through saliva during biting. Swabs should be taken from bitten area, control area, and oral cavity. In case of sexual assaults, oral swab should be taken for semen. Mouthwashes with water can be used to obtain test samples for spermatozoa. It is acceptable to use either cotton tip applicators or cigarette paper to gather this evidence.

Ultraviolet illumination
Bite marks which are not visible by naked eyes may become visible when examined under UV light in a dark room.

This technique will demonstrate invisible bite marks up to 6 months after infliction.

Impression and models
Depending on constitution of the skin, the bite marks can be distorted, this can be problem when analyzing the bite marks. To prevent mistakes by the pattern associated comparison, it is recommended to simulate bites at similar body parts using the study casts of the suspect[6] or using digital technique for a stepwise dynamic comparison.[18,19]

Take two high-quality impressions of each arch. Alginate can be used for making impression, but preferred material is rubber-base and silicon-base impression material due to its dimensional accuracy [Figure 2]. Take registration in dental wax in centric occlusion, edge-to-edge bite, and in protrusive and lateral excursions of the jaws.

Master cast is poured with Type IV dental stone, and duplicate casts should also be made [Figures 3 and 4].

Sample bites
In case of dead victims, bite marks can be excised along with underlying tissue after fixing acrylic stent around bite mark to avoid shrinkage of tissue. The specimen is then stored in 4% formalin.
METHODS OF ANALYSIS OF BITE MARKS

Odontometric triangle method\cite{20,21}

In odontometric triangle method (objective method), a triangle is made on the tracing of bite marks and teeth models by marking three points - A, B, and C. Points A and B are plotted on outermost convex points on the canine teeth. Center of two central incisors is selected as Point C. All three points are joined to form triangle ABC. Lines AB, BC, and CA are measured, and angles a, b, and c are calculated. This is done for both upper and lower jaw teeth model and compared with that of bite marks of wax, apple, and skin. Statistical analysis is carried out, and results are obtained.

Comparison technique

It has two types (1) direct and (2) indirect.\cite{22}

In direct method, models from the suspect can be directly placed over the photograph of the bite mark to demonstrate concordant points [Figures 5 and 6]. Videotape can be used to show slippage of the teeth producing distorted images and to study dynamics of the bite marks. Bite mark and study casts can be compared using three-dimensional (3D) pictures.\cite{19}

Indirect method involves preparation of transparent overlay which is then placed over the scaled 1:1 photographs and comparison is made.\cite{23}

Image perception software procedure\cite{24}

This is a new method of comparing and analyzing photographs of bite mark with overlays of suspected biter’s dentition using image perception software. A photograph of bite mark is opened with image perception software, and a region of interest is then selected. After such selection, colors can be added to different grayscale areas of the image. The colored image of the bite mark is now layered over the original bite mark photograph using Photoshop of Adobe Systems. With image perception software, it is possible to depict a 2D picture as a 3D object.\cite{24}

Other special methods in bite mark analysis are:

**Vectron**

This is used to measure distance between fixed points and angles.

**Stereometric graphic analysis**

This is used to produce counter map of the suspect’s dentition.\cite{24} A stereometric graphic plotting method permits the outline of the tooth mark or the biting edge of a tooth to be registered in great detail in all three dimensions in the form of a contour map.

**Scanning electron microscopic analysis of bite mark wounds**\cite{26-28}

The degree of correlation of a particular set of the teeth with a certain bite mark is proportional to the number of characteristics common to both. However, individual characteristics are much more significant because they are less likely to occur purely by chance in a given population. Since the scanning electron microscope can readily demonstrate individual characteristics when they are present, it can be an extremely useful tool for the forensic odontologist.

The ABFO provides a range of conclusions to describe results of bite mark comparison.\cite{24}

1. Excluded: Discrepancies in bite marks and suspect’s dentition
2. Inconclusive: Insufficient forensic detail to draw any conclusion
3. Possible biter: Teeth like the suspects could be expected to create a mask like the one examined but so could other dentition
4. Probable biter: Suspect most likely made the bite; most people in population would not leave such bite
5. Reasonable medical certainty: Suspect is identified for all practical and reasonable purposes by the bite mark.

![Figure 5](image-url): Comparison of the bite mark and teeth of study cast of the upper jaw of suspect

![Figure 6](image-url): Comparison of the bite mark model and teeth of study cast of the upper jaw of suspect
Difficulties in bite mark analysis

1. Subjective element in fabrication
2. Subjective element in comparison
3. Distortion through skin elasticity, anatomical location, and body positioning is recurring problem
4. Loss of data, contamination.

DISCUSSION

Bite marks are a form of “patterned injury” which means that the configuration is caused by particular object.[29]

Biting is considered to be a primitive type of assault and results when teeth are used as a weapon in an act of dominance or dispersion.[30] Bite marks may be caused by humans or animals; they may be on tissue, food items, or objects. As no two fingers are identical, neither two mouths nor two teeth are exactly identical.[31]

The first person who published an analysis of bite mark case is Sorup. He called the method “odontoscopy,” analogous to the fingerprint identification called “dactyloscopy.” By this method, plaster cast of the teeth of suspect is obtained, dried, and vanished after which the incisal edges and occlusal surfaces are coated with printer’s ink. Upon this inked surface, a sheet of moistened paper is pressed, and print is transferred from it to transparent paper. This print is placed over life-size photograph of the bite mark and compared. Sorup’s method was later criticized.[32]

The most famous bite mark case of the 20th century involved serial murderer Ted Bundy who killed at least 100 women. On January 15, 1978, a case was reported of two young girls who were raped and brutally killed by serial killer Ted Bundy. There was no solid evidence available for conviction. Analysis was conducted for an odd bite mark on the left buttock of victim. The suspect was told to provide dental impression. This was matched with mark found on victim’s body. Ted Bundy was found guilty and was convicted.

Bite mark evidence has played an important role in judicial system. In some criminal cases, bite mark evidence is the only evidence on which a conviction has been achieved.[29] The scientific basis of bite mark analysis is rooted in belief that no two humans have identical dentition in respect to size, shape, and alignment of the teeth. Although bite mark of individual has uniqueness when it comes to analysis, it is complicated by numerous factors.[29] The investigators of bite marks should have knowledge of any mark or bruise which have characteristics which closely resemble the injuries have produced by teeth require substantial information.[33] The forensic value of bites in nonhuman materials is based on nature of material itself and in case of perishable items, how long ago the bite took place. Once teeth impressions are taken, these can be compared against bite mark data and matched for up to 76 comparison factors. These include whorls, indentations, abrasions, striations, distance between cuspids, tooth width and thickness, alignment, and mouth arch.[34] The forensic odontologist is now able to combine information from conventional analysis and pseudo-3D images to investigate the bite mark and attempt to establish its origin with a higher degree of certainty. The availability of additional coloring of selected areas with similar intensity values as well as rendering 2D photographs as pseudo-3D images may enable the researcher to analyze the image more extensively and come to accurate conclusions regarding source of bite.[34] Use of DNA in bite marks was pioneered into eliminate subjectivity associated with conventional analysis.[35,36] However, it is proposed that the presence of nucleic acid degrading enzymes within saliva can readily degrade DNA in living victim as the skin’s temperature accelerates the process.[37]

In bite mark analysis, two simultaneous and opposite paths develop. The inclusive path is one in which the unique features of suspected biter’s dentition show a strong link with the bite mark injury in a tooth-by-tooth and arch-to-arch comparison with pattern recorded in the bitten skin or object. The exclusive path is one in which the suspected biter’s dentition does not show link with the bite mark injury in an arch-to-arch and tooth-by-tooth analysis.[26]

CONCLUSION

The field of bite mark science is expanding, and need for individuals trained and experienced in the recognition, collection, and analysis of this type of evidence is increasing. The conclusion from bite mark analysis can assist crime judicial system; thus, it is an important tool in crime investigation. The serious nature of crimes in which bites are found often dictates that “Forensic Standards” should be established for gathering and interpretation of evidence. Errors in recording, comparison, analysis, and interpretations of bite marks may lead to serious consequences. With recent advances in research, more objective methods of bite mark analysis such as salivary DNA recovery and bacterial genotyping have become mainstay of investigation in such crimes. Further efforts to reduce subjectivity in standard physical techniques are required.

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There are no conflicts of interest.
REFERENCES

INTRODUCTION

Dental caries is the most common oral ailment, affecting a large number of population in urban areas. Enamel demineralization starts when pH drops below 5. This is the beginning of early enamel caries. Subsurface layer gets demineralized during primary stages of demineralization. However, neutralizing the oral pH can oppose demineralization. This process is achieved by remineralization. Remineralization increases amount of calcium and phosphate ions in the immediate environment of the lesion. In remineralization, building up of partly dissolved apatite crystals occurs and further caries process is prevented.

Numerous remineralizing agents such as topical fluoride, stannous fluoride have been used for remineralization. It involves direct delivery of ions to the affected area.

Restriction of initiation of dental caries can be achieved by milk and its products. Casein, calcium, and phosphate provide anticarcinogenic property to milk, giving its protective action. When casein phosphopeptide-amorphous calcium fluoride is applied to oral environment, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex molecule binds readily to enamel, biofilm, and soft tissues and delivers the calcium and phosphate ions strictly where it is needed. Studies done by Patil et al. and Jayarajan et al. reported that CPP-ACP with fluoride (CPP-ACPF) has more potential for remineralization as compared to CPP-ACP.
Recently, calcium sucrose phosphate (CaSP) was reintroduced as Enafix in the Indian market as a remineralizing agent. It decomposes to calcium, phosphate, and sucrose ions, thus resulting in increased rate of remineralization.\[^4\]

This randomized control study focuses on evaluating the enamel remineralizing potential of CPP-ACPF (GC Tooth Mousse Plus, Leuven, Belgium) and CaSP (Enafix, Group Pharmaceuticals Limited, Bengaluru, Karnataka, India) using surface microhardness analysis (Vickers hardness test). Null hypothesis of this study was that both remineralizing agents, CPP-ACPF and Enafix, had similar remineralizing efficacy.

**MATERIALS AND METHODS**

In this study, forty freshly extracted sound maxillary premolars, extracted for orthodontic reasons were used. Teeth with any visible or detectable caries, or any white spot lesions, or any hypoplastic lesions were excluded.\[^5\]

The teeth were cleaned of gross debris. Elimination of microbial growth was achieved by using an autoclave cycle for 40 min.\[^5\]

The teeth were decoronated, and the crown portions were divided into four segments of two buccal and two palatal halves each, using a double-faced diamond disc mounted on a contra-angle handpiece.\[^5\]

Enamel samples were embedded in self-cure acrylic with the enamel surface exposed. These samples were stored in deionized water until further use. A total of forty acrylic slabs embedded with enamel samples were produced and were then randomly divided into four groups of ten samples each [Figure 1 and Table 1].

Demineralizing solution was prepared in the Department of Biochemistry. A digital pH meter (Slope Labtronics, Model LT-11, Panchkula, Haryana, India) was used to check pH during and after preparation of the solution. The composition of demineralizing solution used was as follows:

- 2.2 mM calcium chloride, CaCl\(_2\)·2H\(_2\)O (Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India)
- 2.2 mM monosodium phosphate, NaH\(_2\)PO\(_4\)·7H\(_2\)O (Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India)
- 0.05 M lactic acid, C\(_3\)H\(_6\)O\(_3\) (Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India).

The final pH was adjusted to 4.5 with 50% sodium hydroxide, NaOH (Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India).\[^1\]

All the samples of Groups A, B, and D were then immersed into a glass container containing 50 ml of prepared demineralizing solution for 48 h at 37°C inside a Universal Incubator (Coslab, ISO 9001:2000, Ambala Cantt, Haryana, India). This demineralizing procedure was contemplated to produce a consistent subsurface lesion. After 48 h of incubation in the demineralizing solution, the teeth were washed with deionized water, dried with the help of an air syringe, and placed in different clean glass containers until further evaluation.\[^1\]

The samples in Groups A and B were treated with respective remineralizing agents at every 24 h for 7 days. Samples were rubbed with respective remineralizing agent with the help of polishing cup attached to a contra-angle handpiece for 4 min [Figure 2], washed with deionized water, and then placed in artificial saliva. All samples were placed in the Universal Incubator at 37°C between each remineralizing cycle. In the control groups, samples were only washed with deionized water and placed in artificial saliva. Artificial saliva was renewed every 24 h just before immersion of freshly treated samples.\[^1\]

After 7 cycles of remineralization, the surfaces were assessed for surface microhardness using Vickers microhardness testing machine [Figure 3]. The surface microhardness of the specimens was determined using MITUTOYO microhardness tester (Kawasaki, Japan). A load of 100 g was exercised steadily to the surface of specimens for 10 s using Vickers elongated diamond pyramid indenter under a \( \times 40 \) objective lens. Five indentations were placed on the surface and the average value was considered for each specimen. Accuracy of values of diagonal length of indentation was determined under high magnification of \( \times 400 \). The depth of the indentation was measured through a built-in scaled
microscope and the values were converted to Vickers microhardness values.

**Statistical methods**
The surface microhardness of samples was compared across study groups. The mean and standard deviation of microhardness of samples was obtained for each group and comparison was performed using one-way analysis of variance. The pair-wise comparison of mean microhardness between groups was carried out using Tukey’s post hoc test. The statistical significance was tested at 5%, and the analysis was performed using SPSS 18.0 software (SPSS Inc., IBM, New York, USA).

**RESULTS**
The analysis resulting into \( P < 0.0001 \) indicates statistically significant difference in the mean levels of surface microhardness (SMH) of different materials.

The mean for positive control was the highest 282.35 ± 28.97 MPa while that of negative control was the lowest 198.74 ± 25.46 MPa. In case of materials, CaSP that had a mean value of 234.24 ± 19.05 while CPP-ACPF had the least mean value of 213.91 ± 31.81 MPa [Table 2].

Pair-wise comparison of mean SMH between different study groups reveals that the mean microhardness of positive control group is significantly higher than all other groups as \( P < 0.05 \). The mean microhardness of CaSP (Enafix) was slightly higher than CPP-ACPF. However, the difference was statistically insignificant as \( P < 0.05 \). Yet, its mean difference was statistically significant from that of negative control [Table 3].

In summary, the study reveals that the overall difference of mean microhardness across groups is mainly due to positive and negative control groups. Mean values for two experimental groups are insignificantly different from each other. Thus, the null hypothesis was accepted.

**DISCUSSION**
A subsurface carious lesion of enamel is clinically seen as a white spot lesion. The most prominent feature of a white spot lesion is that there is a subsurface demineralized zone with top layer of intact enamel. However, the mineral content of surface is deficient. As compared to sound enamel, white spot lesion shows a lower microhardness.\(^{[2]}\)

Demineralization process is directly affected by the acidic environment produced by the metabolism of bacteria. During demineralization, calcium, phosphate, fluoride, carbonate, sodium, and magnesium ions diffuse out from the enamel surface into the saliva. More the acidic environment more is the outflow of the calcium ion and phosphate ion. Mineral content of surface is higher than the body of the lesion. As
calcium and phosphate ions diffuse to the exterior, there is more probability of remineralization at the surface.\[^2\]

In the present study, the specimens were placed in the demineralizing solution for 48 h at 37°C in the Universal Incubator. This resulted in a subsurface demineralization.\[^2\]

Materials that are prone to cracking and possess microcrystalline structure are appropriate for microhardness measurement. As there are intact surface and subsurface demineralization, SMH measurement was a suitable technique for this experimental design. Therefore, in the present study, the microhardness values for each specimen were measured.\[^2\]

In the present study, mean for positive control was the highest 282.35 ± 28.97 MPa while that of negative control was the lowest 198 ± 25.46 MPa, which showed that mean microhardness of the sound enamel was highest. Between the both experimental groups, CaSP, i.e., Enafix showed more microhardness (234.24 = 19.05) as compared to CPP-ACPF (213.91 = 31.81) but statistically both are insignificant.

CPP-ACPF is supersaturated solution of amorphous and crystalline calcium phosphate phases. It has added fluoride content. It is a stabilized composition so that spontaneous precipitation of calcium phosphate is stopped. The remineralizing capacity is directly proportional to the levels of free calcium and phosphate ions that are stabilized by CPP.\[^6\]

When CPP-ACPF is applied on the tooth surface, its sticky CPP part readily mixes with enamel and biofilm releasing the calcium and phosphate ions. The free calcium and phosphate ions enter the enamel rods and form the apatite crystals again.\[^3\]

Mehta et al. reported that CPP-ACP molecules need an acidic exposure to get activated and this would separate ACP from the casein. In the present study, the samples underwent demineralization only once. Less value of CPP-ACP in this study might be because the samples were not acid activated when CPP-ACPF was applied on the tooth surface.\[^3\]

This is due to a difference in time between the release of ACP from CPP during the acid challenge and the time required to deposit calcium and phosphate into the lesion during remineralization. Another reason can be due to the short duration of application of the material. Therefore, it is necessary to have a longer period of application to be able to detect deposition of calcium and phosphate in the demineralized lesion.

CaSP is a combination of calcium salts of sucrose phosphate esters, mixed with inorganic calcium. It readily breaks down and releases calcium ions, phosphate ions, and sucrose phosphate ions into saliva. It is composed of 10%–12% calcium (wt%) and 8%–10% phosphorous (wt%). Calcium and phosphate ions in aqueous media form insoluble precipitates. CaSP forms aqueous solutions consisting of high concentration of calcium and phosphate without occurrence of precipitation. It acts as an ideal carrier for calcium and phosphate in water. Enafix acts by adsorption of sucrose phosphate ion rapidly on the enamel surface, thereby reducing the rate of acid dissolution of hydroxyapatite and quick remineralization by calcium and phosphate ion by common ion effect.\[^5\]

This study was aimed to evaluate the effectiveness of newly introduced Enafix, which is an inexpensive substitute for the costly remineralizing agents and is suitable for the Indian economical background.

Drawbacks observed in the study include failure to remineralize artificial caries completely in 7-days time. Hence, the period of application for complete remineralization cannot be described for the remineralizing agents used. Although surface remineralization was confirmed, enamel subsurface remineralization was not evaluated in the study. Within the limitations of this in vitro study, one can conclude that remineralization takes place with the use of CaSP and CPP-ACPF. However, complete remineralization did not occur within 7 days.

**CONCLUSION**

Enafix being a cost effective material as compared to GC Tooth mousse plus, it can be used as an alternative for better remineralization in Indian scenario.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**


INTRODUCTION

Patients seek dental health-care services for different reasons among which include pain, bleeding, swelling, unesthetic appearance, and tooth mobility. The expectation of these patients is that the available dental treatment will be tailored to meet their dental needs and demand.

