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CONTENTS

Case Reports

PRESIDENT'S MESSAGE

Dr. Girish Parmar
President, IACDE
Additional Director (Dental), Govt. of Gujarat
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Ahmedabad

It is rightly said that knowledge multiplies as it gets shared.

As the President of IACDE, it gives me immense pleasure to know that the Karnataka Association of Conservative Dentistry and Endodontics has launched its very own journal.

Karnataka has been the hub of dental education and research in India. With over 37 dental colleges, the highest in India, it is home to some of the best minds of Conservative Dentistry and Endodontics.

I wish the Karnataka Association all the luck and support from my behalf

Let's make the world smile together.

PRESIDENT'S MESSAGE

Dr. Roopa R. Nadig
President, ACE- Karnataka
Dean, Professor and Head of Department,
Dayananda Sagar College of Dental Sciences
Past president, IACDE
Past Dean of Faculty of Dentistry, RGUHS

My dear colleagues,

It is indeed a great joy and satisfaction to see the birth of ACE-Karnataka, a dream come true for me and many others in the fraternity. I am writing this message with a deep sense of responsibility coupled with a fervent desire to serve and nurture the visions of this newly formed association. Thanks also to our team of founding members for unanimously accepting me as the President.

The primordial aim of the association is to propagate scientific enquiry and continuing education by bringing all the members of our fraternity to share and gain, bind and bond, amalgamate and integrate to perpetuate scientific knowledge for the benefit of the community at large.

Associations should provide an environment for learning, innovation, and generation of knowledge. The academic culture of publishing scientific work both original research and clinical work has to be ingrained in every professional. Dissemination of such scientific work is not only a means for scientific progress but also provides a foundation for the evaluation and assessment of their scientific achievements. It is our endeavor to provide a sound platform for the fraternity to publish scientifically sound articles and disseminate, distribution the same for the benefit of all.

I thank Dr. Mithra N. Hegde , the dynamic secretary of the association and the entire editorial team headed by Dr. Vinod Kumar for their stupendous work in bringing out this first ever scientific journal of our association. The association and its editorial team will strive to remain devoted, committed and work in unison with you all towards achieving this objective.

"The illiterate of the 21st century will not be those who cannot read and write, but those who cannot learn, unlearn, and relearn."

— Alvin Toffler

ORIGINAL RESEARCH

Comparative image analysis of the two contemporary endodontic system's ability in maintaining original internal canal anatomy: an in-vitro study

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ABSTRACT

Maintaining the original morphology during endodontic treatment is imperative and as crucial as the other tenets of endodontic management. The aim of the study was to evaluate the Radiographic image analysis and comparison of Rotary and Self Adjusting File system's canal preparation ability in preserving original canal anatomy. 26 freshly extracted non-carious anterior and premolars were disinfected and mounted on wax jig, which in turn was stabilized on sensor positioning system. This was the assembly to standardize the protocol for taking preoperative and postoperative radiographs. The samples were randomly assigned to Group A: Rotary and Group B: Self-adjusting file system. The images were stored and analysed in adobe Photoshop image analysis software. The data obtained was statistically analysed by 't' test and one sample statistical analysis It was observed that all sample had increase in dimension as compared to preoperative root canal morphology. The SAF system had minimal increase in dimensions as compared to the rotary systems values. Further, the original morphology was more or less maintained with SAF as compared to Rotary system which had a tapering configuration. SAF system to a certain extent can maintain the original root canal morphology whereas rotary systems give a more tapered canal configuration outcome. Adobe Photoshop system can be used for image analysis.

Keywords: SAF, rotary, root canal anatomy, canal preparation

INTRODUCTION

One of the determinants of endodontic success is three-dimensional cleaning and shaping by removing inner layers of radicular dentin and thorough debridement. This was traditionally achieved by preparing and enlarging the original root canal dimensions from the first file which binds to three sizes greater enlargement with the ISO numbered files. The rationale was that such preparation, with removal of internal layers, will facilitate the percolation of the irrigant to the complete length and breadth of prepared canal. This was tangible in straight-line canals but in the curved canals, the use of stiff ISO stainless steel files led to deviation of original tooth morphology. The introduction of nickel-titanium files has made the preparation of curved canals easier. The metallurgy of the files with inherent elasticity prevented operator or iatrogenic accidents like deviation or transportation of canals, elbowing or ledge formation, which were routinely encountered with the ISO files. These files were able to keep the files centred and follow the central axis of the root canal.[1,4] The nickel-titanium file's canal preparation, gives beautiful radiographic obturations in canal with a round cross-section, but in oval and elliptical canal there would be portions of internal root surface which are not instrumented, this will not be evident on the radiograph but micro CT studies have found rotary instrumentation wanting in this aspect. [1,5] In tear-shaped canal and flat canals, it is usually the buccal or lingual areas which are left untouched in canal preparation. To include these areas our preparation becomes wider, while maintaining the taper from orifice to apical terminus. [1,6,7]

The self-adjusting file (SAF) is a compressible hollow thin-walled latticed cylindrical file. The file is said to adapt to the original internal root canal morphology and prepare the canal three-dimensionally while maintaining the original root canal morphology longitudinally and in transverse section.[1-8] According to Metzer, SAF utilizes a very unique method of canal preparation, its vibratory adaptive mechanism is claimed to maintain the original morphology and preclude the removal of excessive dentin. With this system, the need for maintaining taper to enhance percolation of irrigant to all nooks and corner is also obviated.[1,8]

Comparison of change in morphology after root canal preparation technique can be quantitatively analysed by CBCT method in vivo. Digital image manipulation to assess change in internal root canal morphology can be brought about by various image analysis software, one of which is Adobe CS software.[9]

METHODOLOGY: 26 freshly extracted non carious teeth: anterior and premolars, were selected were disinfected as per infection protocol. They were then mounted in a jig of modelling wax and positioned on intaglio surface of the sensor positioning system. The outer adhesive surface of the sensor positioning system had the sensor placed securely.

The rim of sensor positioning system had the end of the tube of handheld portable x-ray machine secured with clear adhesive. The portable handheld machine was positioned on

the top of the table. (Fig 1a & b) This whole assembly was created and distance noted so that for each tooth type as well as preoperative and postoperative conditions, the same parameters and distances would be exactly replicated. The preoperative radiograph was taken and image stored. The teeth were randomly divided into 2 groups, depending on the file system. Group A: Prepared by Protaper rotary kit. Group B: Prepared by SAF system.

The apical preparation of all group A was done till F2. The SAF 2mm of 25 mm length was used for wide canals and 1.5mm with 25 mm length for fine canals. The active working length was 18 mm for all teeth. The cleaning and shaping, irrigation protocol was as per standardized endodontic protocol and glide path was achieved. The manufactures instructions were followed for sequencing of each file system. The postoperative radiograph was taken and the image stored. Before measurement, it was ensured the geometric distortion, if any, was nullified by the software tool of Adobe Photoshop CS image analysis software. The pixel intensity was adjusted for titrating the contrast. Thereafter, measurements bisecting the long axis of the teeth were done for both preoperative and postoperative images. Measurements in millimetres were carried out at five different points on the root canal. First, a straight line was dropped on the Mesial (left0 side of root canal approximating the straight portion from orifice to complete root length.ie along the long axis of the tooth. On that straight line, the following levels for measurement were determined. Point A: was at crown root junction, that is the orifice of the root canal; Point E: was at end of root tip. Point D: was calculated by dropping a line from distal aspect of the root canal terminus tip. Where it bisected the straight line it became point D. The remaining portion was divided into two halves and named point B and C respectively. (Fig 2) Thus the measurements were done at five different levels both for preoperative and postoperative images.

Then the image was superimposed and digitally subtracted. The difference between the two superimposed images was calculated at the predetermined levels. The data was compiled and statistically analyzed by using SPSS version 24- IBM software.

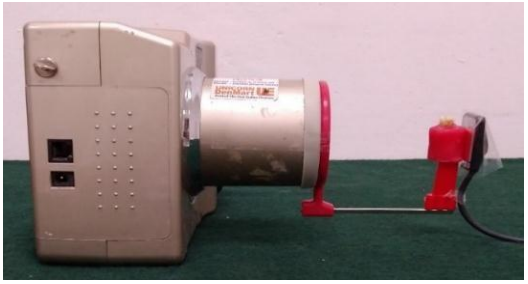


Fig 1: The tooth positioning assembly to standardize the distance and position the tooth preoperatively and postoperatively at same distance.

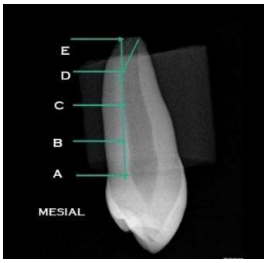


Fig 2: The root canal is measured at five points.

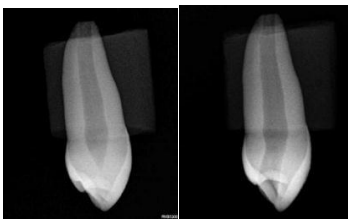


Fig 3: a. Preoperative b. Postoperatively SAF

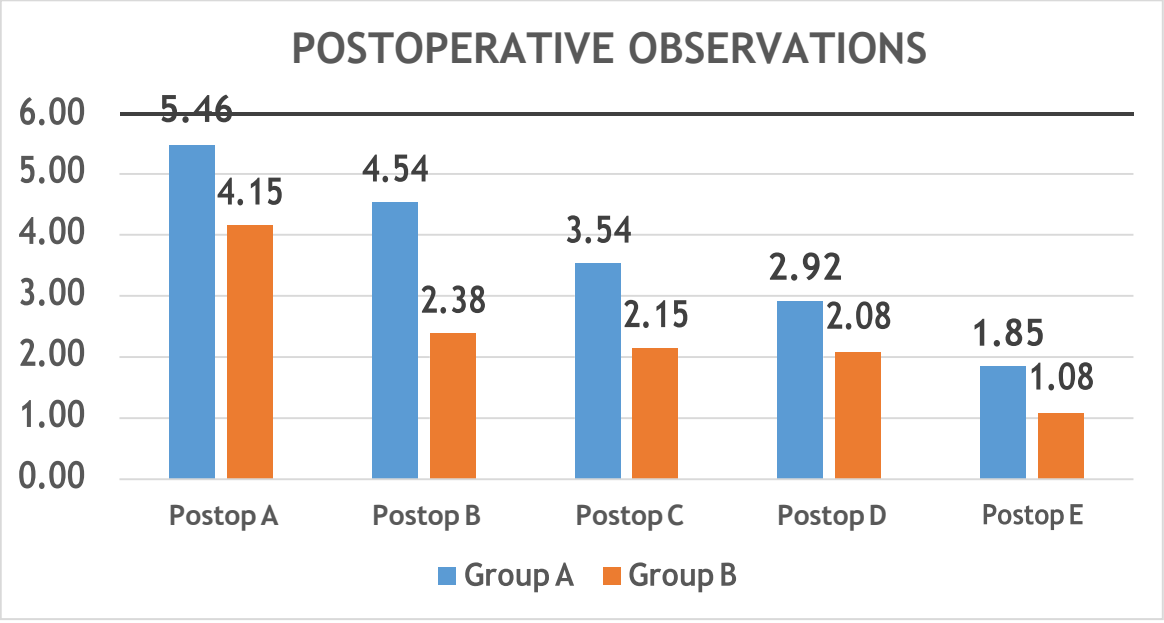
Fig 3 c. Both images superimposed.