On dental consultation, clinical diagnosis is made from the information gathered through history, clinical examination, and investigation. The tentative dental treatment plan is usually formulated following clinical diagnosis, but the definitive treatment plan is arrived at following the detailed discussion between the attending dentist and the patient. The detailed discussion between the attending dentist and the patient is necessary to acknowledge the patient’s rights in treatment decisions. If modification of the tentative dental treatment plan is usually done after the patient has decided their best treatment option in relation to their financial status and availability for treatment under the guidance of the dentist.

However, some dental patients rather than present their complaint or listen to the professional advice from the dentist, request for tooth extraction. The reason for requesting tooth extraction is usually the permanent cessation of pain. This situation is more pronounced among adults and has been documented among patients in different parts of the world.

The objective of this study was to determine the characteristics of patients requesting for tooth extraction in a Nigerian secondary health-care setting. The study was prospective in design and used an interviewer-administered questionnaire which elicited information on age, gender, educational status, dental attendance pattern, tooth extraction experience or that of close relative or friend, reasons for tooth extraction request, and awareness of tooth restoration options was the data collection tool. Data analysis was done using the IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) and P < 0.05 was considered statistically significant.

Results: A total of 137 patients aged between 17 and 65 years that met the inclusion criterion were studied. The majority of the patients who requested for tooth extraction were males, aged between 26 years and 65 years, had tertiary education, and had previously visited the dental clinic. A total of 48.9% and 62.0% of the participants had previous tooth extractions and had relatives that have undergone tooth extractions, respectively. Dental visit pattern was significantly associated with tooth extraction experience. The leading reason for requesting for tooth extraction was a permanent stoppage of pain. Age and gender were not significantly associated with the reasons for requesting tooth extraction.

Conclusion: Data from this study revealed that adult patients of both genders, varying ages, educational attainments, and dental attendance patterns requested for tooth extraction in the studied Nigerian secondary health-care setting, and their leading reason for the request was a permanent stoppage of pain.

Key words: Dental treatment, Nigeria, patient request, tooth extraction
dentist, first, demand specific treatment for their complaint. The demanded treatment may be an appropriate or inappropriate treatment option for their complaint. In some cases, there is no indication for tooth extraction based on dental criteria, but the patient may request for tooth extraction on a psychopathologic basis which includes fear of dental treatment or because of a mental disorder such as posttraumatic stress disorder, a somatoform pain disorder, or a disorder of body image perception. It has been reported that patients who request procedures for themselves that will lead to long-term adverse effects on their function and health are educated and helped to fully understand that their request represents substandard treatment and the attending clinician has the right to refuse the demanded inappropriate treatment after proper evaluation. However, the development of trust in health-care provider may trigger patient into agreeing with the recommendations offered by the clinician.

The influential factors in cases where the demand is appropriate are perceived overwhelming nature of the patient’s complaint, and experience and opinions of significant others. The demand for tooth extraction by dental patients is expected to be high in developing countries because tooth extraction is the most commonly performed dental procedure in developing countries. Previous studies stated that 33%–38.1% of the respondents that had tooth extraction stated that it was their own decision to have tooth extraction or they suggested it to the dentist. Patient desire or preference has been documented as one of the reasons for tooth extraction in the literature, but the affected patients have not been properly characterized. The objective of this study was to determine the characteristics of patients requesting for tooth extraction in a secondary health-care setting in Benin City, Nigeria.

MATERIALS AND METHODS

Ethical consideration
The protocol for this study was reviewed and approval granted by the Ministry of Health, Benin City, Edo State, Nigeria. Written informed consent was obtained from the participants. Participation was voluntary, and no incentive was offered.

Study design/study setting
This prospective study was conducted at the Central Hospital, Benin City between February 2012 and October 2014. This secondary health facility is located in the center of the State capital and renders dental services to all categories of the people in the society.

Inclusion criteria
Patients aged 17-year-old and above who presented to the dental clinic with the presenting complaint of “I want to remove (extract) my tooth” were included in this study.

Exclusion criteria
Patients below 17 years, nonconsenting patients, those referred by other dentists, and other caregivers for tooth extraction were excluded from the study.

Sampling
Every consecutive patient that came to the dental clinic with the presenting complaint of “I want to remove (extract) my tooth” were included in this study until the minimum sample size was achieved.

Data collection tool
Data collection tool was an interviewer-administered questionnaire. The questionnaire elicited information on age, gender, educational status, dental attendance pattern, tooth extraction experience or that of close relative or friend, reasons for tooth extraction request, and awareness of tooth restoration options.

Data analysis
The data were subjected to descriptive and nonparametric statistics using the IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $P < 0.05$.

RESULTS
A total of 137 patients aged between 17 and 65 years that met the inclusion criterion were studied. The majority of the participants were aged between 26 years and 40 years. Males constituted 56.2% of the participants and dominated the 26–40 years age group, but this is not statistically significant [Table 1]. Almost half of the participants had a tertiary level of education and were the first dental clinic attendees. A total of 48.9% and 62.0% of the participants had the previous extractions and had relatives that have undergone tooth extractions, respectively. Older patients had more dental visit frequency and tooth extractions. Dental visit pattern ($P = 0.002$) and tooth extraction experience ($P = 0.000$) were associated with aging. The leading reason for requesting for tooth extraction was a permanent stoppage of pain (63.5%). Other reasons which constituted 15.3% include esthetically displeasing appearance (10) (shape [4], arrangement of tooth [3], broken tooth [3], and additional tooth [1]) masticatory disturbances (3), pain might come back if treated (2), advised by a friend who has had extraction (2) failed restoration (1),

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender, n (%)</th>
<th>Total</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>17-25</td>
<td>21 (27.3)</td>
<td>18 (30.0)</td>
<td>39 (28.5)</td>
</tr>
<tr>
<td>26-40</td>
<td>29 (37.7)</td>
<td>20 (33.3)</td>
<td>49 (35.8)</td>
</tr>
<tr>
<td>41-60</td>
<td>27 (35.1)</td>
<td>22 (36.7)</td>
<td>49 (35.8)</td>
</tr>
<tr>
<td>Total</td>
<td>77 (100.0)</td>
<td>60 (100.0)</td>
<td>137 (100.0)</td>
</tr>
</tbody>
</table>
and no time for root canal therapy (1). More than half (58.4%) of the participants were aware of tooth restoration option. Age and gender were not significantly associated with reasons for requesting tooth extraction [Table 2]. Dental visit pattern was significantly associated with tooth extraction experience \( (P = 0.000) \) [Table 3].

**DISCUSSION**

This study was set to determine the characteristics of patients requesting for tooth extraction in a secondary health facility in Benin City, Nigeria, studied 137 patients aged between 17 and 65 years between February 2012 and October 2014. The older patients constituted more than two-thirds of the population, and this may be explained by the fact that the young- and middle-aged adult Nigerians demand immediate resolution of health issues and challenges because of their high-dependency ratio. The usually relax of esthetic importance of teeth with aging is also a contributory explanation for the age distribution.

The self-acclaimed knowledge, persistence and impatient nature, and the firm belief that their demand is right and wise among men may explain why the participants were more males than females. The less consideration of the effect of tooth loss on orofacial esthetic and function may additionally explain why more male constituted more participants than females despite the documented evidence of more dental visit among females than males in Nigeria. \(^{11-14}\)

**Table 2: Characteristics of participants requesting for tooth extraction**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17-25</td>
<td>26-40</td>
<td>41-65</td>
</tr>
<tr>
<td>Education status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonformal</td>
<td>0</td>
<td>0</td>
<td>2 (4.1)</td>
</tr>
<tr>
<td>Primary</td>
<td>1 (2.6)</td>
<td>4 (8.2)</td>
<td>13 (26.5)</td>
</tr>
<tr>
<td>Secondary</td>
<td>20 (51.3)</td>
<td>16 (32.7)</td>
<td>14 (28.6)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>18 (46.2)</td>
<td>29 (59.2)</td>
<td>20 (44.7)</td>
</tr>
<tr>
<td>Dental visit pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>28 (71.8)</td>
<td>23 (46.9)</td>
<td>13 (26.5)</td>
</tr>
<tr>
<td>Second</td>
<td>9 (23.1)</td>
<td>21 (42.9)</td>
<td>28 (57.1)</td>
</tr>
<tr>
<td>&gt;second</td>
<td>2 (5.1)</td>
<td>5 (10.2)</td>
<td>3 (16.3)</td>
</tr>
<tr>
<td>Tooth extraction experience</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (28.2)</td>
<td>22 (44.9)</td>
<td>34 (69.4)</td>
</tr>
<tr>
<td>No</td>
<td>28 (71.8)</td>
<td>27 (55.1)</td>
<td>15 (30.6)</td>
</tr>
<tr>
<td>Previous extraction of relative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24 (61.5)</td>
<td>36 (73.5)</td>
<td>25 (51.0)</td>
</tr>
<tr>
<td>No</td>
<td>15 (38.5)</td>
<td>13 (26.5)</td>
<td>24 (49.0)</td>
</tr>
<tr>
<td>Awareness of tooth restoration options</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25 (64.1)</td>
<td>29 (59.2)</td>
<td>26 (53.1)</td>
</tr>
<tr>
<td>No</td>
<td>14 (35.9)</td>
<td>19 (39.6)</td>
<td>23 (46.9)</td>
</tr>
<tr>
<td><em>Reason for requesting for tooth extraction</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best tx option</td>
<td>6 (15.4)</td>
<td>16 (32.7)</td>
<td>14 (28.6)</td>
</tr>
<tr>
<td>P</td>
<td>0.169</td>
<td>0.490</td>
<td></td>
</tr>
<tr>
<td>Stop pain finally</td>
<td>24 (61.5)</td>
<td>32 (65.3)</td>
<td>31 (63.3)</td>
</tr>
<tr>
<td>P</td>
<td>0.935</td>
<td>0.300</td>
<td></td>
</tr>
<tr>
<td>Cheapest tx</td>
<td>6 (15.4)</td>
<td>9 (18.4)</td>
<td>6 (12.2)</td>
</tr>
<tr>
<td>P</td>
<td>0.702</td>
<td>0.925</td>
<td></td>
</tr>
<tr>
<td>Only tx for toothache</td>
<td>5 (12.8)</td>
<td>6 (10.2)</td>
<td>3 (6.1)</td>
</tr>
<tr>
<td>P</td>
<td>0.554</td>
<td>0.684</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>6 (15.4)</td>
<td>6 (12.2)</td>
<td>9 (18.4)</td>
</tr>
<tr>
<td>P</td>
<td>0.702</td>
<td>0.925</td>
<td></td>
</tr>
</tbody>
</table>

*Multiple reasons for positive response were depicted on the reason for requesting for tooth extraction. Tx: Treatment*
The availability, accessibility, and affordability of education in metropolitan cities in Nigeria including Benin City explain why majority of the participants had attained secondary and tertiary levels of education. The relatively low- and non-involvement of patients with lower educational attainment in research reported in the literature may also be a contributory explanation.[15] This study did not observe a significant effect of educational status on patients' request for extraction which contrasted with Klock[6] report of the significant effect of educational level on patient preference for tooth extraction.

Close to half of the participants making the request for tooth extraction were the first time dental clinic attendee due to the fact that dental visit in Nigeria is commonly problem oriented rather than prevention oriented. The predominance of an episodic problem-oriented pattern of the dental visit in Nigeria explained why majority of the participants that have visited the dentist previously had extraction as their treatment.[16] This study concurred with this fact as dental visit pattern was significantly associated with tooth extraction experience.

The reasonable proportion of the participants had a close relative that had tooth extraction and close to half of the participants have had tooth extraction who may have informed their opinion about the tooth extraction as a treatment. This may explain why more than a quarter (26.3%) of the participants requested for tooth extraction because they considered it the best treatment option. It was observed that patients aged 26 years and above were found to be regular visitors to the clinic and also had a higher history of previous extractions, but this did not deter them from requesting for further extractions. This contrasts with studies[17,18] done in Scotland, where irregular attendance to the dental clinic was a contributor to high tooth morbidity rates.

In this study, 58.8% of the participants were aware of tooth restoration option which was higher than 40.3% reported by Uti and Sofola.[7] This may be explained by the fact that their study included all patients that had extraction rather than restricting their study participants to only those requesting for tooth extraction. Although the awareness of other treatment options was significantly associated with willingness to have their tooth saved,[7] the adduced reason why tooth extraction was requested for despite this reasonable awareness of tooth restoration option is due to the presence of pain. Pain on presentation has been cited as the most common reason for not wanting to save teeth.[7] The request for extraction because of their belief that it is the cheaper treatment option in this study which was also reported by Uti and Sofola.[7] The economic challenges among a reasonable proportion of Nigerians and economic implication of dental care generally may contribute to this level of tooth extraction request.

The unbearable nature of pain and sleep disturbances have been cited as compelling reasons to visit the dentist[16] and pain observed to be a major reason (47.2%–64.4%) for tooth extraction in studies.[7,8] It is therefore not surprising that the leading reason for requesting for tooth extraction was a permanent stoppage of pain. The permanent stoppage of pain as a reason for tooth extraction request was higher in females than males which explains the lower tendencies of females to tolerate pain than males. It is considered culturally unacceptable for males to openly acknowledge experiencing pain because it is assumed to undermine masculinity. Studies have documented gender effects on pain perceptions which are linked to hormones and cultural beliefs.[19,20] Some patients in this study requested for tooth extraction because they believed it is the only treatment for a toothache. This observation is consistent with the study in Pakistan[21] which reported that the patients because of their traditional belief thought that extraction is the best treatment of diseased teeth.

Esthetic displeasing appearance due to the position or arrangement of the tooth, improperly shaped, and broken tooth were also given as reasons for tooth extraction request. This may be due to the lack of knowledge of the orthodontic treatment of malocclusion and also the long-term consequences of tooth loss. A minor reason for tooth extraction request among the participants was advice by friends because their friends may have had similar problems which were solved by tooth extraction. This is confirmed in a study by Klock[6] who observed that only 0.4% of patients said that family, friends, or colleagues influenced the decision to have their teeth extracted. It has been reported that patients with bad previous experience of dental treatment, particularly restorative treatment, were more in extraction cases as compared to the nonextraction cases.[21] This is consistent with our study in which we observed that patients in the 26–40 years age group preferred extraction to other treatment options because they felt it was the best option.

The finding of this study is limited by utilization of convenience sample of consecutively patients requesting for tooth extraction and nondocumentation of the reasons and number of teeth extracted. However, the findings of this study provide a good insight into treatment demand of dental patients and will serve as oral health intervention baseline tool.
CONCLUSION

Adult patients of both genders, varying ages, educational attainments, and dental attendance patterns request for tooth extraction in the studied Nigerian secondary health-care setting, and their leading reason for the request was a permanent stoppage of pain.

Acknowledgment

The abstract of this study was presented at the 3rd and 4th Annual Scientific Conference of School of Dentistry, University of Benin, Nigeria, that held in August 2015.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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INTRODUCTION

Anxiety is a subjective sensation with a varied degree of perception. Anxiety includes a wide range of strong external stimuli which can be both physiological and psychological problems. As a human being, everyone has an anxiety toward different issues. However, the level of anxiety varies among different age groups. Among them, students at their teenage level are considered to experience increased levels of anxiety when compared to others.

Clinical anxiety is the emotional reaction that health professionals face before delivering health care. This anxiety occurring at the transition from preclinical to clinical stage is specific in health career training. It is known that anxiety can be beneficial or harmful depending on the severity. Minimal anxiety is good for students because it makes them task oriented and aids concentration. However, excessive anxiety, on the other hand, can be very debilitating, increases the risk of suffering illness, decreases learning, impairs performance, and undermines optimal healthcare delivery.

Clinical training for dental students remains undisputedly a vital component of the dental education offering a wide variety of learning. This exposure to the reality of professional practice in the clinic is essential in producing skilled clinicians.

ABSTRACT

Aims: The dental clinical setting is a significant learning environment for dental undergraduates which may induce anxiety, which adversely affects the clinical performance. Hence, the aim of this study was to determine the clinical anxiety-provoking situations among dental students in Chennai.

Subjects and Methods: A questionnaire-based cross-sectional survey was conducted among 375 clinical dental students in Chennai. The questionnaire used in the study consisted of 37 clinical anxiety-provoking situations. The situations were assessed using a 4-point Likert scale. The questionnaire was distributed and the students completed the questionnaire under the supervision of research assistance, who collected the completed questionnaires immediately for evaluation.

Results: Of the 375 respondents, 21% were male and 78.8% were female. About 98.7% of the population reported anxiety with the majority of study population experiencing moderate anxiety. Examination failure (88%), meeting clinical requirement (82.5%), fracturing a tooth while extraction (94%), extracting a wrong tooth (92%), and getting infected by the patients (92.7%) were considered as more common clinical anxiety-provoking situations.

Conclusion: The challenges and stress at different stages of dental education may elicit and increase the level of anxiety which can be prevented by creating a friendly learning environment for better learning with less anxiety and fear.

Key words: Anxiety, dental, Likert scale
However, the learning that occurs in clinical settings presents challenges that may cause students to experience anxiety.\(^4\)

Hence, the objective of this study was to determine the clinical anxiety-provoking situations among dental students in Chennai.

**SUBJECTS AND METHODS**

A cross-sectional study was conducted among final year students and Compulsory Rotary Residential Internship (CRRI) of four randomly selected dental colleges in Chennai regarding clinical anxiety-provoking situations in January 2016. The study was approved by the Institutional Review Board of Priyadarshini Dental College and Hospital, and permission to conduct the study was given by the Ethical Committee of Priyadarshini Dental College and Hospital.

The estimated sample size was 382 based on 90% power with an \(\alpha\) error of 0.05. The sample size was estimated using Fisher’s exact test.

Prior authorization was obtained from the Principal of each Dental College, after brief description about the aim and purpose of the study. The investigators met the head of the department of each specialty and got the necessary permission to contact the students. Each department students were explained about the survey, after which the questionnaire was distributed to the participants.

The questionnaire contained 37 clinical anxiety-provoking situations. The situations were assessed using a 4-point Likert scale from “not anxious,” “slightly anxious,” “fairly anxious,” to “very anxious.” For the purpose of analysis, response was scored 1 for not anxious, 2 for slightly anxious, 3 for fairly anxious, and 4 for very anxious. Demographic variables such as age and gender were also collected.

The students were instructed to give their own answer and discussion was not permitted. After answering, the questionnaire was collected for evaluation.

The data collected were entered into MS Excel sheet and analyzed using Statistical Package for the Social Science (Version 21.0 Armonk, NY: IBM Corp.) Software version 21.0. Mann–Whitney test for gender difference was also applied. \(P < 0.05\) was considered statistically significant.

**RESULTS**

The study participants included 385 dental students, of which 81 (44.4%) were male and 304 (55.6%) were female. The age group of participants ranged from 20 to 25 years. Figure 1 shows the more common clinical anxiety-provoking situations among dental students. Figure 2 shows how anxious are study subjects to examination failure. Figure 3 shows the anxiety level of the study subjects while extracting a tooth. Table 1 shows the level of clinical anxiety-provoking situations among dental students in Chennai.

**DISCUSSION**

Medical school has been long recognized as involving numerous stressors that can affect the well-being of the
student. The mental status of medical students has been an important issue to be taken under consideration, reported in 1956.[5] Clinical training includes learning clinical procedures, completion of quotas coupled with attending lectures, and studying for examinations require dental students to work harder adding overall stress and anxiety.

Identification of potential problems is important in dental education programs as it might give students, faculty, and administrators an opportunity to take precautionary measures to prevent clinical anxiety and stress.[6]

In this study, extracting a wrong tooth, getting infected by patients, examination failure, and fracturing a tooth while extraction are the top most clinical anxiety-provoking situations which is similar to the study conducted by Kieser and Herbison,[7] in New Zealand dental students.

It has been established from the previous study on clinical medical and nursing students that fear of making mistakes is one of the significant causes of anxiety.[8]

In developing countries, tooth extraction is the common dental procedure performed in dental clinics. In this study, about 44.4% of study population experience severe anxiety while tooth extraction which is more commonly associated with extracting a wrong tooth and fracturing a tooth during extraction which is similar to study conducted by Obasiagbon et al.[4] among Nigerian clinical dental students. This may be due to the presence of complications from this procedure which may be considered simple by the general public.

In the present study, the completion of clinical cases and quotas are considered as an anxiety-provoking situation by 82.3% of the study population, which is similar to the study conducted by Sekhon et al.[9] among Dental College students in India.

The completion of clinical cases and quota is one of the requirements for qualification of examination; hence, study population may consider completion of clinical cases and quotas as a clinical anxiety-provoking situation.

The possibility of dental professional getting infected by patients during the care delivery procedures induces anxiety among 91.2% of the study population which is similar to the studies conducted by Moss and McManus[10] among students of London and Kieser and Herbison[7] among dental students in New Zealand. This may be due to the lack of knowledge or practice of students in infection prevention measures.

About 31.3% of the study population reported moderate anxiety while treating a child patient which is similar to the study conducted by Telang et al.[11] in which 31% of Malaysian dental students reported anxiety. This may be due to the lack of confidence and skills regarding management of child patients and lack of patient cooperation.

There has been a stigma, negative attitudes, and various forms of discrimination toward psychiatric patients among different categories of healthcare workers. About 40% of the study population experience severe anxiety while treating a psychiatric patient; this may be due to poor knowledge and awareness about psychiatric conditions and their management. This is similar to the study conducted by Kalaa et al.[12] among dental students in Turkey.

To the best of our knowledge, this is the first of its kind in Chennai to determine clinical anxiety-provoking situations among dental

| Table 1: Clinical anxiety‑provoking situations among dental students in Chennai |
|-------------------------------------------------|-----------------|-----------------|
| Variables                                       | Frequency (n)  | Percentage      |
| Examination failure                            |                |                 |
| Not anxious                                     | 47             | 12.2            |
| Slightly anxious                                | 96             | 24.9            |
| Fairly anxious                                  | 113            | 29.4            |
| Very anxious                                    | 125            | 32.5            |
| Treating psychiatric patient                    |                |                 |
| Not anxious                                     | 30             | 7.8             |
| Slightly anxious                                | 129            | 33.5            |
| Fairly anxious                                  | 142            | 36.9            |
| Very anxious                                    | 83             | 21.6            |
| Fracturing a tooth                              |                |                 |
| Not anxious                                     | 36             | 9.4             |
| Slightly anxious                                | 137            | 35.6            |
| Fairly anxious                                  | 126            | 32.7            |
| Very anxious                                    | 85             | 22.1            |
| Extracting a wrong tooth                       |                |                 |
| Not anxious                                     | 25             | 6.5             |
| Slightly anxious                                | 84             | 21.8            |
| Fairly anxious                                  | 104            | 27.0            |
| Very anxious                                    | 171            | 44.4            |
| Accidental pulp exposure                        |                |                 |
| Not anxious                                     | 36             | 9.4             |
| Slightly anxious                                | 124            | 32.2            |
| Fairly anxious                                  | 136            | 35.3            |
| Very anxious                                    | 89             | 23.1            |
| Getting infected by patients                    |                |                 |
| Not anxious                                     | 33             | 8.6             |
| Slightly anxious                                | 82             | 21.3            |
| Fairly anxious                                  | 117            | 30.4            |
| Very anxious                                    | 152            | 39.5            |
It is shown that the highest anxiety was due to the lack of confidence and fear of making mistakes which might cause disappointment and stress among students. If the sources can be identified and targeted, early barriers to learning can be minimized.

CONCLUSION

Data from this study revealed that clinical anxiety of moderate severity is more prevalent among dental students. This may be due to challenges and stress at different stages of dental education. However minimal anxiety is good for students as it aids in concentration, alertness, etc. Excessive anxiety on other hand can affect the overall performance of students. This can be prevented by creating a supportive learning environment to enhance student confidence level which helps in better learning with less anxiety and fear.

Recommendations

- Introduction of orientation course like practical application of infection prevention measures which may help students during clinical training
- Student confidence can be enhanced by more interactive classes regarding skills and management of patients
- In case of quota-based system in education, it can be shifted from quantity of cases to quality of cases.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

**Questionnaire: Priyadarshini Dental College and Hospital clinical anxiety among dental students**

Age/gender: ______
Year of course: ______

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<tr>
<th>Questions</th>
<th>(1) Not anxious</th>
<th>(2) Slightly anxious</th>
<th>(3) Fairly anxious</th>
<th>(4) Very anxious</th>
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<td>4. Admitting not knowing something to consultant</td>
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<td>7. Joining theater team</td>
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<td>18. Making diagnosis</td>
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<td>19. Telling patient that you do not know the diagnosis</td>
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<td>36. Fear of patient's satisfaction with denture</td>
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<td>37. Getting infected by patient</td>
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Oral Health Status and Treatment Needs among Health-care Workers in Shimla District, Himachal Pradesh India

Shailee Fotedar, Vikas Fotedar¹, Vinay Bhardwaj, Shelja Vashisht, Kavita Manchand²

Department of Public Health Dentistry, H. P. Govt. Dental College, ¹Department of Radiation Oncology, RCC, IGMC, Shimla, Himachal Pradesh, ²Department of Public Health Dentistry, Awadh Dental College and Hospital, Jamshedpur, Jharkhand, India

ABSTRACT

Objectives: To assess oral health status and treatment needs of the health workers in Shimla district, Himachal Pradesh.

Materials and Methods: A cross-sectional study was conducted among health workers in Shimla district, Himachal Pradesh. A sample of 130 was selected by selecting three blocks randomly to get a required sample size of 126. Clinical recordings were done according to the World Health Organization diagnostic criteria 1997. The data were analyzed using SPSS package, Chicago, IL, version 16.0. The statistical tests used were t-test and ANOVA. A P < 0.05 was considered to be statistically significant.

Results: The mean age of the study population was 48.42 ± 5.94 with a range of 25–57. The prevalence of dental caries in the present study was 80%. The mean decayed, missing, and filled teeth were 6 ± 5.42 and were higher in females and the age group of 55–64 years. Only 17 (13.1%) had healthy periodontium and calculus was found in 49 (47.7%) subjects. The need for prosthesis in upper jaw and lower jaw was for 33 (25.3%) and 61 (46.9%), respectively.

Conclusion: The caries experience of the population was quite high. Hence, there is a need for treatment camps, and regular follow-up checkups for the health workers in Shimla district.

Key words: Dental caries, health workers, treatment needs

INTRODUCTION

Health is one of the most valuable assets one can possess. Oral health is now recognized as equally important in relation to general health.¹ Oral health means more than healthy teeth. The World Health Organization (WHO) has a definition of good oral health: “Oral health means being free of chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft lip and palate, periodontal (gum) disease, tooth decay and tooth loss, and other diseases and disorders that affect the mouth and oral cavity.”¹² Several oral diseases have important side effects on general health, while systemic conditions may show a mutual influence on oral health. Therefore, oral health care needs to be addressed by a multi-professional approach and should be integrated into comprehensive health-promoting strategies and practices.¹² The WHO too has urged its member states to consider mechanisms to incorporate the essential oral health services into the existing primary health-care system, with emphasis placed on disease prevention and health promotion for the poor and the disadvantaged populations.¹³

“Primary health care,” rural health infrastructure has been designed to cover rural population through subcenters, primary health centers (PHC) and community health centers (CHCs).¹³ As on March 31, 2014, there are 152,326 subcenters, 25,020 PHCs and 5363 CHCs functioning in India. In Shimla district, there are 89 PHC’s, 8 CHC’s, 14 civil hospitals and 313 subcenters.¹⁴ Primary health-care workers posted here might play a key role in oral health promotion in those areas where there is a deficiency of dental professionals.

It has been shown that rural Indian community, who constitute more than 70% of the Indian population, has a low

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level of oral health awareness and practice when compared to urban. Oral health status of health workers will reflect their knowledge and attitude toward oral health to a great extent. They can influence the community as they can extend health education at the first contact in the community and hence should possess good oral health.

Literature on the oral health knowledge and oral health status of health-care workers in India is almost nonexistent. Hence, this study was carried out to assess oral health status and treatment needs of the health workers in Shimla district.

**MATERIALS AND METHODS**

A cross-sectional study was conducted among multipurpose health-care workers (both males and females) in Shimla district, Himachal Pradesh, India from February 2016 to May 2016. The necessary ethical clearance was sought from the Ethical Committee of H. P. Government Dental College and Hospital, Shimla. Prior permission was taken from the Chief Medical Officer, Shimla district to conduct the oral health examination. Informed consent was also taken from each subject before recording oral health.

A pilot study was conducted on thirty subjects which gave us a prevalence rate of 91%. To estimate this proportion with a 95% confidence interval of proportion and error of 5% the sample size required was 126 subjects using the formula

\[ n_0 = \frac{z^2 \times pq}{d^2} \]

where: \( n_0 \) is the sample size, \( z \) is the value for the selected alpha level, \( p \) is the estimated proportion of an attribute that is present in the population, \( q \) is \( 1 - p \), \( d \) is the acceptable margin of error for proportion being estimated. For administrative purposes, Shimla district is divided into nine developmental blocks, namely Mashobra, Rampur, Jubbal, Rohru, Theog, Chopal, Basantpur, Nankhari and Chuara. Hence, three blocks namely Mashobra, Rampur and Mathiana were selected randomly to get the required sample size.

**Inclusion criteria**
The health workers present on the day of visit.

**Exclusion criteria**
Those who refused for oral health examination.

A Type III clinical examination was conducted. A single trained examiner who was calibrated in the department conducted all the examinations. Intraexaminer calibration was done by examining 15 subjects followed by their reexamination after 10 days which resulted in a kappa value of 0.74. The examiner was assisted by an alert and cooperative recording assistant. Data were collected using a modified the WHO Performa. The data were analyzed using SPSS version 16 (SPSS., Chicago, IL). The test of significance used was t-test and ANOVA. \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

Out of total 130 subjects, there were 60 (46.2%) males and 70 (53.8%) females. The mean age of the study population was 48.42 ± 5.94 with a range of 25–57. Most of them were in the age group of 45–54 years. Most of the participants 62 (47.7%) had a qualification of matriculation followed by 12th pass 32 (24.6%). Toothbrush was used by 97.7% and toothpaste by 121 (93.1%). The frequency of brushing twice was reported by 79 (60.8%) [Table 1].

Extraoral examination shows 3 (2.3%) of subjects had lymph node enlargement, 2 (1.5%) had ulceration on their face. Temporomandibular joint (TMJ) symptoms were reported by 15 (11.5%). TMJ clicking was present in 26 (20%) and TMJ tenderness in 19 (14.6%). Intraorally, leukoplakia was found in 2 (1.6%) located in buccal mucosa and alveolar abscess in 8 (6.2%).

The prevalence of dental caries in the present study was 80%. The mean decayed, missing, and filled teeth (DMFT) were 6 ± 5.42. The mean DMFT was higher among females 6.43 ± 6.01 than males 5.50 ± 4.64, but the difference was not statistically significant. The mean of various age groups is given in table and the difference among the groups was statistically significant \( (P = 0.03) \) [Table 2]. Mean number of DMFT was 2.05 ± 1.96, 3.24 ± 4.38, 0.99 ± 1.79.

![Table 1: Demographic profile of the subjects](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
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<tr>
<td>Male</td>
<td>60 (46.2)</td>
</tr>
<tr>
<td>Female</td>
<td>70 (53.8)</td>
</tr>
<tr>
<td>Age groups (years)</td>
<td></td>
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<tr>
<td>25–34</td>
<td>4 (3.07)</td>
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<td>35–44</td>
<td>12 (9.2)</td>
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<tr>
<td>45–54</td>
<td>94 (70.7)</td>
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<td>55–64</td>
<td>20 (15.3)</td>
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<tr>
<td>Level of education</td>
<td></td>
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<tr>
<td>10th pass</td>
<td>62 (47.7)</td>
</tr>
<tr>
<td>12th pass</td>
<td>32 (24.6)</td>
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<tr>
<td>Graduation</td>
<td>23 (17.7)</td>
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<td>Postgraduation</td>
<td>13 (10.0)</td>
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<td>Oral hygiene aid used</td>
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<tr>
<td>Toothbrush</td>
<td>127 (97.7)</td>
</tr>
<tr>
<td>Finger</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>Tree stick</td>
<td>0</td>
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<tr>
<td>Oral hygiene material used</td>
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<tr>
<td>Toothpaste</td>
<td>121 (93.1)</td>
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<tr>
<td>Tooth powder</td>
<td>9 (6.9)</td>
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<tr>
<td>Charcoal</td>
<td>0</td>
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<tr>
<td>Salt</td>
<td>0</td>
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<tr>
<td>Frequency of brushing</td>
<td></td>
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<tr>
<td>Once</td>
<td>51 (39.2)</td>
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<tr>
<td>Twice</td>
<td>79 (60.8)</td>
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respectively. Mean number of subjects requiring one surface restoration, two surface restoration, pulp care, and extraction was 0.29, 0.65, 0.42, and 0.54.

Only 17 (13.1%) had healthy periodontium. Calculus was found in 49 (47.7%) followed by deep pockets in 29 (22.5%). Mean number of sextants was 1.83, 1.54, 1.52, 0.32, 0.42, for healthy, bleeding, calculus, shallow pockets, and deep pockets, respectively. Community periodontal index score was not significantly different between the genders \( (P = 0.084) \) and brushing frequency \( (P = 0.785) \) [Table 3].

Subjects having prosthesis in upper arch and lower arch were same, i.e., 14 (10.2%). The need for prosthesis in upper jaw and lower jaw was 33 (25.3%) and 61 (46.9%), respectively. The need for prosthesis was almost same for males and females \( (P = 0.241) \) [Table 4].

Only 13 (10%) subjects had dental fluorosis. Moderate fluorosis was found in 5 (3.8%) followed by very mild in 4 (3.1%).

**DISCUSSION**

India is a vast country with the majority of people living in rural areas. Following the Alma Ata Declaration of 1978 on the appropriateness of “primary health care,” rural health infrastructure has been designed to cover rural population through subcenters, PHCs and CHCs. As oral health is an integral component of general health, oral health care of the necessity has to be delivered through primary health-care infrastructure. Primary health-care workers might play a key role in promoting oral health in such areas. Hence, the health of these workers should be also of utmost concern to us. Good oral health is important because the experience of pain, problems with eating, chewing, smiling, and communication due to missing, discolored, or damaged teeth have a major impact on people’s daily lives and well-being. The prevalence and recurrences of these impacts, restrict activities at work, and at home causing millions of work hours to be lost each year throughout the world.

The present study intended to provide information about the oral health status, treatment needs of health workers in Shimla.

In the present study, use of toothbrush and toothpaste was a universal finding. The frequency of brushing twice here was reported by 60.8%, which is higher than 52.1% as reported by Kaur et al. and 20% as reported by Baseer et al.

The prevalence of dental caries in the present study was 80% which is <97.2% as reported by Aggnur et al. The mean DMFT in the present study was 6.0 which is in line with 5.02 as reported by Aggnur et al. The mean DMFT was higher among females than males which may be because the flow rates of saliva and compositional analysis have been shown to be generally less protective in women than in men. Alternatively, women have been the family member with the responsibility of food preparation. This would allow easier access to foods and snacks outside of mealtime, which provide bacteria in their oral flora with more substrate for caries development. The mean DMFT increased with age.

The mean of decayed teeth was highest in the younger age group (3.25) and lowest in older age group (1.92) while the mean missing teeth were highest in older age group (4.92) and mean missing teeth was 0.0 in the age group of 25–34. This is mostly because of lack of knowledge about various

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**Table 2:** Mean decayed, missing, and filled teeth according to age, gender and brushing frequency

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SD</th>
<th>P</th>
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<td>Age group</td>
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<tr>
<td>25–34</td>
<td>3.75±2.87</td>
<td>0.03*</td>
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<tr>
<td>35–44</td>
<td>5.20±5.05</td>
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<tr>
<td>45–54</td>
<td>5.97±5.52</td>
<td></td>
</tr>
<tr>
<td>55–64</td>
<td>8.33±5.66</td>
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<tr>
<td>Total</td>
<td>6.00±5.42</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
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<td>5.50±4.64</td>
<td>0.91</td>
</tr>
<tr>
<td>Female</td>
<td>6.43±6.01</td>
<td></td>
</tr>
<tr>
<td>Brushing frequency</td>
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restorative treatments in older age groups and lack of availability in those areas. The maximum treatment need was for two surface restoration in 57 (43.8%) followed by root canal therapy in 35 (26.9%), followed by extraction in 33 (25.3%) surface restoration.

In the present study, 13.1% had healthy periodontium which is higher than 2.2% and calculus was found in 47.7% which is also lower than 80.4% as reported by Aggnur et al.[13] This means that though periodontal health was better in our population, but still they lacked knowledge about the maintenance of oral hygiene or oral prophylaxis has not been availed.

The need for prosthesis was higher in lower arch than upper arch, and it was same for both the males and females.

The most prevalent intraoral condition was abscess followed by leukoplakia and ulceration which is in contrast to the findings of national oral health survey where the most prevalent condition was ulceration followed by leukoplakia. All the subjects with leukoplakia in our study had a history of smoking for more than 15 years.