RESULTS

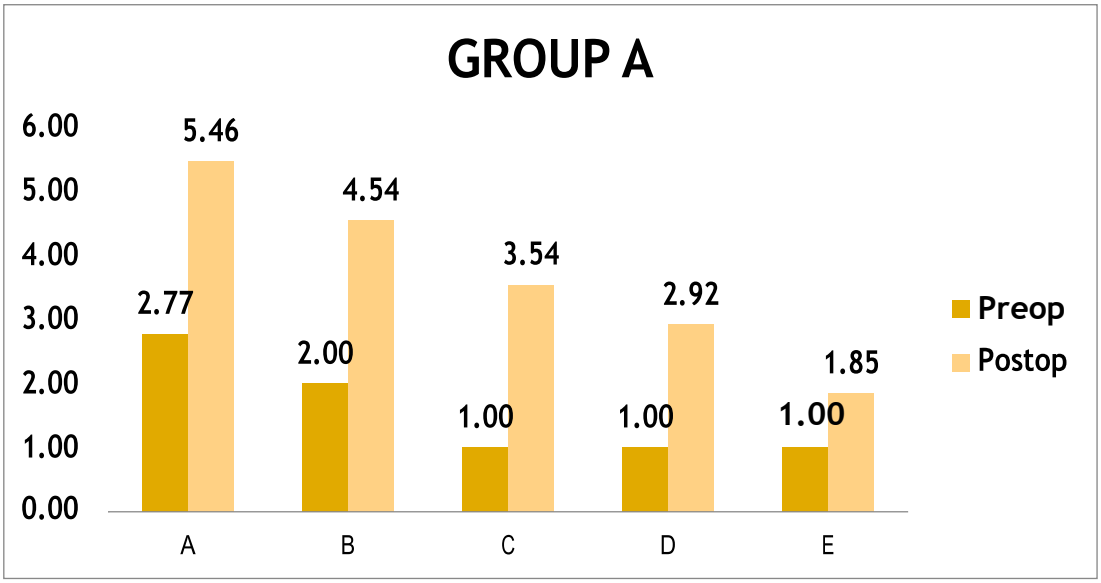
The statistical analysis was done. Student's t-test was applied for each reading at the five levels for preoperative as well as postoperative values in both the groups. For levels in groups where preoperative was more or less constant, one- sample statistical analysis was done. It was observed that there was marked deviation in the Rotary group from the original morphology and the preparation was tapering in the SAF group there was less of deviation and the preparation was similar in shape to original morphology but slightly larger in dimension if the points were to be traced. (Table 1; Graph 1 & Graph 2 A& B)

T-TEST						
	Group	N	Mean	Std. Deviation	t-value	p-value
PREOPERATIVE A	Group A	13	2.77	0.60	0	1
	Group B	13	2.77	0.60		
POSTOPERATIVE A	Group A	13	5.46	0.66	6.208	<0.001
	Group B	13	4.15	0.38		
POSTOPERATIVE B	Group A	13	4.54	0.52	10.711	<0.001
	Group B	13	2.38	0.51		
POSTOPERATIVE C	Group A	13	3.54	0.52	7.794	<0.001
	Group B	13	2.15	0.38		
POSTOPERATIVE D	Group A	13	2.92	0.64	4.371	<0.001
	Group B	13	2.08	0.28		
POSTOPERATIVE E	Group A	13	1.85	0.38	5.941	<0.001
	Group B	13	1.08	0.28		

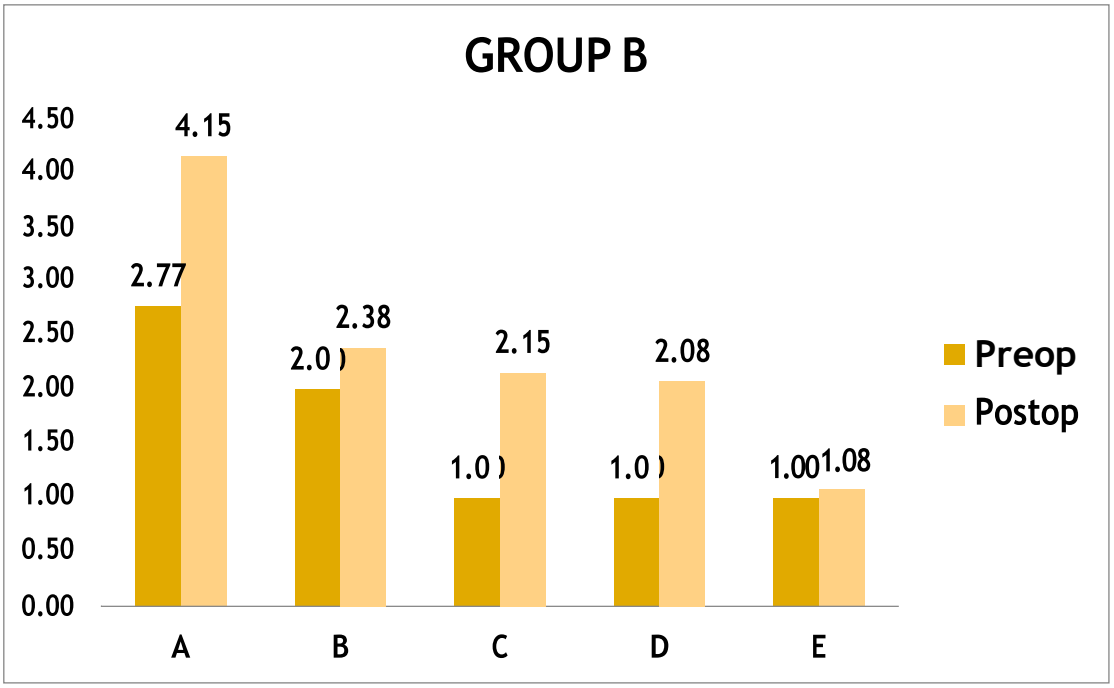
Table 1: The comparison between preoperative and postoperative values of both Groups.



GRAPH 1: Comparison between Groups of postoperative values at each level of assessment.



Graph 2A: Comparison between preoperative and postoperative values in GROUP A



Graph 2B: Comparison between preoperative and postoperative values in GROUP B

DISCUSSION

The essence of endodontics is to conserve the original canal anatomy while striving to achieve a continuous tapered preparation.[10] It relates to the capability of the file to uniformly prepare all the walls of the root canal, thus warranting that the central axis of the prepared canal coincide with that of the original canal, this is crucial more so ever in radicular curvatures.[11] Centering ability is one of the most worthwhile quality which is inherently dependent on the metallurgy of the file as well its design. The internal root canal morphology also will have a impact on the centering ability of the endodontic instrument.[12-15]

Casual or callous approach towards the original internal anatomy has led to greater incidence of transportation of the apical foramen, elbows, zipping, dentinal ledges; perforations: supra crestal, furcal, strip perforations, and separated instruments.[14-17] The fundamental anatomical and morphological features of the root canal system like flat canals, elliptical canals, ovoid or ovate canals isthmuses and intracanal communications create challenges for thorough cleaning and shaping and debridement.[17-20] The round geometric cross-section of the file is bound to leave some parts of buccal, lingual, mesial and distal surfaces which are not touched by the files. Thus, we are relying heavily on our irrigation systems to compensate for the complete cleaning and shaping.

Overzealous indiscriminate use of rotary instrumentation systems have resulted in procedural errors leading to failures.[21-25] Thus, oscillatory or reciprocating system were explored. [17-19] The shaping of simulated canals was more centered using a reciprocating motion when compared to continuous rotation but was more time consuming and require a dedicated endomotor.[17-19] In this study, it was observed that the Group A i.e. the rotary preparation group exhibited more wider and larger canal preparation than that of the original root canal outline. The preparation was tapering and the extent of preparation was markedly wider in coronal third, corresponding to the taper of SX file from the Protaper series. The magnitude of deviation of the canals prepared with rotary system as compared to original dimension was statistically significant. (Table 1, Graph 1A, 2A)

SAF system is cylinder-shaped nickel-titanium abrasive lattice that adapts to the original transverse section of the root canal. The motion is vibratory adapted as opposed to the 360-degree rotation.[25-27] The adaptive vibratory motion in a circumferential plane will gradually abrade the dentinal surface of the root canal, the resultant preparation will have the same shape and form of the original canal but the diameter would have marginally increased. (Fig 3C). This is valid not just for round canals but also for flat canals or egg-shaped canals. According to Metzger et al, the straightening of the canal is also prevented as there is stiff inner metal core and the hollow SAF file is highly pliable and the canal preparation is only dependent on the abrasive action of the lattice of the file. SAF incorporates an irrigation source through its hollow lattice design, allowing simultaneous irrigation and disinfection of the canal.[25-29] This study proved that the SAF system not only leads to smaller root canal preparations compared to rotary system but

the resultant preparation was slightly enlarged image of the original. The preparation was wider in coronal region but at level B, C, D, E it was similar to original root canal internal morphology and the SAF preparation more ore less followed the original root canal. As compared to SAF, the rotary file system led to deviation in original radicular internal anatomy and also the preparation was much larger and was a tapering configuration. (Table 1, Graph1, 2B)