CONCLUSION

The study was carried out on a population of 130 subjects. The prevalence of dental caries was quite high among the population especially the females and young age group. The treatment need was highest for two surface restorations, followed by root canal therapy. About half of the population had calculus. The need for prosthesis was higher than the prosthesis present, and it was almost same for both males and females. Hence, it is hereby recommended that the treatment camps should be arranged for them followed by regular checkup at a 6 month interval. Health education should be given to them to enhance their knowledge and practices toward oral health care for which will serve the community in the long way. Further studies are recommended in this area as very limited previous data is available.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

Reliability of Kvaal’s and Cameriere’s Methods of Age Estimation in a Specific Populace in Central India

Abhishek Singh Nayyar, H. C. Gayatri, Milind V. Naphade, Ujjwala M. Naphade, Abdul Qahar Qureshi, Farooque Iqbal Siddiqui, Shaikh Shahed Anwar, Pooja Rasik Shroff

Department of Oral Medicine and Radiology, Saraswati Dhanwantari Dental College and Hospital and Post-Graduate Research Institute, Parbhani, ‡Department of Oral and Maxillofacial Surgery, VYWS Dental College and Hospital, ‡Consultant Oral and Maxillofacial Pathologist, ‡Consultant Oral and Maxillofacial Surgeon, Amravati, Maharashtra, ‡Consultant Oral Medicine and Radiologist, Bangalore, Karnataka, India

ABSTRACT

Context: Comparison between antemortem and postmortem dental records and radiographs produces results with a high degree of reliability and relative simplicity. Kvaal et al. introduced an age estimation method by indirectly measuring secondary dentin deposition on radiographs. Cameriere et al., later, put forth a method based on radiographic estimation of pulp/tooth area ratio (AR) in canines. The purpose of the present study was to assess the reliability of various teeth in Kvaal’s and Cameriere’s methods of age estimation in a specific populace in Central India. Materials and Methods: One hundred and ten patients aged between 15 and 75 years were selected, and the variables P = complete pulp length/root length (from enamel-cementum junction [ECJ] to root apex), r = complete pulp length/complete tooth length, a = complete pulp length/root width at ECJ level, b = pulp/root width at midpoint level between ECJ level and mid-root level, and c = pulp/root width at mid-root level and pulp/tooth AR were recorded as devised in Kvaal’s and Cameriere’s methods of age estimation, respectively. Statistical Analysis: Statistical analysis was performed with SPSS (version 10.5) package. Mean comparison of morphological variables was carried out using Student’s t-test. Intra- and inter-observer reproducibility of measurements was studied using the concordance correlation coefficient. Results: Comparing between Kvaal’s and Cameriere’s methods, results of the latter method were found to be more accurate in predicting age. Conclusion: Kvaal’s method showed varying results for different teeth, but Cameriere’s method was more consistent, accurate and showed less variability.

Key words: Age estimation, Cameriere’s method, Kvaal’s method

INTRODUCTION

Aging refers to irreversible and inevitable changes that occur with time, which encompasses all aspects of human life, namely, anatomic, physiologic, and psychological.[1] Aging, in forensic context, is necessary both for the dead and the living. For the dead, it is principally to aid in the identification process by creating a biological profile which can, then, be compared to missing individuals while in case of the living, the aim is to solve judicial or, civil problems concerning age of minors in questions of adoption and/or, immutability and for adults, to solve judicial or, civil problems for individuals lacking valid documents for identification.[2] Although several parts of the body can be used for age estimation, the poor condition of the remains, particularly, in severe crashes or, fires, in cases of those recently dead, and due to moisture and burial conditions, in cases of historic subjects, make, many parts of the body, unusable.[3] Despite these problems, in the last few years, the literature has provided several skeletal and dental methods for assessing age. Most of them apply many age indicators related to degenerative changes in the skeleton.[8] Teeth can survive in most of the conditions, encountered at death, and during decomposition, even when

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Corresponding Author: Dr. Abhishek Singh Nayyar,
44, Behind Singla Nursing Home, New Friends’ Colony,
Model Town, Panipat - 132 103, Haryana, India.
E-mail: singhabhishek.rims@gmail.com

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the body is exposed to extreme forces and/or temperatures. The specific composition and shape of teeth are a source for many different hereditary and acquired characteristics. Thus, they can serve as reliable biomarkers of aging. It is also observed that tooth development is not perceptibly affected by diseases, drugs, as well as the endocrine status as compared to the bones, consequently making them the preferred tissue in forensic and archaeological investigations. Once a tooth is fully mineralized and erupted, it forms a very stable entity. Both the developmental and regressive changes affecting the teeth can be related to chronological age of the individual. In the last few years, forensic odontology has shown increasing interest in search for optimal age estimation methods in individuals using dental tissues/teeth as evidence. These methods are broadly classified as morphologic and radiologic methods. The former are further subclassified as clinical, histologic, and biochemical methods which include methods analyzing the various forms of tooth modification such as wear, dentin transparency, tooth cementum annulations, racemization of aspartic acid, and apposition of secondary dentin. However, all these methods have one or the other limitation restricting their usage on a mass scale. Furthermore, radiography, being a nondestructive method, plays a vital role in forensic odontology to uncover the hidden facts, which cannot be seen by means of physical examination. Dental examination and comparison between antemortem and postmortem dental records and radiographs produce results with a high degree of reliability and relative simplicity. Radiographic age estimation, using teeth, relies on developmental stages of teeth especially in children, while in adults, continuous deposition of secondary dentin throughout the life depicted by reduction in pulp area can be employed. Several age estimation methods exploit changes undergone by the teeth during the lifetime, but most are destructive warranting extraction of teeth. These methods, therefore, cannot be used in living individuals and in cases where it is not acceptable to extract teeth for various reasons. Radiography, thus, plays an important role in such cases. Dental pulp is a mesenchymal tissue surrounded by a pulp canal. Outside the pulp, there are some odontoblastic lines, which release dentin during the subject’s life and reduce the size of the pulp canal. Changes in its size caused by the apposition of secondary dentin are the best morphometric parameters for estimating age in adult subjects as it is a continuous process that takes place throughout the life of an individual. This apposition of secondary dentin can be indirectly measured by the reduction in pulp size on the radiographs. In 1925, Bodecker established that the apposition of secondary dentin correlated with age. Kvaal et al. introduced an age estimation method by indirectly measuring secondary dentin deposition on radiographs and proposed a number of length and width measurements of tooth and pulp. Later, Cameriere et al., put forth a similar method based on radiographic estimation of pulp/tooth area ratio (AR) in canines. The purpose of the present study was to assess the reliability of various teeth in Kvaal’s and Cameriere’s methods of age estimation in a specific population.

MATERIALS AND METHODS

Source of data
A total number of 110 patients, 59 males and 51 females, aged between 15 and 75 years, were selected from the outpatient department. Seven age groups (Group I–VII) were formed with both males and female patients. Group I consisted of 15 patients with age between 15 and 20 years, Group II (age 20–30 years) with 47 patients, Group III (age 30–40 years) with 23 patients, Group IV (age 40–50 years) with 15 patients, Group V (age 50–60 years) with 3 patients, Group VI (age 60–70 years) with 4 patients, and Group VII (age 70–75 years) with 3 patients. The permission to conduct the present study was obtained from the Institutional Ethics Committee. All the patients gave an informed consent prior to being included into the study.

Selection criteria

Inclusion criteria

• Patients aged between 15 and 75 years
• The selected teeth were the right or left maxillary central incisor, lateral incisor, and second premolar and right or left mandibular lateral incisor, canine, and first premolar which had fully erupted into the oral cavity
• The roots of the teeth were fully formed
• Individuals were of ethnic origin from Central India (history confirmed up to two generations).

Exclusion criteria

• Teeth with any pathology, such as caries or periodontitis or periapical lesions, that would alter the surface area of the tooth
• Patients with a history of trauma with or without fracture of teeth
• Teeth with any prosthetic rehabilitations and orthodontic appliances
• Fractured teeth
• Severely attrited teeth secondary to parafunctional habits
• Teeth with any developmental anomaly.

After clinical examination, patients who fitted into the inclusion criteria were subjected to digital intraoral radiographs.

Methodology

Intraoral radiography

Patients were selected according to the decided inclusion and exclusion criteria. After selection of the patients, their consent was taken for radiographic examination. All the guidelines were followed as per the ALARA principle while subjecting the patients to digital intraoral radiographs. The radiographic examination was carried out with the
help of X mind X-ray system, 70 kv, 8 mA, 0.425 kVA, 2 mm aluminum filter manufactured by SATELEC (India) Private Limited; DIGORA Optime DXR-50 5001, Digital Imaging System with Windows 2.8 Digital Imaging Program. PSP Digital Sensor (DIGORA Optime DXR-50 5001, Digital Imaging System) with size 2 sensor (31 mm × 41 mm) and Film holding instrument: RINN-Greene Stabe Disposable Film Holder were used with AutoCAD 2007 software (Autodesk Inc., San Rafael, CA, USA) for taking digital intraoral radiographs.

**Positioning of the patient**

- For maxillary teeth: The patient’s head was positioned upright with the sagittal plane vertical and the occlusal plane horizontal
- For mandibular teeth: The patient’s head was tilted back slightly to compensate for the change in occlusal plane, when the mouth was opened.

Measurement of teeth by Kvaal’s method is shown in Figure 1 wherein the following morphological variables were recorded.

- \( p \): complete pulp length/root length (from enamel-cementum junction [ECJ] to root apex)
- \( r \): complete pulp length/complete tooth length
- \( a \): complete pulp length/root width at ECJ level
- \( b \): pulp/root width at midpoint level between ECJ level and mid-root level
- \( c \): pulp/root width at mid-root level.

Measurement of teeth by Cameriere’s method is shown in Figure 2 wherein the pulp/tooth area ratio (AR) was recorded.

Measurements were made by a second observer to prevent any inter-observer bias. The morphological variables, chronological age, and subject’s gender were entered in a Microsoft Excel Spreadsheet for use as predictive variables for age estimation. Correlation coefficients were evaluated between chronological age and morphological variables. Estimated age was obtained using morphological variables for each tooth.

**Statistical analysis**

Statistical analysis was performed with SPSS (version 10.5, SPSS Inc., Chicago, USA) package. Mean comparison of morphological variables was carried out using Student’s t-test. Intra- and inter-observer reproducibility of measurements was studied using the concordance correlation coefficient.

**RESULTS**

The chronological age of adults was estimated based on the measurements of the different said variables of the various teeth including maxillary central incisor, lateral incisor, and second premolar and mandibular lateral incisor, canine, and first premolar which were numbered 1–6, respectively, on images of digital intraoral radiographs from derived regression equations. The demographic data of the patients are presented in Table 1 and Graph 1. There was no significant difference observed between morphological variables amongst the males and females indicating gender did not influence the estimation of chronological age [Tables 2-6]. In Tooth # 4 (mandibular lateral incisor), however, morphological variables, \( b \) = pulp/root width at midpoint level between ECJ level and mid-root level and width (W), showed a weakly positive correlation between gender and chronological age [Table 7]. Comparison of the readings of the two observers did not reveal any statistical significance [Table 8]. Karl Pearson’s correlation coefficients between age and morphological variables showed that the variables \( P \) = complete pulp length/root length (from ECJ-root apex), \( r \) = complete pulp length/complete tooth length, mean (M), length (L) and pulp/tooth area ratio (AR) correlated significantly with age with variable \( P \) = complete pulp length/root length (from ECJ-root apex) correlating the best amongst...
them. The ratios between width measurements (a = complete pulp length/root width at ECJ level, b = pulp/root width at midpoint level between ECJ level and mid-root level, and c = pulp/root width at mid-root level) correlated least with age and were not found to be statistically significant and therefore excluded from further statistical analysis. Variable \( P = \) complete pulp length/root length (from ECJ to root apex) had highest \( P \) value of \(-0.920\) for central incisor and \(-0.951\) for mandibular first premolar [Table 9]. The scatter plot graph between predicted age and chronological age showed that the resultant values were equally distributed along the line. Comparing between Kvaal’s and Cameriere’s methods, results of the latter method were found to be more accurate in predicting age. The scatter plot graph between predicted versus estimated age showed better distribution in Cameriere’s method than Kvaal’s method [Graphs 2-4; with Graph 2 showing comparative analysis between Kvaal’s and Cameriere’s methods, Graph 3 for Kvaal’s method, and Graph 4 for Cameriere’s method].

**DISCUSSION**

In 1995, Kvaal et al.\([6]\) presented a method for age estimation which was based on investigation of periapical radiographs while Paewinsky et al.\([11]\) verified the applicability of this method on orthopantomographs. Cameriere et al., in 2004, for the first time conducted a preliminary study to evaluate the variations in pulp/tooth AR as an indicator of age and their method of age estimation seemed promising.\([7]\) While the authors obtained high levels of accuracy in age prediction, they advised that future research should investigate “the

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</tr>
<tr>
<td>III</td>
<td>30.1–40</td>
<td>12 (52.2)</td>
<td>11 (47.8)</td>
</tr>
<tr>
<td>IV</td>
<td>40.1–50</td>
<td>10 (66.7)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>V</td>
<td>50.1–60</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>VI</td>
<td>60.1–70</td>
<td>2 (50.0)</td>
<td>2 (50.0)</td>
</tr>
<tr>
<td>VII</td>
<td>≥70</td>
<td>3 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>59 (53.60)</td>
<td>51 (46.40)</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of the morphological variables among males and females**

<table>
<thead>
<tr>
<th>Statistics for tooth # 1 (maxillary central incisor)</th>
<th>Gender</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Male</td>
<td>59</td>
<td>33.416</td>
<td>15.105</td>
<td>1.047</td>
<td>0.297</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>30.671</td>
<td>11.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root length from ECJ to root apex (p)</td>
<td>Male</td>
<td>59</td>
<td>1.175</td>
<td>0.123</td>
<td>-0.803</td>
<td>0.424</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>1.192</td>
<td>0.104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/complete tooth length (r)</td>
<td>Male</td>
<td>59</td>
<td>0.803</td>
<td>0.066</td>
<td>-0.388</td>
<td>0.699</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.808</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root width at ECJ level (a)</td>
<td>Male</td>
<td>59</td>
<td>0.265</td>
<td>0.058</td>
<td>0.786</td>
<td>0.434</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.256</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at midpoint level between ECJ level and mid-root level (b)</td>
<td>Male</td>
<td>59</td>
<td>0.221</td>
<td>0.052</td>
<td>-1.231</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.233</td>
<td>0.052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at mid-root level (c)</td>
<td>Male</td>
<td>59</td>
<td>0.218</td>
<td>0.267</td>
<td>-0.537</td>
<td>0.592</td>
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<td></td>
<td>Female</td>
<td>51</td>
<td>0.248</td>
<td>0.325</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (M)</td>
<td>Male</td>
<td>59</td>
<td>0.536</td>
<td>0.074</td>
<td>-0.759</td>
<td>0.449</td>
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<tr>
<td></td>
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<td>51</td>
<td>0.548</td>
<td>0.082</td>
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<tr>
<td>Width (W)</td>
<td>Male</td>
<td>59</td>
<td>0.219</td>
<td>0.141</td>
<td>-0.720</td>
<td>0.473</td>
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<tr>
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<td>Female</td>
<td>51</td>
<td>0.241</td>
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<td></td>
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<tr>
<td>Length (L)</td>
<td>Male</td>
<td>59</td>
<td>0.989</td>
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<td>0.488</td>
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<td>Female</td>
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<td>1.000</td>
<td>0.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference between width and length (W – L)</td>
<td>Male</td>
<td>59</td>
<td>-0.769</td>
<td>0.157</td>
<td>-0.325</td>
<td>0.746</td>
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<tr>
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<td>Female</td>
<td>51</td>
<td>-0.759</td>
<td>0.174</td>
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</tr>
<tr>
<td>Pulp/tooth (AR)</td>
<td>Male</td>
<td>59</td>
<td>0.248</td>
<td>0.363</td>
<td>0.358</td>
<td>0.721</td>
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<td>Female</td>
<td>51</td>
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<td>0.278</td>
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</table>

Statistical analysis: Student’s t-test (unpaired); statistically significant at \(P<0.05\). ECJ: Enamel-cementum junction, AR: Area ratio, SD: Standard deviation
### Table 3: Comparison of the morphological variables among males and females

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Male</td>
<td>59</td>
<td>33.416</td>
<td>15.105</td>
<td>1.047</td>
<td>0.297</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>30.671</td>
<td>11.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root length from ECJ to root apex (p)</td>
<td>Male</td>
<td>59</td>
<td>1.214</td>
<td>0.197</td>
<td>-0.369</td>
<td>0.713</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>1.226</td>
<td>0.156</td>
<td></td>
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</tr>
<tr>
<td>Complete pulp length/complete tooth length (r)</td>
<td>Male</td>
<td>59</td>
<td>0.785</td>
<td>0.131</td>
<td>0.726</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.769</td>
<td>0.096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root width at ECJ level (a)</td>
<td>Male</td>
<td>59</td>
<td>0.235</td>
<td>0.057</td>
<td>0.574</td>
<td>0.567</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.229</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at midpoint level between ECJ level and mid-root level (b)</td>
<td>Male</td>
<td>59</td>
<td>0.204</td>
<td>0.050</td>
<td>-0.63</td>
<td>0.530</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.210</td>
<td>0.045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at mid-root level (c)</td>
<td>Male</td>
<td>59</td>
<td>0.166</td>
<td>0.046</td>
<td>-0.032</td>
<td>0.975</td>
</tr>
<tr>
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<td>Female</td>
<td>51</td>
<td>0.167</td>
<td>0.054</td>
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<tr>
<td>Mean (M)</td>
<td>Male</td>
<td>59</td>
<td>0.521</td>
<td>0.069</td>
<td>0.059</td>
<td>0.933</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.520</td>
<td>0.051</td>
<td></td>
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<tr>
<td>Width (W)</td>
<td>Male</td>
<td>59</td>
<td>0.185</td>
<td>0.037</td>
<td>-0.398</td>
<td>0.692</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.188</td>
<td>0.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (L)</td>
<td>Male</td>
<td>59</td>
<td>0.999</td>
<td>0.152</td>
<td>0.066</td>
<td>0.947</td>
</tr>
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<td></td>
<td>Female</td>
<td>51</td>
<td>0.998</td>
<td>0.118</td>
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<td></td>
</tr>
<tr>
<td>Difference between width and length (W – L)</td>
<td>Male</td>
<td>59</td>
<td>-0.814</td>
<td>0.151</td>
<td>-0.177</td>
<td>0.860</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>-0.809</td>
<td>0.127</td>
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</tr>
<tr>
<td>Pulp/tooth AR</td>
<td>Male</td>
<td>59</td>
<td>0.147</td>
<td>0.049</td>
<td>-0.587</td>
<td>0.559</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.152</td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis: Student’s t-test (unpaired); statistically significant at P<0.05. ECJ: Enamel-cementum junction, AR: Area ratio, SD: Standard deviation

### Table 4: Comparison of the morphological variables amongst males and females

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>n</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Male</td>
<td>59</td>
<td>33.416</td>
<td>15.105</td>
<td>1.047</td>
<td>0.297</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>30.671</td>
<td>11.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root length from ECJ - root apex (p)</td>
<td>Male</td>
<td>59</td>
<td>1.036</td>
<td>0.123</td>
<td>-0.361</td>
<td>0.719</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>1.044</td>
<td>0.101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/complete tooth length (r)</td>
<td>Male</td>
<td>59</td>
<td>0.641</td>
<td>0.075</td>
<td>-0.657</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.689</td>
<td>0.204</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root width at enamel-cementum junction (ECJ) level (a)</td>
<td>Male</td>
<td>59</td>
<td>0.214</td>
<td>0.199</td>
<td>1.181</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.180</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at midpoint level between ECJ level and mid-root level (b)</td>
<td>Male</td>
<td>59</td>
<td>0.186</td>
<td>0.056</td>
<td>1.004</td>
<td>0.318</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.176</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at mid-root level (c)</td>
<td>Male</td>
<td>59</td>
<td>0.163</td>
<td>0.126</td>
<td>-0.373</td>
<td>0.710</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.176</td>
<td>0.214</td>
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<td></td>
</tr>
<tr>
<td>Mean (M)</td>
<td>Male</td>
<td>59</td>
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<td></td>
<td>Female</td>
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<td>0.070</td>
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<tr>
<td>Width (W)</td>
<td>Male</td>
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<td>0.176</td>
<td>0.109</td>
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</tr>
<tr>
<td>Length (L)</td>
<td>Male</td>
<td>59</td>
<td>0.839</td>
<td>0.091</td>
<td>-1.382</td>
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<tr>
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<td>51</td>
<td>0.866</td>
<td>0.118</td>
<td></td>
<td></td>
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<tr>
<td>Difference between width and length (W–L)</td>
<td>Male</td>
<td>59</td>
<td>-0.664</td>
<td>0.103</td>
<td>1.087</td>
<td>0.279</td>
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<tr>
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<td>Female</td>
<td>51</td>
<td>-0.691</td>
<td>0.151</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/tooth area ratio (AR)</td>
<td>Male</td>
<td>59</td>
<td>0.099</td>
<td>0.152</td>
<td>-0.118</td>
<td>0.906</td>
</tr>
<tr>
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<td>51</td>
<td>0.102</td>
<td>0.161</td>
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</tr>
</tbody>
</table>

Statistical Analysis: Student’s t-test (Unpaired); Statistically significant at P<0.05
Table 5: Comparison of the morphological variables among males and females

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Male</td>
<td>59</td>
<td>33.416</td>
<td>15.105</td>
<td>1.047</td>
<td>0.297</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>30.671</td>
<td>11.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root length from ECJ to root apex (p)</td>
<td>Male</td>
<td>59</td>
<td>0.984</td>
<td>0.244</td>
<td>-1.253</td>
<td>0.297</td>
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<tr>
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<td>Female</td>
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<td>1.031</td>
<td>0.116</td>
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<tr>
<td>Complete pulp length/complete tooth length (r)</td>
<td>Male</td>
<td>59</td>
<td>0.695</td>
<td>0.164</td>
<td>-1.878</td>
<td>0.063</td>
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<tr>
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<td>Female</td>
<td>51</td>
<td>0.742</td>
<td>0.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete pulp length/root width at ECJ level (a)</td>
<td>Male</td>
<td>59</td>
<td>0.245</td>
<td>0.342</td>
<td>1.106</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.192</td>
<td>0.044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at midpoint level between ECJ level and mid-root level (b)</td>
<td>Male</td>
<td>59</td>
<td>0.203</td>
<td>0.050</td>
<td>-0.553</td>
<td>0.581</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.216</td>
<td>0.171</td>
<td></td>
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</tr>
<tr>
<td>Pulp/root width at mid-root level (c)</td>
<td>Male</td>
<td>59</td>
<td>0.161</td>
<td>0.053</td>
<td>0.07</td>
<td>0.944</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.161</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (M)</td>
<td>Male</td>
<td>59</td>
<td>0.458</td>
<td>0.107</td>
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<td>0.530</td>
</tr>
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<td>Female</td>
<td>51</td>
<td>0.468</td>
<td>0.056</td>
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<td></td>
</tr>
<tr>
<td>Width (W)</td>
<td>Male</td>
<td>59</td>
<td>0.182</td>
<td>0.042</td>
<td>-0.462</td>
<td>0.652</td>
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<td>Female</td>
<td>51</td>
<td>0.188</td>
<td>0.093</td>
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<tr>
<td>Length (L)</td>
<td>Male</td>
<td>59</td>
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<td>0.201</td>
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<td>0.156</td>
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<td>1.000</td>
<td>0.836</td>
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<tr>
<td>Difference between width and length (W – L)</td>
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<td>-0.658</td>
<td>0.204</td>
<td>1.346</td>
<td>0.181</td>
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<td></td>
<td>Female</td>
<td>51</td>
<td>-0.812</td>
<td>0.853</td>
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</tr>
<tr>
<td>Pulp/tooth AR</td>
<td>Male</td>
<td>59</td>
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<td>0.032</td>
<td>-0.781</td>
<td>0.436</td>
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<td>Female</td>
<td>51</td>
<td>0.120</td>
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</tr>
</tbody>
</table>

Statistical analysis: Student's t-test (unpaired); statistically significant at P<0.05. ECJ: Enamel-cementum junction, AR: Area ratio, SD: Standard deviation

Table 6: Comparison of the morphological variables among males and females

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Male</td>
<td>59</td>
<td>33.416</td>
<td>15.105</td>
<td>1.047</td>
<td>0.297</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>30.671</td>
<td>11.892</td>
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<td>Complete pulp length/root length from ECJ to root apex (p)</td>
<td>Male</td>
<td>59</td>
<td>1.054</td>
<td>0.157</td>
<td>-0.536</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>1.069</td>
<td>0.124</td>
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<tr>
<td>Complete pulp length/complete tooth length (r)</td>
<td>Male</td>
<td>59</td>
<td>0.687</td>
<td>0.096</td>
<td>-0.277</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.691</td>
<td>0.067</td>
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<tr>
<td>Complete pulp length/root width at ECJ level (a)</td>
<td>Male</td>
<td>59</td>
<td>0.193</td>
<td>0.062</td>
<td>-1.666</td>
<td>0.999</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.251</td>
<td>0.261</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp/root width at midpoint level between ECJ level and mid-root level (b)</td>
<td>Male</td>
<td>59</td>
<td>0.197</td>
<td>0.059</td>
<td>-1.452</td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.212</td>
<td>0.052</td>
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</tr>
<tr>
<td>Pulp/root width at mid-root level (c)</td>
<td>Male</td>
<td>59</td>
<td>0.160</td>
<td>0.068</td>
<td>-0.871</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.179</td>
<td>0.156</td>
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<tr>
<td>Mean (M)</td>
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<td>59</td>
<td>0.458</td>
<td>0.058</td>
<td>-1.796</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.481</td>
<td>0.073</td>
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</tr>
<tr>
<td>Width (W)</td>
<td>Male</td>
<td>59</td>
<td>0.178</td>
<td>0.051</td>
<td>-1.347</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.196</td>
<td>0.083</td>
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<tr>
<td>Length (L)</td>
<td>Male</td>
<td>59</td>
<td>0.871</td>
<td>0.121</td>
<td>-0.46</td>
<td>0.646</td>
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<td></td>
<td>Female</td>
<td>51</td>
<td>0.880</td>
<td>0.090</td>
<td></td>
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<tr>
<td>Difference between width and length (W – L)</td>
<td>Male</td>
<td>59</td>
<td>-0.692</td>
<td>0.121</td>
<td>-0.34</td>
<td>0.735</td>
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<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>-0.684</td>
<td>0.126</td>
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<tr>
<td>Pulp/tooth AR</td>
<td>Male</td>
<td>59</td>
<td>0.113</td>
<td>0.036</td>
<td>-0.475</td>
<td>0.636</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
<td>0.116</td>
<td>0.029</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis: Student's t-test (unpaired); statistically significant at P<0.05. ECJ: Enamel-cementum junction, AR: Area ratio, SD: Standard deviation
effect of race and culture in model parameters.” Indeed, other researchers have, also, advocated the verification of age estimation methods on independent samples and some have concluded that best results are derived when population-specific formulas are used. Babshet et al. found that Cameriere’s formula, based on the Italian population, is not as applicable to the Indian population as was the case of naive population. The purpose of the present study was to assess the reliability of various teeth in Kvaal’s and Cameriere’s methods of age estimation in a specific populace in Central
India based on the relationship between chronological age and measurement of different variables, as used in Kvaal’s and Cameriere’s methods, of selected teeth, using digital intra-oral periapical radiographs (IOPARs). The study sample included, 110 patients aged between 15-75 years, only of Central India origin with at least last two generations residing in the location of the study. This was done to ensure ethnic uniformity of the study sample, considering that the development of teeth varies among populations and that it is genetically determined. Digital radiography was selected because of less radiation exposure than the conventional film-based radiography. Since Kvaal et al. did not find significant differences between teeth from the left or the right side of the jaw, teeth from either left or right side were processed depending on whichever were best suited for measurements. Ratios between the teeth and pulp size were calculated. This procedure helped to reduce the effect of the possible variation in magnification and angulation of the intraoral radiographs. The present study revealed that gender had no significant influence on the morphological variables of teeth, except for morphological variables, $b = \frac{\text{pulp/root width at midpoint level between ECJ level and mid-root level}}{\text{width (W)}}$, which showed a weakly positive correlation between gender and chronological age for mandibular lateral

![Graph 2: Scatter plot of predicted age versus chronological age by Kvaal’s and Cameriere’s methods](image)

![Graph 3: Scatter plot of predicted age versus chronological age by Kvaal’s method](image)

### Table 9: Correlation between age and morphological variables

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tooth # 1 (maxillary central incisor)</th>
<th>Tooth # 2 (maxillary lateral incisor)</th>
<th>Tooth # 3 (maxillary second premolar)</th>
<th>Tooth # 4 (mandibular lateral incisor)</th>
<th>Tooth # 5 (mandibular canine)</th>
<th>Tooth # 6 (mandibular first premolar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete pulp length/root length from ECJ to root apex ($p$)</td>
<td>Correlation coefficient</td>
<td>-0.920*</td>
<td>0.881*</td>
<td>0.903*</td>
<td>0.639*</td>
<td>0.895*</td>
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<tr>
<td></td>
<td>$P$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Complete pulp length/complete tooth length ($r$)</td>
<td>Correlation coefficient</td>
<td>-0.764*</td>
<td>0.619*</td>
<td>0.234*</td>
<td>0.919*</td>
<td>0.758*</td>
</tr>
<tr>
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<td>$P$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.014</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Complete pulp length/root width at ECJ level ($a$)</td>
<td>Correlation coefficient</td>
<td>-0.106</td>
<td>-0.150</td>
<td>0.285*</td>
<td>-0.031</td>
<td>-0.008</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.272</td>
<td>0.119</td>
<td>0.003</td>
<td>0.747</td>
<td>0.936</td>
</tr>
<tr>
<td>Pulp/root width at midpoint level between ECJ level and mid-root level ($b$)</td>
<td>Correlation coefficient</td>
<td>-0.216*</td>
<td>0.191*</td>
<td>-0.038</td>
<td>-0.084</td>
<td>-0.021</td>
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<tr>
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<td>$P$</td>
<td>0.023</td>
<td>0.045</td>
<td>0.692</td>
<td>0.385</td>
<td>0.828</td>
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<tr>
<td>Pulp/root width at mid-root level ($c$)</td>
<td>Correlation coefficient</td>
<td>-0.111</td>
<td>-0.061</td>
<td>-0.107</td>
<td>0.125</td>
<td>-0.104</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.247</td>
<td>0.526</td>
<td>0.267</td>
<td>0.193</td>
<td>0.279</td>
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<tr>
<td>Mean ($M$)</td>
<td>Correlation coefficient</td>
<td>-0.520*</td>
<td>0.812*</td>
<td>0.359*</td>
<td>0.602*</td>
<td>0.657*</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Width ($W$)</td>
<td>Correlation coefficient</td>
<td>-0.142</td>
<td>-0.153</td>
<td>-0.112</td>
<td>0.022</td>
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</tr>
<tr>
<td></td>
<td>$P$</td>
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<td>0.110</td>
<td>0.246</td>
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<tr>
<td>Length ($L$)</td>
<td>Correlation coefficient</td>
<td>-0.917*</td>
<td>0.835*</td>
<td>0.655*</td>
<td>-0.787</td>
<td>-0.341</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Difference between width and length ($W - L$)</td>
<td>Correlation coefficient</td>
<td>0.327*</td>
<td>0.776*</td>
<td>0.457*</td>
<td>0.661*</td>
<td>0.326*</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Pulp/tooth AR</td>
<td>Correlation coefficient</td>
<td>-0.348*</td>
<td>0.915*</td>
<td>0.332*</td>
<td>0.927*</td>
<td>0.912*</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Statistical analysis: Karl Pearson’s correlation coefficient, *Correlation coefficients statistically significant at $P < 0.05$. ECJ: Enamel-cementum junction, AR: Area ratio
incisor, similar to the findings of the studies conducted by Kvaal et al.,[6] and Cameriere et al.[7] Few other studies by Jeevan et al.[13] and Cameriere et al.[14,15] also, showed similar results. In the present study, width ratios \( a = \text{complete pulp length/root width at enamel-cementum junction (ECJ) level}, \)
\[ b = \text{pulp/root width at midpoint level between ECJ level and mid-root level} \]
\[ c = \text{pulp/root width at mid-root level} \]
did not show significant correlation with age. In addition, there were no significant differences between inter- and intra-observer measurements in the present study, similar to the studies, conducted by Kvaal et al.[6] Cameriere et al.,[7,14,15] Paewinsky et al.,[11] Jeevan et al.,[13] and Zaheer et al.[16] In the present study, Pearson’s correlation coefficients between chronological age and morphological variables showed that the variables \( P = \text{complete pulp length/root length (from ECJ to root apex)}, \)
\[ r = \text{complete pulp length/complete tooth length, mean (M), length (L), and pulp/tooth AR correlated significantly with age with variable} \]
\[ p = \text{complete pulp length/root width at enamel-cementum junction (ECJ) level}, \]
\[ b = \text{pulp/root width at midpoint level between ECJ level and mid-root level} \]
\[ c = \text{pulp/root width at mid-root level} \]

### CONCLUSION

In the present study, individual teeth correlated best with chronological age than all the teeth taken together. In addition, statistical analysis indicated that gender did not show any correlation with chronological age. Of all the morphological variables, variables \( P = \text{complete pulp length/root length (from ECJ to root apex)}, \)
\[ r = \text{complete pulp length/complete tooth length, mean (M), length (L), and pulp/tooth AR correlated significantly with age with variable} \]
\[ p = \text{complete pulp length/root length (from ECJ to root apex)} \]
\[ r = \text{complete pulp length/complete tooth length, mean (M), length (L), and pulp/tooth AR correlated significantly with age with variable} \]
\[ b = \text{pulp/root width at midpoint level between ECJ level and mid-root level} \]
\[ c = \text{pulp/root width at mid-root level} \]

### Limitations of the study

- Though the results of the study are promising, they cannot be generalized to other populations. Therefore, separate regression equations were formulated;
- Rotated teeth, decayed teeth or, teeth with any prosthesis were excluded from the study. If the individual has any of the mentioned conditions, then this method cannot be employed to estimate the age, as these conditions alter the tooth surface area.

### Need for future studies

- The efficacy of these methods should be further confirmed or validated using a larger sample size from population of different racial and ethnic origin;
- Future studies can be taken up to estimate the age using other single rooted teeth and multirooted teeth to determine the completeness of these methods.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.
REFERENCES

INTRODUCTION

Pemphigus, life-threatening illness affects only 1–5 patients per million populations per year. Cell junctions (desmosomes) are best visualized using either conventional or freeze-fracture electron microscopy. This is the first study in itself to document cellular morphologic details in patients of pemphigus vulgaris (PV) by scanning electron microscopy (SEM) in Indian population. This study was carried out with the aim of studying the cellular morphology and changes in the cell structures in patients of PV by SEM. Materials and Methods: The study consisted of four smears each from six patients belonging to the age group of 20–45 years comprising of two males and four females diagnosed for PV. The slides were then viewed under SEM. Results: The pathologic cell showed uneven cell boundaries with irregular arrangement of ridges forming a complex pattern; also the irregular elevations of plasma membrane formed a “plaque” like appearance on the cell boundaries. Conclusion: This study has added new observations related to the cellular changes and morphology seen in patients of PV, and numerous hypotheses are also stated to correlate with the possible etiopathogenesis of PV. In future, these observations may be useful in learning the pathogenesis of the disease even in the absence of frank lesions of the disease.

Key words: Epithelial cells, microridges, pemphigus vulgaris, scanning electron microscopy

ABSTRACT

Aim: Pemphigus, life-threatening illness affects only 1-5 patients per million populations per year. Cell junctions (desmosomes) are best visualized using either conventional or freeze‑fracture electron microscopy. This is the first study in itself to document cellular morphologic details in patients of pemphigus vulgaris (PV) by scanning electron microscopy (SEM) in Indian population. This study was carried out with the aim of studying the cellular morphology and changes in the cell structures in patients of PV by SEM. Materials and Methods: The study consisted of four smears each from six patients belonging to the age group of 20–45 years comprising of two males and four females diagnosed for PV. The slides were then viewed under SEM. Results: The pathologic cell showed uneven cell boundaries with irregular arrangement of ridges forming a complex pattern; also the irregular elevations of plasma membrane formed a “plaque” like appearance on the cell boundaries. Conclusion: This study has added new observations related to the cellular changes and morphology seen in patients of PV, and numerous hypotheses are also stated to correlate with the possible etiopathogenesis of PV. In future, these observations may be useful in learning the pathogenesis of the disease even in the absence of frank lesions of the disease.

Key words: Epithelial cells, microridges, pemphigus vulgaris, scanning electron microscopy

INTRODUCTION

Pemphigus, life-threatening illness affects only 1–5 patients per million populations per year. Cell junctions (desmosomes) are best visualized using either conventional or freeze-fracture electron microscopy, which reveals that the interacting plasma membranes (and often the underlying cytoplasm and the intervening intercellular space as well). The importance of desmosomal junctions is demonstrated by some forms of the potentially fatal skin disease like pemphigus. Affected individuals make antibodies against one of their own desmosomal cadherin proteins. These antibodies bind to and disrupt the desmosomes that hold their skin epithelial cells (keratinocytes) together. This results in a severe blistering of the skin, with leakage of body fluids into the loosened epithelium.

PV is an autoimmune skin disease characterized by intraepithelial blisters on the skin and mucous membranes.
and pathogenic antiepithelial autoantibodies which recognize
the desmosomal glycoprotein desmoglein-3 (Dsg3) (1–3).[4] The
term desmosome was later coined by Josef Schaffer in 1920 and
is derived from the Greek words “desmo,” meaning bond or
fastening, and “soma,” meaning body (Wells 2005; Calkins and
Setzer 2007). The blisters in PV are located in the suprabasilar
regions of the epidermis and are formed by a process of
keratinocyte cell-cell detachment known as acantholysis[5]
depending on the presence and distribution of Dsg3.