The analysis of deviation requires an image analysis tool which gives a reproducible result. CBCT imaging owing to low effective radiation, rapid scans and sub millimetric dimensions of data being analysed, is considered an important diagnostic, adjunct.[29-30] It is an obligatory diagnostic tool to asses and ratify remaining dentin thickness, apical transportation, curvature of radicular portion, and centering of canal, as it provides requisite information and documentation of the same in both oblique and orthogonal planes. Radiographic images, on the other hand, are compromised due to their two-dimensional nature and inherent issues like distortion, noise. CBCT are expensive and hence an alternative,[30-32] economical method is sought in which an assembly can be set up to position the mounted tooth in such a way that both the two images can be analyzed, superimposed and the difference subtracted, as is demonstrated in this study. (Fig 1, 2 , 3a-3c) Carvalho et al has advocated the use of Adobe Photoshop for radiographic image analysis. [9] In this study, all IOPA radiographs were taken with the same handheld x-ray machine kept at the same position on the table. The sensor positioning system along with mounted jig and tooth assembly was kept at the same position for preoperative and postoperative evaluation. This was in confirmation to studies done by Duckworth et al[33] and Yoshioka[34] et al. Thereafter the Adobe Photoshop CS image analysis system was used to superimpose and calculate the increase and change in dimensions of the prepared canal by subtraction method. The two images can be compared and difference in preparation calculated by using that very command in the software's toolbox. This image analysis Photoshop edition is an excellent tool for digital handling and correcting and manipulating the two images.[9]

CONCLUSION

Within the limitation of the study it was observed that root canal preparation done with self-adjusting file system resulted in marginally larger preparation than the original tooth anatomy and maintained the original shape of the root canal. Rotary system resulted in much larger preparation than the preoperative and there was a huge deviation from original shape. The rotary preparations were observed to be of more gradual tapering configuration. Adobe Photoshop image analysis system is an interesting tool to analyze the preparation designs. However, for evaluating the centering ability of file, and the volumetric change, the relevant CBCT analysis needs to be included. In the event of absence of CBCT facility, the Photoshop image analysis can be an economical viable option.

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ORIGINAL RESEARCH

Comparative evaluation of the antimicrobial activity of the triple antibiotic solution, triple antibiotic paste, chlorhexidine and sodium hypochlorite against enterococcus faecalis: *an in vitro study*

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ABSTRACT:

Disinfection of the root canals is an important step for the success of regeneration and revascularization procedures in Endodontics. Enterococcus faecalis is most commonly responsible for failures in endodontic treatment. Combinations of various antibiotics and irrigants have been tried for its eradication. Recently triple antibiotic paste has been found to be very effective for the eradication of enterococcus faecalis from the root canal. The aim of this study is to check the efficacy of triple antibiotic solution, 2% Chlorhexidine, 5.25% Sodium hypochlorite as an irrigant against Enterococcus faecalis. E Faecalis (ATCC 29212) was maintained by using LB medium. The antibacterial efficacy was checked by agar diffusion test. Microbial growth inhibition zone was measured for each irrigant used.Triple antibiotic paste showed the greatest zone of inhibition followed by triple antibiotic solution, 2% Chlorhexidine, 5.25% Sodium hypochlorite. Triple antibiotic solution can be considered as a better irrigant for root canal disinfection compared to Chlorhexidine and Sodium hypochlorite.

Keywords: Triple antibiotic paste, Enterococcus faecalis, root canal irrigants, anti-microbial efficacy

INTRODUCTION

Regenerative endodontics aims to develop procedures in which the damaged tooth structures are regenerated from the donor or autologous cells rather the replacement with artificial materials, so they are biologically based procedures.[1] Success of these procedures is dependent on the disinfection of the immature, infected root canal. Many intracanal medicaments, irrigants and combination of antibiotics have been used to achieve this. As early as 1951, Grossman was the first one to introduce PBSC paste to endodontics which was the first antibiotic combination used for disinfection .[2]

Triple antibiotic paste (TAP), consists of Ciprofloxacin, Metronidazole and Tetracycline in the ratio of 1:1:1 and has been recommended for various endodontic procedures. A combination of these antibiotics was introduced into the root canal, primarily for the immature permanent tooth with pulpal necrosis for the regeneration or revascularization purposes.[3] Hoshino and colleagues were the first ones who explored the efficiency of TAP for the eradication of bacteria from the root canals.[4] TAP has been evaluated by the researchers to eliminate E. coli from the infected dentine.[5] These drugs can penetrate upto the cement dentinal junction, helping in complete eradication of bacteria and hence improving the prognosis of the treatment. The inclusion of blood clot in the canal space along with TAP improved the revascularization outcome in necrotic infected immature dog root canal systems.[6]

But TAP has a disadvantage of causing demineralization of dentin structure which decreases the microhardness of dentin in comparison to calcium hydroxide, thus making it prone to fracture. [7,8]

Enterococcus faecalis is the most persistent bacteria found in endodontically failed cases in deciduous and permanent dentition.[9] It is a Gram-positive, facultative anaerobic bacteria, which is resistant to various disinfection procedures.[10] The existence of *E. faecalis* in large proportions in endodontically treated teeth could be attributed to its proficiency to survive chemomechanical preparations and retreatment procedures as well as its the dexterity to invade the root canal during treatment and to re-appear after obturation.[11] Infection control procedures are essential to modern dentistry and have an impact on clinical outcomes.[12]

The most effectual mechanism to eliminate *E. faecalis* is the use of either 5.25% (w/v) sodium hypochlorite, which is a strong antimicrobial agent with tissue dissolving capacity or 2% (w/v) chlorhexidine which has high substantivity.[13]

Though Sodium hypochlorite is considered as a gold standard irrigant in nonsurgical endodontics, it has to be used with caution due to tissue toxicity, risk of subcutaneous emphysema, allergic potential, and disagreeable smell and bitter taste.[14]

Chlorhexidine (CHX) digluconate is a strong antibacterial irrigant and is effective against *E. faecalis*. Depending on its concentration, it shows both bacteriostatic and

bactericidal action.[15] Minimizing the contact time of antibiotic within the root canal can decrease the cytotoxic effect on the vital cells, thereby establishing a need for a safe irrigant.[16] Herbal irrigants are also being researched in endodontics due to their easy availability, cost effectiveness, increased shelf life, low toxicity and lack of microbial resistance.[17] Chlorhexidine seems to act by adsorbing onto the cell wall of microorganisms resulting in leakage of intracellular components.[18]

Hence, the objective of this *in vitro* study was to check the efficacy of triple antibiotic solution as an irrigant against *E. faecalis*.

Materials and Methods

1. To prepare triple antibiotic paste, ciprofloxacin, metronidazole & doxycycline were mixed in the ratio of 1:1:1. 100mg of this powder was mixed with 100ml of distilled water and 8g of methylcellulose powder was added to make a paste.

2. Triple Antibiotic solution:

Ciprofloxacin, metronidazole, doxycycline were mixed in the ratio of 1:1:1. 100mg of this powder was mixed in 100ml of distilled water. The prepared solution was used within 24 hours.
Bacterial culture of *Enterococcus faecalis* was obtained from the American Tissue Type Culture Collection and was maintained by using nutrient agar medium. The bacterial culture was further maintained by sub-culturing regularly on the same medium and was preserved at 4^{o}C before its application in the experiment.

Anti-microbial activity analysis-

The antibacterial activity against *E. faecalis* was checked using the Agar diffusion test. The culture of *E.faecalis* from overnight grown broth was spread on Luria Bertani agar (LB-agar) plates and 10mm diameter wells were made using sterile cork borer. Approximately 40 µl of Triple Antibiotic solution, Triple Antibiotic paste suspension, 2% Chlorhexidine and 5.25% NaOCl were placed with micropipettes into the wells and left at room temperature for 2 hours.

The plates were placed in an incubator at 37°C for overnight. Microbial growth inhibition zone formed by each irrigant was measured in cms.

Results

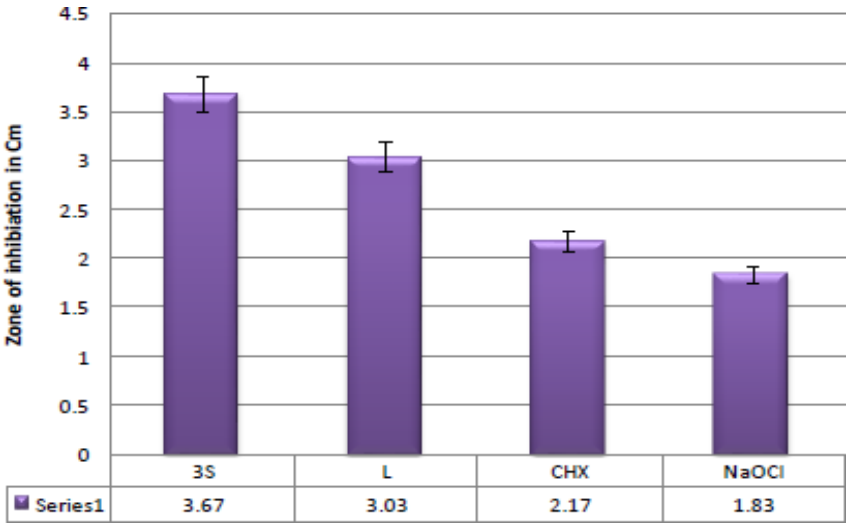

Fig1: Effect of irrigant on zone of microbial inhibition

This study assessed the antibacterial effect of Triple Antibiotic solution, Triple Antibiotic paste suspension with other synthetic irrigants on *E.faecalis* using agar diffusion method. As observed, all the four irrigants had antibacterial effects on *E.faecalis.*

Triple Antibiotic paste suspension showed highest zone of microbial inhibition (3.67 ± 0.33cm) followed by Triple Antibiotic solution (3.03 ± 0.53 cm) when compared with 5.25 % NaOCl and 2% Chlorhexidine which were 1.83 cm & 2.17 cm respectively.

DISCUSSION

Recently, there has been a shift in the endodontic management of permanent immature teeth. More research is being carried out on vital pulp therapy and regenerative endodontics.[19, 20] As instrumentation of the thin, weak walls of the immature tooth pose a risk of fracture, root canal irrigants play a pivotal role in the disinfection protocol, thus paving a way to successful healing. The search for a potent antibacterial irrigant which could be safely used in such teeth has always been there. This study was done to check the effectiveness of triple antibiotic irrigating solution & paste, Chlorhexidine and Sodium hypochlorite against E. faecalis.