Extensive search of literature reveals few electron microscopic
studies carried out on the PV. This is the first study in itself
to document cellular morphologic details in patients of PV
by scanning electron microscopy (SEM) in Indian population.
Hence, the present study was carried out with the aim of
studying the cellular morphology and changes in the cell
structures in patients of PV by SEM.

MATERIALS AND METHODS

This study was conducted according to the institutional
and ethical rules concerning research. Patients diagnosed
clinicopathologically and through immunohistochemistry
for Dsg3 for PV by Department of Oral Pathology and
Microbiology, Sharad Pawar Dental College and Hospital were
considered for the study. The study consisted of four smears
each from six patients diagnosed for PV. The patients belong
to the age group of 20–45 years comprising of two males and
four females. The physiologic cell population was taken from
an apparently healthy person with no vesiculobullous disorder.
Cytologic smears were obtained from the oral, buccal lesions
of each patient by a wooden spatula. Specific glass slides of
1 cm × 1 cm were prepared, and smears were spread on these
glass slides, fixed immediately in 2% glutaraldehyde for SEM
analysis. The slides were then viewed under SEM microscope,
VNIT Nagpur at X500, X2700, and X6000 respectively after
electroplating and observations were made accordingly.

RESULTS

The smears were viewed under SEM at X500, X2700, and
X6000. A normal physiologic cell showed uniform cell
boundaries at X500. At X2700 uniform arrangement of
microridges with shallow vallies was evident. Nuclear space
was evident. Regular architecture of plasma membrane with
uniform arrangement of microridges was seen. The pathologic
cell showed uneven cell boundaries. Few vallies were also
appreciated on the cell surface. The irregular arrangement of
ridges forming a complex pattern was observed [Figure 1]. At
places, the irregular elevations of plasma membrane formed a
“plaque” like appearance on the cell boundaries [Figure 2].

DISCUSSION

Cellular evaluation of smeared oral cavity scrape samples
plays a well-known role in the diagnosis of the majority of the
oral lesions, including viral infection; because the method is
a simple and inexpensive laboratory procedure. In this study,
the pathologic cell showed uneven cell boundaries with few
vallies on the cell surface along with irregular arrangement of
ridges forming a complex pattern was observed. Our findings
are in accordance with the findings of authors like Kobayashi
et al.[6] Cellular diagnosis introduced by Kobayashi et al.[6] is
useful for the rapid demonstration of acantholytic epidermal
cells in the bullae of PV. For this purpose, a smear is taken
from the underside of the roof of the mouth and the base of
the recently opened bulla. Although few investigations have
applied exfoliative cytology to the diagnosis of pemphigus,
they have noted that the cells are mostly single, but scraping
yields a higher proportion of loosely attached cell clusters
with prominent nucleoli and hyperchromatic nuclei.[6] Atypical
acantholytic changes, which could often be misinterpreted
as malignant cells, were sufficiently distinctive to avoid an
erroneous diagnosis of a tumor.[6]

In regard to the surface topography of the oral cell
samples, Kobayashi et al. first reported on the cell surface
microfeatures in PV.[6] They suggested that cells change in
their plasma membrane due to the destruction of their
organelles and nuclei.[6] Their SEM appearance was similar
to our cell picture, with stubby ridges. The topographic
features of single acantholytic cells appeared somewhat
different from those of the loosely attached cell clusters.
There are numerous linear microridges with longitudinal
elevations of the plasma membrane. Recognition of the
acantholytic nature on SEM seem to depend primarily
on the demonstration of the cell changes in their plasma
membrane due to destruction of their organelles and
nuclei.[6] It might be due to the dissolution of desmosomes
provokes alteration, including dystrophy in microvilli.[3]
Our observations [Tables 1 and 2] are in accordance to
these findings. At places, the irregular elevations of plasma

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>X500</th>
<th>X2700</th>
<th>X6000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Single cell showing uniform cell boundaries</td>
<td>Uniform arrangement of microridges with shallow vallies</td>
<td>Regular architecture of plasma membrane uniform arrangement of microridges</td>
</tr>
<tr>
<td></td>
<td>Nuclear space is evident</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Arrangements of microridges on the cellular surface of a cell in pemphigus vulgaris at different magnifications

Table 1: Observations regarding morphology of normal physiologic cell under scanning electron microscopy
membrane forms a “plaque” like appearance on the cell boundaries [Figure 2]. This typical finding has not been reported in the previously reported cases on PV. A bundle of keratin intermediate filaments is attached to the surface of each plaque.[5] Transmembrane adhesion proteins of the cadherin family bind to the plaques and interact through their extracellular domains to hold the adjacent membranes together by a Ca²⁺-dependent mechanism.[5] However, since SEM is generally limited to the examination of surface morphology, demonstration of other diagnostic morphologic features, such as cell-to-cell adhesion changes (e.g., desmosome junction) is not possible.

Over the past 10 years, progress in molecular biologic research has evolved gradually. Pemphigus is a class of diseases in which autoantibodies target the desmosomal cadherins, predominantly Dsg1 and Dsg3.[5] Although great strides have been made in identifying the pemphigus antigens, it remains unclear exactly how pemphigus antibodies cause loss of adhesion. One likely explanation is that antibody binding to the Dsg extracellular domain simply blocks adhesion by steric hindrance.[6,7] However, this hypothesis is called into question by several observations. For example, PV IgG is unable to induce acantholysis in plakoglobin-null cells.[5,7] In addition, human keratinocytes treated with PV IgG at 4°C do not show loss of cell-cell adhesion until the cells are shifted to 37°C, suggesting that keratinocyte responses are required in order for the antibodies to cause loss of adhesion.[8] Other studies have implicated a role for signal transduction in mediating PV-induced acantholysis.[8]

Pemphigus IgG binding has been shown to cause activation of numerous cell signaling pathways. Work by the Rubenstein laboratory has illustrated that PV IgG binding induced phosphorylation of heat shock protein 27 via p38 mitogen-activating protein kinase (p38MAPK).[9-11] p38MAPK has also been shown to be involved in phosphorylation of Dsg3 in response to PV IgG.[12] Furthermore, inhibition of p38MAPK activity prevents keratin retraction, actin reorganization and formation of epidermal blisters in a mouse model.[9] Similarly, signaling through the c-Myc pathway has also been implicated in PV pathogenesis.[5] These studies suggest that either the activation of signaling pathways on autoantibody binding to the desmosomal cadherins causes loss of adhesion, or alternatively, that manipulation of intracellular signaling pathways can bolster baseline adhesion strength and prevent blistering caused by pemphigus IgG.

A number of observations have also illustrated that pemphigus IgG binding may interfere with the normal turnover of the Dsgs. Early studies found that PV IgG was

![Figure 2: The “plaque” like structure observed on the cellular surface of a cell in pemphigus vulgaris](image)

**Table 2: Observations regarding morphology of pathologic cells of pemphigus vulgaris under scanning electron microscopy**

<table>
<thead>
<tr>
<th>Type of sample (pemphigus)</th>
<th>X500</th>
<th>X2700</th>
<th>X6000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Single acantholytic cell with uneven cell boundaries Few vallies are also appreciated on the cell surface</td>
<td>Uniform stubby microridges forming a network</td>
<td>Few ridges are oriented longitudinal to the cell surface in between irregular arrangement of ridges</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Single acantholytic cell with uneven cell boundaries</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample 3</td>
<td>Single acantholytic cell with uneven cell boundaries</td>
<td>Uniform stubby microridges forming a network</td>
<td>Irregular arrangement of microridges</td>
</tr>
<tr>
<td>Sample 4</td>
<td>Single acantholytic cell with uneven cell boundaries</td>
<td>-</td>
<td>Irregular arrangement of ridges forming a complex pattern</td>
</tr>
<tr>
<td>Sample 5</td>
<td>Single acantholytic cell with uneven cell boundaries. Few vallies are also appreciated on the cell surface</td>
<td>-</td>
<td>At places, the irregular elevations of plasma membrane forms a “plaque” like appearance on the cell boundaries</td>
</tr>
<tr>
<td>Sample 6</td>
<td>Single acantholytic cell with uneven cell boundaries</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
internalized after binding to the surface of keratinocytes.[13-15] Experiments have shown that PV IgG binding results in clathrin-independent endocytosis of Dsg3 and subsequent routing of the cadherin to a lysosomal compartment for degradation.[8,16] Furthermore, blocking Dsg3 endocytosis or upregulating Dsg3 biosynthesis by exogenously expressing Dsg3 prevents keratinocytic loss of adhesion in response to PV IgG.[5,16]

Work performed by Payne and Kitajima also lends support to the impact of PV IgG on desmosomal assembly, as PV IgG causes internalization of newly synthesized pools of Dsg3.[15,17] Altogether, these findings suggest that pemphigus IgG disrupts the normal turnover and assembly of desmosomes. In fact, a growing body of evidence suggests that regulation of post-Golgi trafficking of cadherins is a key mechanism by which cell adhesion is regulated during development and disease.[18,19] New evolution with newer techniques will throw more light on the mechanism of etiopathogenesis of PV.

Few other diseases associated with loss of desmosomes or loss of Dsg4 are associated with defective hair follicle differentiation,[20] whereas Dsg1 haploinsufficiency leads to striate palmoplantar keratoderma, an epidermal thickening disease.[21] The localized impact of these mutations in Dsg1 and Dsg4 on the skin is consistent with the tissue expression patterns of these genes.[9] In contrast, mutations in Dsg2 result in arrhythmogenic right ventricular cardiomyopathy.[5,22]

This study has added new observations regarding the cellular changes and morphology seen in patients of PV. This is the first study in itself to document cellular morphologic details by SEM. Numerous hypotheses are also stated to correlate with the possible mechanism of etiopathogenesis of PV. In future, these observations may be useful in diagnosing early cases of PV even in the absence of frank lesions of this dreadful disease.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

ARTIFACTS IN ORAL BIOPSY SPECIMENS: A COMPARISON OF SCALPEL, PUNCH, AND LASER BIOPSIES

Sarita Yanduri, Garima Pandey, Veerendra B. Kumar, S. Suma, M. G. Madhura

Department of Oral and Maxillofacial Pathology, D. A. Pandu Memorial R. V. Dental College and Hospital, Bengaluru, Karnataka, India

ABSTRACT

Background: Biopsy is an important tool which aids in the diagnosis of lesions ranging from simple non-neoplastic growths to malignancies. The choice of technique depends on factors such as anatomic site and morphology of the lesion. Oral biopsies can be performed using different techniques, in which scalpel biopsy is the conventional method. Punch biopsies are also quite popular because they are safe and can be rapidly performed. However, the above techniques cannot provide hemostasis which is required on highly vascular tissues. As a result, lasers have been gaining popularity. Nevertheless, laser-tissue interactions may produce some artifactual changes, especially at the margins, such as thermal damage and coagulation which may impair the histopathological diagnosis. Aim: The aim of this study was to compare artifacts produced in scalpel, punch, and laser biopsies and to determine the most effective tool of the three in terms of oral biopsies. Materials and Methods: Thirty hematoxylin and eosin stained sections, ten each of conventional scalpel, punch, and laser biopsies, were retrieved from the archives and analyzed for artifacts under a light microscope. Results: There was no statistically significant difference between the three techniques in terms of orientation, crush, and hemorrhage. Both horizontal and vertical splits were seen in scalpel biopsies (70%) while none were present in the other two techniques. Loss of epithelium was maximum with laser biopsies, and thermal damage was also exclusively seen in this technique. Conclusion: Punch biopsy revealed the least number of artifacts. Laser, compared to scalpel and punch, produces artifacts which may render the margins of the lesions uninterpretable.

Key words: Artifacts, laser, oral biopsy, punch, scalpel

INTRODUCTION

It is an accepted fact that biopsy is the gold standard procedure for the diagnosis of lesions ranging from simple non-neoplastic growths to malignancies.[1-3] Selection of the biopsy technique depends on multiple factors such as anatomic site and morphology of the lesion. [2,4] Scalpel biopsy is the conventional method of biopsy, but more often, the patient is left with a slow healing wound or with a wound breakdown.[4,5] The punch biopsies are also quite popular in oral units because they are safe and can be rapidly performed.[4] The use of laser has gained popularity and has advantages over a scalpel in that it can instantly disinfect the surgical wound and has a hemostatic effect.[6] Irrespective of the technique of biopsy, there is always a possibility of producing artifacts as the specimens removed from the oral cavity are often small.[1] These artifacts may result in alteration of normal morphologic and cytologic features thus interfering with arriving at a diagnosis.[7] It is essential to have adequate knowledge and an understanding of artifacts so that appropriate precautionary measures can be taken to avoid or minimize their occurrence.[1,8]

Very few studies have compared the artifacts produced between scalpel and punch biopsies, and these have revealed that the punch biopsy produces the least number of artifacts.[4,5] Although artifacts can be produced in all the three techniques, studies have shown that there are few artifacts that are exclusively seen in laser biopsies.
and these are attributed to the heat produced during the procedure.\(^6\)

Since there are no studies to compare the artifacts produced by scalpel, punch, and laser biopsies, this study aimed to do the same and to determine which is the most effective tool of the three in terms of oral biopsies.

**MATERIALS AND METHODS**

A retrospective study was conducted wherein a total of thirty hematoxylin and eosin stained slides (ten scalpel biopsies, ten punch biopsies, ten laser biopsies) were randomly selected from the archives. All the biopsies had been carried out as routine procedures for the purpose of diagnosis of various lesions [Table 1]. Sections were evaluated by two oral pathologists blinded to the type of biopsy procedure used. After establishing the diagnosis, the sections were analyzed for the presence of various artifacts under a light microscope under different magnifications (×4, ×10, ×40) and scoring was done according to the histological pro forma provided. Artifacts such as orientation, loss of epithelium, curling, crush, hemorrhage, split, fragmentation, pseudocyst, and vacuolation were analyzed for their presence or absence. In addition, artifacts produced by laser biopsy such as thermal damage, inter- and intra-epithelial edema, trichocariosis, and hyperchromatic nuclei were also analyzed. To assess the thermal damage at both the epithelial and connective tissue levels, photomicrographs of the representative sites were taken using ProgRes CapturePro 2.5 (Jenoptik, Germany) and depth of the maximum thermal damage was measured using Image-Pro Express 6.0 software (Media Cybernetics Inc., USA). The results were statistically analyzed using the Chi-square test and Kolmogorov–Smirnov test. \(P < 0.05\) was considered to be statistically significant.

**RESULTS**

When all thirty samples were considered, statistically significant findings were found in relation to artifacts such as loss of epithelium \(P = 0.02\) and split \(P = 0.001\). Loss of epithelium was maximum with the laser biopsies (70%) followed by scalpel (40%) and then punch biopsy (10%) [Figure 1]. Both horizontal and vertical splits were seen in scalpel biopsies (70%) while none were present in the other two techniques [Figure 2].

No statistically significant differences in terms of orientation, crush, hemorrhage, pseudocyst formation, and vacuolation were identified [Figure 3]. Curling was maximum (50%) in punch biopsies and fragmentation was observed in a majority of all sections (scalpel biopsy – 100%, punch biopsy – 80%, and laser – 70%) [Figure 4]. However, these results were not statistically significant. The artifacts encountered in the histopathological examination are listed in detail in Table 2.

Few artifacts were found to be exclusively seen in only laser biopsies. Thermal damage was evident in the connective tissue of all cases and ranged from 65.59 to 374.62 µm with a mean value of 153.02 µm [Figure 5]. However, it could be assessed in the epithelium in only one case (41.05 µm). Other artifacts such as trichocariosis were found in all sections of laser biopsies; intercellular edema (10%), intracellular edema (3%), and hyperchromatic nuclei (60%) were also observed [Figure 6 and Table 3].

**DISCUSSION**

An appropriate biopsy needs to be representative of the lesion. To achieve this, three major factors, namely, selection of the biopsy site, the type of biopsy, and finally adequate submission of the specimen to the laboratory will determine the quality of the biopsy specimen.\(^9\,10\) High vascularity and limited access to the oral cavity do not allow adequate biopsies. Thus, the possibility of producing artifacts is enhanced in such cases.\(^9\,10\,11\)

Oral biopsies by virtue of often being small may be subjected to tissue distortion by even the most minimal
compression. This may lead to the production of squeeze artifacts such as crush, hemorrhage, splits, fragmentation, and pseudocysts.[11,12]

In the present study, split artifacts were present only in scalpel biopsy. Horizontal splits were found in 40% of cases, and vertical splits were found in 30% of sections of scalpel biopsy (P = 0.001). Split artifacts have often been attributed to multiple cuts caused by improper use of the blade. Other reasons could be due to excessive force used during traction by sutures.[5,11] As suture traction was not used in any of the present cases, this could explain the absence of this artifact in punch and laser biopsies.