TAP provides a sterile condition in the root canal which helps in the healing and repair of periapical tissues.[21] Various authors who have been studying the use of nanomaterials for regeneration of pulp, recommend triple antibiotic paste for disinfection.[22] TAP has been used in a new biological obturation technique called ‑SealBio‖, in which it was placed for one or more sittings to achieve disinfection in permanent teeth.[23] The effects of systemic use of antibiotics is dependent on the patients compliance, absorption by the

various body systems and a proper blood supply to the infected area, which is likely to be very less in necrotic teeth.[24] Long term placement of TAP can cause decrease in microhardness of dentin[7], therefore using it as an irrigant can prevent this. Ciprofloxacin has a wide antibacterial spectrum and excellent tissue penetration. Metronidazole has a very strong antimicrobial action against anaerobes. It permeates into the bacterial membrane, binds to the DNA, causes the disruption of the helical structure and cell death. Initially Minocycline was used in TAP but it caused discoloration so doxycycline was used in the study which does not cause discoloration of teeth. Berkhoff JA, et al. stated that TAP remains in dentine, whereas most $Ca(OH)_2$ is eliminated after endodontic irrigation. [25]

CONCLUSION

From the results of this in vitro study, it can be concluded that triple antibiotic solution showed an excellent spectrum of antibacterial activity against E.faecalis. Following this experiment, it can be observed that the solution is equally effective as 5.25% NaOCl and 2 % Chlorhexidine, which are the most preferred root canal irrigants in endodontics. Thus, triple antibiotic solution can be used to overcome the hurdle of toxic effects of synthetic irrigants used in the disinfection of root canals and the difficult removal of TAP.

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ORIGINAL RESEARCH

Cytotoxicity effects of surfactant endodontic irrigants with MTAD on permanent and primary cell lines

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ABSTRACT

Commercially available endodontic irrigants contain surfactants and have been claimed to have better antimicrobial efficacy than their traditional counterparts. Although antibacterial efficacy is a prime factor; toxicity of these products need to be understood in clinical endodontics. The aim of this study was to evaluate cytotoxicity of two independent surfactants with Biopure MTAD on two different cell lines. Serial dilutions of surfactants (0.5% Cetrimide – CTR; 1% sodium dodecyl sulfate – SDS) were prepared respectively and evaluated against control groups (0.9% normal saline – NS , MTAD). Primary cell lines (HGF) and permanent cell line (HeLa) were treated with the test irrigants. Cytotoxicity was evaluated by trypan blue assay. 20µl of the test irrigants were treated with 30µl of the cell suspension for a contact time of 5 minutes. Viability percentage of the respective cell suspensions was evaluated under inverted microscope.On HeLa cell line NS showed highest viable cells, followed by CTR; while MTAD resulted in non viable cells. CTR and NS showed reduced viability scores on HGF significantly; on the contrary MTAD although found to be cytotoxic showed higher viable cells than CTR. SDS was found to be severely cytotoxic on both cell lines. The cytoplasmic membrane of the cells is the key target for cellular cytotoxicity with primary cell line (HGF) being more sensitive than continuous cell line (HeLa); suggesting variation in cellular membrane composition. All irrigants were found to be cytotoxic and exhibit different modes of action.

KEYWORDS: HeLa, HGF, MTAD, SURFACTANT

INTRODUCTION

Irrigants play a vital role in chemomechanical debridement and disinfection of the root canal system. Traditional irrigants, sodium hypochlorite (NaOCl) and ethylene diamine tetra acetic acid (EDTA) find frequent application in endodontic literature. On the contrary chlorhexidine digluconate (CHX), iodine potassium iodide (IKI) find limited applications as well. None of the above meets all the ideal requirements; have certain limitations to their clinical applications and thus commercial irrigants have been on the rise in dental markets. [1]

CHX Plus (2% CHX with surface modifiers), Chlor-XTRA (6%NaOCl with surface modifiers); Vista Dental Products, Racine, W.I and Cetrehexidin (0.2% CHX with 0.2% cetrimide) Vebas, San Giuliano, Milan, Italy; incorporate surfactants to the traditional endodontic irrigants. [2] Biopure MTAD (Dentsply, Tulsa) which consists of tetracycline isomer (doxycycline), an acid (citric acid) and a detergent (tween 80); has been claimed to have superior antibacterial properties when used as a final rinse.[3]

Surfactants/surface modifiers are organic compounds that are soluble in both organic solvents and water and play significant role in enhancing the antibacterial efficacy .[4] Positively charged molecule CTR has significant antimicrobial action due to its stability and solubility in water.[5] Anionic alkyl sulfate such as SDS possess significant antimicrobial property due to its low surface tension and lubricant ability; thereby enhancing the flushing action and debridement the root canal system. [6]

Cytotoxic substances on contact with living tissues can lead to cell injury, impair cell proliferation and repair, irritation, degeneration or tissue necrosis depending on their frequency and concentration of use .[7] Risk of mutations within target cells leading to DNA damage potential may diminish the self-repairing potential of tissues. [8] Various tests that measure cytotoxicity, mutagenicity and genotoxicity in view of current clinical application of substances have been recognized as reliable indicators of carcinogenicity in future. [9]

Although most studies have evaluated cytotoxicity of traditional irrigants; [10] toxicity of commercial irrigants that contain surfactants are less reported. The underlying mechanisms of these surfactants need to be understood to enable better application in irrigants. Thus the purpose of this study was to evaluate the cytotoxicity of surfactants CTR and SDS on two different cell lines by trypan blue exclusion assay.