Areas of fragmentation were found to be maximum in scalpel biopsies (100%), followed by punch (80%) and laser biopsies (70%). However, results were not statistically significant. Fragmentation might be due to excessive stretching of tissue during a biopsy procedure or may be attributed to the use of scissors at the base of tissue for releasing the core of the tissue.[1,5,7]

Sometimes, due to the inappropriate utilization of a toothed forceps, the surface epithelium may be forced through the connective tissue and form a small pseudocyst. However, the total absence of pseudocysts in all cases of the present study can likewise be explained due to the use of blunt forceps during handling.[1,13] Use of a blunt forceps instead of a toothed forceps and grasping the tissue away from the main site of interest may help avoid most of the compression zones and perforation.[2]

Poor orientation was found in only one case, and none of the cases showed vacuolation. Some authors have suggested that by placing the epithelial surface down on a piece of card (usually held with suture) before immersion in fixative reduces the chance of poor orientation.[4,5,13] Epithelial vacuolation may be generated by intralesional injection of anesthetic solution. Therefore, the anesthetic solution should be injected 3–4 mm away from the lesional tissue at four regions, i.e., top, bottom, left, and right of the lesion.[7,12,13]
In the present study, it was noticed that the least number of artifacts were associated with punch biopsies. This is similar to the findings of Moule et al. and Meghna et al., who compared artifacts in specimens of punch and scalpel biopsy.\textsuperscript{[4,5]} It was also seen that loss of epithelium was found in only 10% of cases of punch biopsy while split artifact was completely absent. The minimal number of artifacts seen in this technique along with the fact that it is a simple and quick procedure, gives high-quality samples, causes minimal discomfort to patients without remarkable esthetic sequelae making it a popular choice in dental practice. However, it is well known that punch biopsy has its own limitations such as limited area of application, especially in the region of the soft palate, maxillary tuberosity, or floor of the mouth, due to the lack of firm tissue fixation or support. It also cannot be used for nodules or growths and is not applicable for deep lesions.\textsuperscript{[4,5]}

Scalpel biopsy, on the other hand, is a gold standard procedure and can be used in all the above conditions where a punch biopsy is not applicable. It also has further advantages of ease to use, accuracy, and minimal damage to the surrounding tissue. However, scalpels cannot provide the hemostasis that is helpful for use on the highly vascular tissue.\textsuperscript{[6]}

Hemostasis is best provided by laser biopsy which has several other advantages such as minimal postoperative swelling, scarring, improved wound healing, and less postoperative pain.\textsuperscript{[6,14]} It has a sealing effect on vessels smaller than 500 \( \mu \text{m} \) in diameter so prevents occult

### Table 2: List of artifacts encountered on histopathological examination

<table>
<thead>
<tr>
<th>Artifacts</th>
<th>Scalpel (n=10), n (%)</th>
<th>Punch (n=10), n (%)</th>
<th>Laser (n=10), n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orientation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>9 (90)</td>
<td>9 (90)</td>
<td>10 (100)</td>
<td>0.585</td>
</tr>
<tr>
<td>Poor</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Epithelium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preserved</td>
<td>6 (60)</td>
<td>9 (90)</td>
<td>3 (30)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Partial or complete loss</td>
<td>4 (40)</td>
<td>1 (10)</td>
<td>7 (70)</td>
<td></td>
</tr>
<tr>
<td><strong>Curling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>9 (90)</td>
<td>5 (50)</td>
<td>7 (70)</td>
<td>0.179</td>
</tr>
<tr>
<td>Complete specimen</td>
<td>0</td>
<td>2 (20)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Localized</td>
<td>1 (10)</td>
<td>3 (30)</td>
<td>3 (30)</td>
<td></td>
</tr>
<tr>
<td><strong>Crush</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>9 (90)</td>
<td>7 (70)</td>
<td>7 (70)</td>
<td>0.214</td>
</tr>
<tr>
<td>Base</td>
<td>0</td>
<td>0</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>Specimen</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>0</td>
<td>2 (20)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Hemorrhage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>8 (80)</td>
<td>8 (80)</td>
<td>6 (60)</td>
<td>0.498</td>
</tr>
<tr>
<td>Base</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Specimen</td>
<td>0</td>
<td>1 (10)</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>1 (10)</td>
<td>0</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td><strong>Split</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3 (30)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Horizontal</td>
<td>4 (40)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td>3 (30)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Fragmentation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>2 (20)</td>
<td>3 (30)</td>
<td>0.408</td>
</tr>
<tr>
<td>Superficial</td>
<td>2 (20)</td>
<td>3 (30)</td>
<td>4 (40)</td>
<td></td>
</tr>
<tr>
<td>Deep</td>
<td>4 (40)</td>
<td>3 (30)</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>4 (40)</td>
<td>2 (20)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td><strong>Pseudocyst</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Vacuolation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

\*Statistically significant (P <0.05)
micrometastasis. Laser has been recommended to treat benign oral lesions, such as fibromas, papillomas, gingival hyperplasia, hemangiomas, aphthous ulcers, mucosal frenula or ankyloglossia, and even premalignant lesions such as oral leukoplakias. Factors that determine the initial tissue effect include the laser wavelength, laser power, the available laser waveform (continuous wave, chopped, and pulsed beams), and tissue thermal properties.

One major disadvantage of the use of laser is with respect to the damage which occurs at the margins of the lesion. The damage is thermal in nature and occurs due to tissue protein coagulation, which microscopically appears as a broad band of basophilic coagulum giving an amorphous appearance to the epithelium and connective tissue. Epithelial cells, as seen in the present study, may also appear detached, fusiform, hyperchromatic, and undergo vacuolar degeneration, making it useless for diagnosis, especially if the specimen is small.

Assessment of margins for the presence or absence of epithelial dysplasia and invasion is extremely important in the histopathological evaluation of premalignant and malignant lesions. In laser biopsies, the thermal damage to the epithelium may make the margins uninterpretable. In addition, the marginal artifacts such as crush, trichocariosis, and hyperchromatic nuclei may actually simulate dysplasia and may lead to a misdiagnosis. It has also been noticed that the heat emitted from the laser may cause a separation and subsequent loss of epithelium, thus further affecting margin interpretation. In the present study, 70% of cases showed loss of epithelium.

Thermal damage was noticed in the lamina propria as well, but the depth (mean = 153.02 µm) of thermal damage was far lesser and within the tolerable limits of the margin clearance allowed for oral squamous cell carcinoma specimens. Thus, we agreed with the findings of Makki et al. that laser biopsies preserve the ability to interpret invasive malignancy but make an assessment of the presence or absence of dysplasia difficult.

Suter et al. recommended that the use of a CO2 laser statistically reduced the amount of thermal damage as compared to a diode laser and thus stated that the CO2 laser is used for potentially malignant and malignant disorders. Further, the use of low power settings of the laser may help to a certain extent reduce the possibility of separation of the epithelium and its subsequent loss during tissue processing.

**CONCLUSION**

The findings of the present study highlight that the choice of the instrument for biopsy depends not only on the ease with which the biopsy can be performed but also on the number of artifacts that may be produced as a result of the instrument usage. To the best of our knowledge, no study to date has compared artifacts produced by scalpel, punch, and laser in oral biopsies.

The study shows that all the three techniques can produce artifacts which can be greatly reduced by proper handling of the tissue during the biopsy procedure. Punch biopsy reveals the least number of artifacts but it has its own limitations in usage. Laser provides the best hemostasis compared to scalpel and punch but produces artifacts which may render the margins of the lesions uninterpretable. Thus, we suggest that scalpel or punch biopsies be used for incisional biopsies and relatively large specimens where sufficient margins can be obtained.

We also recommend that more studies with larger sample size be carried out to confirm these findings. Additional parameters such as determining the artifacts produced by...
different types of lasers and addition of immunohistochemical markers may help further enhance our knowledge on this topic.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

Autologous Platelet Concentrate as a Potential Regenerative Biomaterial in the Treatment of Endo-perio Lesion

Pratibha Shashikumar, Swet Nisha

Department of Periodontology and Oral Implantology, JSS Dental College and Hospital, JSS University, Mysore, Karnataka, India

ABSTRACT
Endo-perio lesions are common clinical finding in the day-to-day dentistry. The treatment approach with respect to periodontal regeneration still remains point at issue. Endodontic treatment not always leads to complete healing of periapical and periodontal tissue. In literature, surgical periodontal intervention along with the use of bone grafts, guided tissue regeneration has been attempted for regeneration. Recently, use of platelet concentrates such as platelet-rich fibrin (PRF) releasing growth factors is extensively used in dentistry for periodontal regeneration. This case report aims at evaluating the efficacy of PRF as regenerative biomaterial in the treatment of Endo-perio lesion.

Key words: Endo-perio lesions, growth factors, periodontal regeneration, platelet-rich fibrin

INTRODUCTION
Dental pulp and the periodontium are related in health as well as in disease process. Although both are ectomesenchymal in origin, the pulp originates from the dental papilla and the periodontal ligament from the dental follicle. As the tooth matures, a communication between the pulp and periodontium exists via dentinal tubules, lateral and accessory canals, and apical foramen. In disease state, it can communicate through deep periodontal pockets, developmental grooves, and dentinal tubules. This connections can serve as portal of communication in disease state.

The physiological state transforms into pathology in case any infectious source invades the pulpal or periodontal territory. As with any inflammatory process, the host immune – infectious agent interaction begins and inflammation sets in. The pulpal chamber is closed, and therefore, the spread of infection occurs only through the portal of communications and continues beyond the pulp into the periodontium and results in endo-perio lesions.

Etiologic factors, such as microorganisms, and other contributing factors, such as trauma, root resorption, perforation, and dental malformations, play an important role in the development and progression of endodontic-periodontal lesions.

CASE REPORT
A 52-year-old female reported to the Department of Periodontology and Oral Implantology with a chief complaint of recurrent pain and pus discharge in lower front tooth. The pain was moderate in nature, localized, and aggravated on chewing food. The patient was systemically healthy with no relevant medical history. The patient presented with the previous history of trauma to the tooth 18 years back following which patient had noticed mobility of tooth.

On clinical examination, periodontal abscess with sinus track opening with respect to mandibular left central incisors was noted [Figure 1]. No extraoral swelling was seen. Generalized
debris and calculus were present. Pocket depth of 8 mm was seen on distolabial aspect of tooth, and Grade II mobility was noted. The tooth was tender on palpation and percussion, and draining sinus was present on the labial aspect of the tooth.

On evaluation of the intraoral periapical radiograph of the mandibular left central incisor, both crestal and periapical bone loss were evident [Figure 2]. Electric pulp testing elicited a negative response. Therefore, considering the dental history, clinical tests and radiographs, the diagnosis of this case was “true combined endo-perio lesion.”[1]

The initial phase of treatment comprised drainage of abscess. Scaling and root planning were completed, and the tooth was splinted with fiber mesh reinforced composite. Root canal therapy was done, and the patient was followed up for 3 months. At the end of 3 months, satisfactory obturation was noted, but periodontal pocket depth of 8 mm still persisted, and periodontal surgery was planned.

**Surgical procedure**

The surgical area was anesthetized using lignocaine with adrenaline 1:200,000. A mucoperiosteal flap was raised in relation to 31, 32, 33, 41, and 42. After reflection, open flap debridement was done at the defect area. Dehiscence extending up to apical third of the root was noted with periapical defect on labial and lingual aspect of 31 [Figure 3]. The defect area was covered with platelet-rich fibrin (PRF) coagulam.

**Platelet-rich fibrin preparation**

PRF was produced by taking 10 ml of patient’s whole blood drawn intravenously. The tube was centrifuged immediately at 3000 revolutions per minute for 10 min. After centrifugation, the PRF coagulam was removed from the tube using sterile tweezers, separated from the red blood cell base using scissors, and placed in the defect area [Figure 4].

The primary soft tissue closure of the flap was done with nonresorbable black silk (3–0) suture using continuous sling suture. Periodontal dressing was given.

**Postoperative instructions**

The patient was instructed rinsing with 0.12% chlorhexidine mouthwash twice daily and proper oral hygiene maintenance. Medication prescribed were, capsule amoxicillin ~500 mg thrice daily for 5 days, and tablet aceclofenac 100 mg, paracetamol 650 mg twice daily for 3 days. The sutures were removed 10 days after surgery, and the patient was advised to brush at the surgical site using a postsurgical brush for 2 weeks. The patient was put on regular recall at 1, 3, 6 and 9, 12 months. After 3 months of periodontal flap surgery, the temporary splint was removed, and mobility was assessed. The mobility was reduced to Grade I. After 12 months; the probing depth was found to be reduced by 4 mm, and the sinus tract had healed completely [Figure 5]. The postoperative radiograph showed a substantial bone fill in the crestal and periapical area [Figure 6].

**DISCUSSION**

A definite interrelation exists between the presence of periodontal lesions and the pulpal tissue status. The pulpal periodontal syndrome as described by Seltzer...
et al.[2] still continues to be controversial issue in relation to its definition, classification, etiology, or sequence of treatment planning. The main goal of endodontic or periodontal therapy is the eradication of infectious source from the root canal and periodontium, respectively. The role of an endodontic infection as a local modifying risk factor of periodontal disease has been studied in retrospective clinical studies on periodontitis-prone patients. Single-rooted teeth with an endodontic infection evident as a periapical radiolucency are significantly correlated to deeper periodontal pockets.[3]

Healing phase depends not only on the removal of infective agents but also host immune response. Addition of regenerative material such as PRF may enhance the healing phase and act as a scaffold to hold the blood clot in initial stages and later in bone formation.

When a tooth is having combined endo-perio lesion, the initial phase of treatment would be periodontal Phase I therapy – scaling and root planning, to reduce the microbial load. Endodontic therapy should precede any further periodontal treatment. When the toxic material from the root canal is removed, reattachment of the soft tissue after periodontal surgery is improved.[4] If the pulpal content is removed before periodontal surgery, the problem of sensitivity sometimes observed after periodontal treatment can be avoided.[4] The same treatment protocol was followed in this case with additional regenerative approach; that is use of PRF.

Various materials have been developed as an aid in regenerative therapy still autologous origin remains the gold standard. There is reduced risk of cross-contamination; it is cost-effective, and success rate is high when autologous material is used as regenerative material as compared to others alloplastic, synthetic materials. In quest of autologous materials, Choukren et al. developed PRF in 2000 at France.

PRF belongs to a new generation of platelet concentrates, containing all constituents of a blood sample favorable to healing and immunity. The slow polymerization mode confers to the PRF membrane a particularly favorable physiologic architecture to support the healing process.[5]

It is of high interest to note that the PRF matrix enmeshes glycosaminoglycans (heparin, hyaluronic acid) from blood and platelets which helps in cell migration in healing process. Platelet cytokines play a fundamental role in initial healing mechanisms owing to their capacity to stimulate cell migration and proliferation and induce fibrin matrix remodeling as well as secretion of cicatricial collagen matrix.[6] These properties of PRF make it a novel biomaterial for periodontal regenerative therapy.

Several studies have combined the regenerative potential of PRF and different bone grafts in the treatment of endo-perio lesions. A case report by Yu-Chao et al. on clinical application of platelet-rich fibrin as the sole grafting material in periodontal intrabony defects showed to be an effective modality of regenerative treatment for periodontal intrabony defects.[7] In the present case report, only PRF without any bone graft was used, and substantial bone fill was seen after 12 months.
The successful outcome of surgical periodontal therapy aims at regeneration of lost periodontal tissues. Alloplastic materials act as osteoconductive material and in developing countries like India cost is a major issue while providing treatment to the patients in day-to-day basis. Use of PRF eliminates the cost issue and provides affordable treatment and is an autologous biomaterial having favorable regenerative potential. Hence, it can be successfully used in the treatment of periodontal defects associated with endo-perio lesions.

CONCLUSION

Sequential planning is critical to successful treatment of endo-perio lesions PRF can be considered as cost-effective, promising regenerative biomaterial in the treatment of endo-perio lesions.

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Conflicts of interest
There are no conflicts of interest.

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Asymmetric Extractions for an Asymmetric Malocclusion

Christopher Lawrence Tan Soon Lee, Kirti Saxena

Department of Orthodontics, Klinik Pergigian Jalan Gambut, Kuantan, Pahang, Malaysia

ABSTRACT

Some patients with Class II subdivision malocclusions have Class I characteristics on one side and Class II characteristics on the other. The resulting asymmetric occlusal relationship complicates orthodontic treatment. Traditionally, four premolars are extracted in such cases to achieve a bilateral Class I molar relation which needs patient compliance with the use of Class II elastics. The present case report shows one such Class II subdivision malocclusion case which was treated by asymmetric extractions. This approach is independent of the patient compliance and saved one premolar, with no compromise in dental occlusion or esthetics.

Key words: Class II subdivision malocclusion, extraction of three premolars, midline shift

INTRODUCTION

The studies show that Class II subdivision malocclusion is primarily caused by distal positioning of the mandibular first molar in relation to the maxillary first molar, on the Class II side. Secondly, it can be consequent to mesial positioning of the maxillary first molar, in relation to the mandibular first molar, on the Class II side. As a result, most Class II subdivision malocclusion patients present with mandibular dental midline displaced toward the Class II side associated with the maxillary dental midline coincident to the midsagittal plane or with a mild deviation, which requires asymmetric orthodontic approaches.

The present case report shows one such Class II subdivision malocclusion case which was treated by asymmetric extractions. First premolars were extracted in the upper arch and the first premolar on the Class I side was extracted in the lower arch. This approach is independent of patient compliance and saved one premolar along with good posttreatment occlusion and a slight improvement of the profile.

CASE REPORT

A 16-year-old Malay boy complained of corner teeth sticking out in the upper arch. Extra oral examination showed a mildly convex profile with posterior divergence, competent lips, an average nasolabial angle, and lower facial height with no gross asymmetry. Intraoral examination showed all 28 teeth present with moderate crowding in both arches and a lower midline shift to the left of midfacial axis with acceptable overjet and overbite. The molar and canine relation on the right side were in Class I occlusion, whereas on the left side were in Class II occlusion with Class II division 2 incisor relation [Figure 1a].

Cephalometric examination showed a mild Class II skeletal relation, proclined lower incisors with average growth pattern [Table 1]. All third molars were visible on orthopantomogram [Figure 1b] with satisfactory oral hygiene.

Treatment objectives were

- To align arches
- To establish balanced occlusal relationship bilaterally
- To maintain correct overjet and overbite
- To improve the profile
- To constitute a good esthetic smile with correct centerlines.
Treatment options/alternatives

- Lower molar protraction (Class II side) with alignment along with all four first premolar extractions
- Lower molar protraction (Class II side) with alignment along with first premolar extractions in the upper arch, the first premolar extraction on Class I side and second premolar extraction on Class II side in lower arch
- Extraction of two premolars in the upper arch to correct crowding and one premolar in the lower arch on Class I side to correct midline and crowding.

The patient chose the third option as only three premolars were being extracted and was willing to accept a Class II molar relation on the left side.

Table 1: Cephalometric findings

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Cephalometric norms for Malays[^10]</th>
<th>Cephalometric values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNA angle (°)</td>
<td>84±3</td>
<td>85</td>
</tr>
<tr>
<td>SNB angle (°)</td>
<td>81±4</td>
<td>80</td>
</tr>
<tr>
<td>ANB angle (°)</td>
<td>3±2</td>
<td>5</td>
</tr>
<tr>
<td>Upper incisor to maxillary plane angle (°)</td>
<td>114±6</td>
<td>108</td>
</tr>
<tr>
<td>Lower incisor to mandibular plane angle (°)</td>
<td>97±6</td>
<td>92</td>
</tr>
<tr>
<td>Maxillo-mandibular angle (°)</td>
<td>26±5</td>
<td>28</td>
</tr>
<tr>
<td>Facial proportion (%)</td>
<td>55</td>
<td>56</td>
</tr>
<tr>
<td>Interincisal angle (°)</td>
<td>124±8</td>
<td>133</td>
</tr>
</tbody>
</table>

Figure 1: (a) Pretreatment extraoral and intraoral snaps. (b) Pretreatment orthopantomogram and cephalogram

Treatment progress

Anchorage reinforcement was needed to prevent mesial molar movement in the upper arch; this was achieved by cementing a Nance button [Figure 2] as the treatment involved the extraction of the first premolars in the upper arch to correct the crowding. Treatment started with the placement of 0.022˝ Roth prescription brackets and molar tubes. Leveling and alignment were done using 0.012˝ Ni-Ti and 0.017˝ × 0.025˝ Ni-Ti wires. The patient was monitored and recalled every 6 weeks to check progress. Finally, 0.017˝ × 0.025˝ stainless steel wire in the upper arch and 0.019˝ × 0.025˝ stainless steel archwires were placed in the lower arch.

The upper and lower anteriors were then consolidated together, and en-masse retraction was performed using active tie-backs [Figure 2]. Final space closure was done using power-chain. Once the occlusion settled, the appliance was debonded. Retention was achieved with Modified Hawley retainers in both arches.

RESULTS

The time taken for leveling and aligning was 7 months and 11 months for space closure and settling. On the right side, the Class I molar relation was maintained, whereas on the left side, the Class II molar relation showed better intercuspation. At the end of treatment, a proper overjet and overbite were maintained with coincident midlines and a slight improvement of the profile was seen [Figure 3].

DISCUSSION

Patients with Class II subdivision malocclusions have Class I characteristics on one side and Class II characteristics on the other. The resulting asymmetric occlusal relationship complicates orthodontic treatment. In most patients with Class II subdivision malocclusion, the maxillary dental midline is coincident to the midsagittal plane or has a minimal deviation whereas the mandibular dental midline is usually displaced toward the Class II side.[^1,^2,^9] In this case, the decision of upper first premolar extraction was made to relieve the crowding and align the canines into the arch which addressed the patient’s chief complaint. In the lower arch, there was an option of extracting two premolars or a single premolar. If two premolars been extracted:

- Correction of center lines would need use of anterior cross elastics

Figure 2: Mid-treatment snaps showing a Nance button and active tiebacks
CONCLUSION

Comparatively lesser compliance needed from the asymmetric extraction approach seems to be superior to the alternative requirement of Class II elastics. In comparison to the extraction of four premolars, extracting three premolars are more acceptable to the patients. In the present case, we achieved the goals of profile improvement and clinically as well as functionally acceptable occlusion. Finally, a proper case selection and treatment plan are must to achieve optimum treatment results.

Declararion 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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Esthetic Management of a Recurrent Gingival Fibroma

Sue Ann Loe, Vivek Vijay Gupta, Srinivas Sulugodu Ramachandra
Faculty of Dentistry, SEGi University, Selangor, Malaysia

ABSTRACT
Peripheral reactive lesions of gingiva are common lesions of the oral cavity that dentists come across in daily life. Among these are focal fibrous hyperplasia, pyogenic granuloma, peripheral giant cell granuloma, and peripheral ossifying fibroma. Localized irritation fibroma is a gingival growth, usually arising from interdental papilla due to the presence of some chronic irritation due to dental calculus or other iatrogenic factors. This article reports a case of a 39-year-old male with a recurrent irritation fibroma in the maxillary anterior gingiva treated by excision. The presence of growth in the esthetic maxillary anterior segment and the possibility of unesthetic defect due to excision of the growth have been discussed. The importance of treating the chronic infectious irritational factor and regular follow-up to prevent long-term recurrence of the growth has been discussed.

Key words: Chronic periodontitis, esthetics, excision, irritation fibroma, maintenance therapy

INTRODUCTION
Irritation fibroma is the most common tumor-like and submucosal reactive lesion in the oral cavity.[1] The presence of low-grade irritation or injury from mastication, food lodgement, presence of calculus, and other iatrogenic factors such as broken tooth and overhanging restorations are usually the etiologic factors for the development for these irritation fibromas. Chronic mechanical irritation can be seen very commonly due to the presence of dental calculus in moderate to deep periodontal pockets, which cannot be maintained by the patient using normal oral hygiene aids.[1] Hence, among the treatment options in such cases are the pocket reduction therapy and nonsurgical periodontal maintenance therapy. Inability to remove the dental calculus causing chronic mechanical irritation completely or accumulation of such local factors again can lead to the recurrence of irritation fibromas. Surgical excision of irritation fibroma is required which can be more challenging in anterior region due to esthetic concerns.[2]

As slight excess of gingival tissue is required to be excised to prevent the recurrence of the lesion, it might lead to gingival recession or a gingival defect which could be unacceptable in the anterior region or might require an additional root coverage procedures.[2]

This article reports esthetic management of a case with recurrent gingival fibroma in the maxillary anterior region.

CASE REPORT
A 39-year-old male patient reported to the Faculty of Dentistry, SEGi University, for cleaning his teeth. Clinical examination revealed abundance of plaque and calculus deposits, especially in the mandibular anterior region. Intraoral examination also revealed a localized gingival growth present around right maxillary central and lateral incisors [Figure 1]. Gingival growth was sessile, well-defined, firm in consistency and nontender on palpation with a size of 1 cm × 1 cm. The gingival overgrowth was pale pink. The patient provided with a history of similar growth in the same region and was surgically excised by a local dentist. The growth had recurred over the past couple of years. The patient was medically fit as there was no history of any systemic disease. A basic periodontal examination (BPE) was carried out, and BPE score of 3 was obtained for all the

Corresponding Author: Dr. Srinivas Sulugodu Ramachandra, Faculty of Dentistry, SEGi University, No. 9, Jalan Teknologi, Taman Sains, Kota Damansara, Selangor, Malaysia. E-mail: periosrinivas@gmail.com

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maxillary and mandibular sextants. Detailed periodontal charting revealed periodontal pocket of 6 mm around the right maxillary central incisor. An orthopantomogram (OPG) and intraoral periapical (IOPA) radiography in relation to maxillary anteriors were advised. OPG revealed generalized interproximal bone loss. IOPA showed interproximal bone loss up to coronal one-third of the root [Figure 2]. Based on the clinical features and history, a provisional diagnosis of fibroma, peripheral ossifying fibroma, was made. A treatment plan involving scaling and polishing, root debridement in teeth with residual periodontal pockets, and excision of the gingival growth was formulated. The patient was explained about the growth and the proposed treatment plan. The patient was concerned about the esthetics in the upper front region due to possible loss of gingiva during excision. An informed consent was obtained from the patient.

Nonsurgical periodontal therapy in the form of scaling and root debridement was initiated. Thorough removal of all the supra- and sub-gingival calculus was carried out. The patient was recalled after 10 days for review and excision of the growth [Figure 3 inset]. Excision of the gingival growth was done under local anesthesia using B.P blade no 15 [Figure 3]. Care was excised not to sacrifice unnecessary portions of the attached gingiva which would result in an unesthetic defect in the anterior gingiva. However, a small uninvolved portion of the gingiva around the growth was removed so that chances of recurrence are decreased. Hemostasis was achieved using pressure packs. Small specks of subgingival calculus were noticed beneath the gingival growth. Remnants of subgingival calculus were removed using curettes. Periodontal dressing was placed to cover the surgical area. Postoperative instructions were given, and the patient was advised to use oral analgesics if needed. The excised tissue was sent for histopathological examination.

Histological examination revealed that the growth contained abundance of acellular, dense, collagenous fibrous tissue covered with stratified squamous epithelium was noticed [Figure 4]. The collagen bundles were arranged in radiating haphazard pattern, and the overlying epithelium appeared...

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**Figure 1:** Clinical image showing localized gingival growth around right maxillary central and lateral incisors. Abundance of local deposits can be appreciated, especially in the mandibular anterior region. Inset shows a sessile growth attached to the attached gingiva and extending up to coronal one-third of maxillary right central incisor

**Figure 2:** Orthopantomogram showing generalized interproximal bone loss. Inset shows intraoral periapical radiograph with crestal interproximal bone loss

**Figure 3:** Clinical image after excision of the growth. Calculus present beneath the growth can be appreciated. Inset shows the growth during the follow-up after completion of Phase I therapy

**Figure 4:** Histomicrograph shows abundant acellular, collagenous fibrous tissue with stratified squamous hyperplastic epithelium (×10). Inset showing scattered chronic inflammatory cells with no giant cells or ossifying tissues (×40)
Loe, et al.: Recurrent gingival fibroma


Hyperplastic. Scattered chronic inflammatory cell infiltrates are seen in the subepithelium [Figure 4 inset]. No giant cells or ossifying tissues were found even on a higher magnification. The patient was examined 2 weeks later with the area around 11 showing signs of normal healing and minimal gingival recession. Three months later, area showed uneventful healing [Figure 5]. The patient was put on periodontal maintenance therapy to prevent the recurrence of the lesion. At 6 months follow-up, there has been no recurrence of the growth [Figure 6].

DISCUSSION

Fibromas are reactive hyperplasia of fibrous connective tissue in response to local irritation or trauma.[3] They are most commonly seen in the fourth to sixth decades of life, with a male: female ratio of approximately 1:2. Buccal mucosa along the occlusal line, labial mucosa, tongue, and gingiva are the most common intraoral sites.[3] Oral hygiene of the oral cavity in this case was poor with abundance of plaque and calculus. However, the growth was localized in the maxillary anterior region as a deep periodontal pocket of 6 mm was noticed in the maxillary right central incisor. The presence of chronic infection (subgingival plaque and calculus in the periodontal pocket) as an irritating factor could be the reason for localization of the gingival growth in the right maxillary anterior region. The gingival growth in this case was a recurrent growth. The growth had been excised around 5 years back by a local dentist. Incomplete excision of the growth and incomplete removal of the chronic irritating factors could be the reasons for recurrence of the growth. Hence, complete excision of the growth with removal of the chronic irritating factors was carried out. Thorough scaling and root debridement, followed by removal of the residual calculus after excision of the growth, ensured removal of all chronic irritational factors. This highlights the importance of removal of all the chronic infections in such cases. Vitality testing was also performed to ensure that possible chronic endodontic infection is to be ruled out as a cause for recurrence of the growth.

Gingival growths can be sessile or pedunculated. Sessile growths are attached to the underlying tissue with a wide base whereas the pedunculated lesion is attached to the underlying tissue by a stalk or pedicle. The technique/method of excision is dependent on whether the growth is sessile or pedunculated. Pedunculated growths are excised at the base of the stalk/pedicle whereas the sessile growths are excised from the base with some amount of normal tissue. Both sessile and pedunculated lesions flatten out due to pressure from the cheeks, tongue, and lips. The growth in this case was sessile and was attached to the gingiva in the maxillary anterior region. Excision of gingival growths with their base attached to the gingiva has the risk of creating unesthetic defect in the gingiva. This defect could result in a niche for future plaque accumulation or could simply be unesthetic.[2,4,5] Conservative and careful excisions of the lesion will save avoid the unesthetic defects, but carries the risk of chances of recurrence.[2,4,5]

Histologically, dense mass of fibrous connective tissue covered by was noticed epithelium. Atrophy of the rete ridges because of the underlying fibrous mass is seen.[3] As various types of reactive lesions of gingiva can present a similar clinical picture, only the histological examination showing specific cells such as giant cells or some mineralized tissue can help us reach the final diagnosis.[4] In the present case of localized reactive gingival overgrowth, collagen bundles were arranged in radiating haphazard pattern and the overlying epithelium appears hyperplastic. Scattered chronic inflammatory cell infiltrates are seen in the subepithelium. However, no giant cells or ossifying tissues were found even on a higher magnification.

Pyogenic granuloma occurs most frequently on the gingiva and that has a strong tendency to recur after simple excision if the associated irritant is not removed. Sometimes, it might be difficult to identify the causative agent; however, lesion’s proximity to the gingiva suggests local irritating factors playing an important role in their occurrence.[7] Long-standing pyogenic granulomas can convert into fibromas showing a
reactive fibrogenesis to wall off the chronic long-standing inflammation.[8] In this case, it is possible that the lesion would have initially started as pyogenic granuloma and later converted to fibroma. Following thorough scaling and root planing with proper oral hygiene instructions, the patient has maintained good oral hygiene, and there was no evidence of recurrence after 6 months.

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There are no conflicts of interest.

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Minimally Invasive Treatment of White Spot Lesions

Kanika Yadav, Ida de Noronha de Ataide, Marina Fernandes, Rajan Lambor
Department of Conservative Dentistry and Endodontics, Goa Dental College and Hospital, Bambolim, Goa, India

ABSTRACT
This case report elaborates the management of white spot lesion (WSL) using air microabrasion as minimally invasive treatment modality. Air microabrasion can remove the outermost layer of enamel to manage superficial discolorations and incipient carious lesions. A WSL on the maxillary left incisor was treated conservatively with air microabrasion technique. This technique might be an alternative to chemical microabrasion, macroabrasion, and invasive restorative treatments for the management of WSLs.

Key words: Air microabrasion, demineralization, white spot

INTRODUCTION
White spot lesions (WSLs) are incipient carious lesions characterized by subsurface demineralization with an intact enamel surface. It results due to dissolution of hydroxyapatite from the enamel prisms by acidogenic microflora.[1] Predisposing factors are inadequate oral hygiene, plaque accumulation, and frequent ingestion of fermentable carbohydrates. White spots may also be seen after removal of orthodontic bands and brackets.[2] Incipient carious lesions may vary in clinical appearance ranging from opaque white to yellow brown discolorations. Opaque white appearance of such lesions is due to the internal porosities created by the loss of mineral which alters the refractive index of enamel.[3]

CASE REPORT
A 19-year-old female patient reported to the Department of Conservative Dentistry and Endodontics with a chief complaint of localized discoloration of the upper left central incisor. The patient’s medical and dental history was noncontributory. On intraoral examination, a WSL on incisal third of the maxillary left central incisor was evident. Lesion was opaque, chalky, matte in appearance, had yellow brown discoloration at the center, and was rough on probing. Incisal edge of the tooth was chipped off due to demineralized defect [Figure 1]. Tooth responded normally to electric pulp test and cold test. Lesion was diagnosed as incipient carious lesion (WSL) after clinical and radiographic examination. After discussing different treatment modalities with the patient, minimally invasive treatment was adopted. Treatment plan outlined was conservative tooth preparation using air microabrasion to remove the WSL.

The tooth #21 was cleaned with a rubber cup and prophylaxis paste. Following this, tooth was isolated with rubber dam application. Impression putty was used to further isolate the lesion to prevent the abrasion of adjacent sound tooth structure [Figure 2]. Air abrasion unit (Microetcher II Ea Danville Materials, CA, USA) was assembled by connecting the abrasive reservoir containing aluminum oxide particles to the handpiece and tubing to the airline of the dental unit [Figure 3]. The tip of the air abrasion handpiece was positioned at a distance of 2 mm from the lesion, and a stream of aluminum oxide particles was directed towards the lesion [Figure 4]. Complete removal of the WSL revealed a more extensive involvement [Figure 5]. Therefore, a composite resin restoration was placed in the cavity prepared by air abrasion. Cavity was etched with 37%
phosphoric acid (Conditioner 36, Dentsply DeTrey, Konstanz, Germany) for 15 s and rinsed with distilled water. The cavity was blot dried and total etch adhesive (Prime and Bond NT, Dentsply Caulk, Milford, DE, USA) was applied with the applicator tip. Excess primer was removed with a gentle air stream and light cured for 20 s with a halogen curing light (Woodpecker LED Curing Light; Guilin Woodpecker Medical Instrument Co, Ltd, Guangxi, China). Nanofilled composite (Ceram X, Dentsply DeTrey) was used to restore the defect. Finishing and polishing of the restoration were carried out [Figure 6]. Three month follow-up shows intact restoration and no progression of WSL [Figure 7].

**DISCUSSION**

WSLs are incipient carious lesions with subsurface demineralization and a relatively intact enamel surface. These lesions are also called “surface-softened defects.” Clinically, lesions are opaque white in appearance initially.
which may turn yellow brown with the progression of the lesion. On probing, lesions are softer than the adjacent sound enamel. WSLs can be active or inactive (arrested). Active lesions have rough, chalky surface and may progress to caries under acid attack, whereas arrested lesions are smooth and shiny which remains constant or may remineralize under favorable oral conditions. In this case, lesion was dull/matte in appearance and rough, chalky on tactile examination indicating an active lesion. These lesions should be distinguished from fluorosed teeth. Fluorosis is more generalized and diffuse in outline unlike WSL which is localized and well defined.

Early diagnosis of incipient lesions is important to institute preventive measures for caries control. Traditional diagnostic methods such as visual, tactile, and radiographic examination are inaccurate to detect incipient lesions. Recent methods for caries detection such as DIAGNOdent, quantitative laser fluorescence, optical coherence tomography, and digital imaging fiberoptic transillumination are more accurate and reliable for diagnosing early carious lesions.

Air abrasion dentistry has evolved over a period as a means of providing a truly conservative preparation for preservation of a maximal sound tooth structure. Air abrasion removes the tooth structure using a stream of aluminum oxide particles generated from compressed air. Air abrasion preparations are comfortable and usually can be done without use of local anesthesia. Tooth structure removal with air abrasion unit depends on various parameters such as the air pressure, abrading particle size, particles flow rate, nozzle diameter of the handpiece, nozzle angulation, distance from tooth, and time of exposure.

Air pressure ranges from 40 to 160 pounds/square inch. The recommended levels are at 100 psi for cutting and 80 psi for surface etching. The most common particle sizes are 27 or 50 µm in diameter, with an approximate flow rate of 2.5 g/min. The speed of the abrasive particles when they hit the tooth depends on gas pressure, nozzle diameter, particle size, and distance from the surface. Typical operating distances from the tooth range from 0.5 to 2 mm. It is best to hold the nozzle tip at a 30° to 60° angle to deflect the flow of particles away from the field instead of deflecting back into the oncoming stream.

Various air abrasion systems such as PrepMaster or EtchMaster (Groman Inc, Florida, US), PrepStart and PrepAir (Danville Engineering, CA, USA), or CrystalMark (CrystalMark Inc, CA, US) work on the same principle. Recently, bioactive glass has been tried and evaluated as an alternative to aluminum oxide particles for ultraconservative cavity preparation.

Various applications of air microabrasion are as follows:
- Removal of superficial enamel defects
- Cleaning fissures and surface preparation for sealant
- Preparation for preventive resin restorations
- Removal and repair of composite, glass ionomer cement, porcelain restorations
- Small cavity preparations
- Surface preparation for abrasion and abrasion
- Removal of pits and fissure surface stain
- Cleaning and preparation of castings
- Detection of pits and fissure caries.

Air microabrasion has several advantages over conventional cavity preparation such as no discomfort due to vibration, lesser need for local anesthesia, and conservation of the tooth structure. Limitations to its use are as follows: it cannot be used to remove amalgam restorations, not effective for removal of gross caries, cannot be used in conjunction with magnification devices as rebound particles could damage the lenses, and does not obviate the need for acid etching. Air microabrasion is contraindicated in patients with allergy to dust, asthma, and chronic pulmonary disease.

Resurgence of air abrasive technology with newer restorative materials has given a new dimension to “minimally invasive dentistry.” This case report highlights the management of WSL with minimally invasive treatment option. Furthermore, it provides a brief review of etiology, clinical features, diagnosis, and various treatment options for incipient carious lesions.

CONCLUSION
The dynamic balance between remineralization and demineralization determines the progression of WSLs. Prompt diagnosis and treatment of such lesions arrest its progression to caries. With an increase in demand for minimally invasive and less expensive treatment for esthetic cases, air microabrasion may be considered a suitable treatment option.

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Conflicts of interest
There are no conflicts of interest.

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Review Article
Bite mark analysis: Chasing the bite!

In the recent years, numbers of suspected bite mark cases examined by forensic odontologist are increasing. Human bite mark analysis is most demanding and complicated part of forensic dentistry, invol...

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