MATERIALS AND METHODS

Preparation of irrigants and cell line

This in vitro study was performed in central research laboratory, A.B Shetty Memorial Institute of Dental Sciences (NITTE University). 0.5% cetrimide (CTR), Himedia; 1% sodium dodecyl sulfate (SDS), Merck were prepared by serial dilution in distilled water.

Biopure MTAD (Tulsa Dentsply) and 0.9% normal saline served as control. HeLa (Henrietta Lacks) and HGF (Human Gingival Fibroblast) cell lines were obtained from Manipal life sciences, Manipal ; in a controlled humidified atmosphere of 5% CO_2/95% air .

Cell culture and storage

The cells were cultured and maintained in a growth medium containing the following constituents: Dulbecco's modified Eagle's medium (Himedia) with 25 mmol/L glucose, 1mmol/L pyruvate, 4.02 mmol/L L-alanyl-glutamine and 10% fetal calf serum (Sigma Aldrich).Individaul cells were obtained with 0.15% trypsin (Himedia) for 5 min, following which 2 ml of complete medium was added and the cells were centrifuged at 1000 rpm (180 g) for 5 min. Cell suspension was loaded and standardized in 96 well microtitre plates (Himedia) with a mean density of $1x10^4$ cells per well.

Cytotoxicity Evaluation

Cytotoxicity was assessed using trypan blue dye. 20µl of the test irrigants were treated with 30µl of the cell suspension for a contact time of 5 minutes. 50µl tryphan blue dye (0.05%) was added and cytotoxicity after a period of 5 minutes. Viabilty percentage of the cell suspension was assessed by loading into Neubauer chamber and cell count performed under inverted microscope (Olympus, India). Nonviable cells take up the stain and appear blue; while viable cells have a clear cytoplasm. Vitality of cells expressed in percentage was calculated using the formula (% viability = Average number of viable cells / Total number of cells X 100). [11]

RESULTS

The viability scores of irrigants on HeLa cell line were as follows; 0.5% CTR (68%), 1%SDS (0%), 0.9% saline (93.5%) and 100% MTAD (0%). The viability percentage obtained is summarized in figure 1. The viability percentage of irrigants on HGF obtained were; 0.5% CTR (21%), 1%SDS (0%), 0.9% saline (66.25%) and 100% MTAD (32.5%) as seen in figure 2. Although decreased viability scores was seen in HGF by CTR and saline; an upward trend was seen for MTAD thus confirming that target cell line are highly sensitive than non-target cell lines.

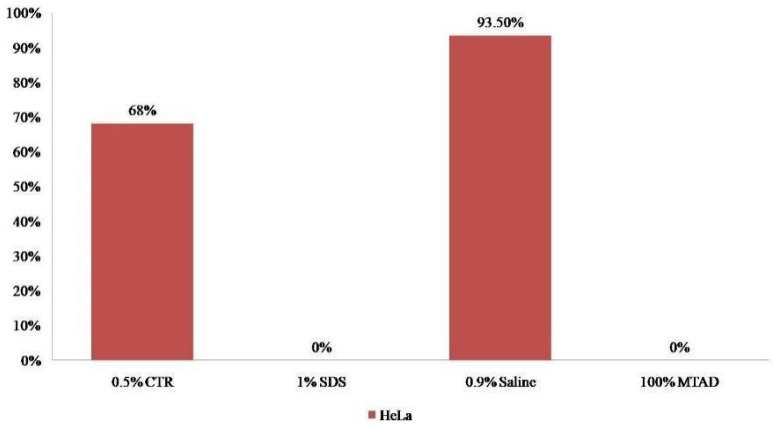

Figure 1 Viability percentage of HeLa cells with surfactants and MTAD

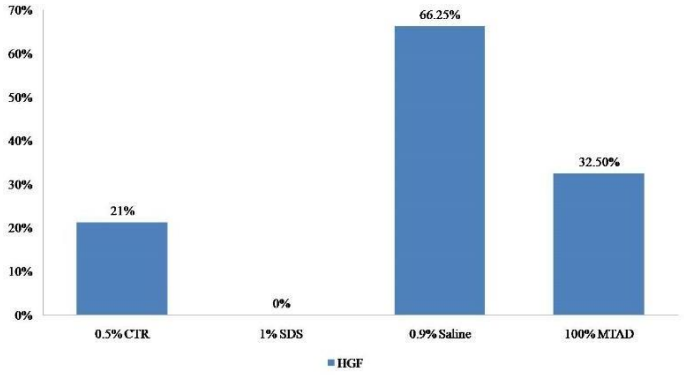

Figure 2: Viability percentage of HGF cells with surfactants and MTAD

DISCUSSION

This study evaluated the cytotoxicity of two experimental independent surfactants (CTR, SDS) and a commercial irrigant (MTAD) on two different cell lines. This study evaluated the trypan blue exclusion assay to qualitatively and quantitatively assess the degree of cytotoxicity of irrigants. To best of our knowledge such an attempt has not yet been done to report the cytotoxicity of these surfactant irrigants on different cell lines simultaneously.

In vitro studies are relatively simple, inexpensive and as such great amount of information can be obtained under controlled conditions to reveal underlying mechanism of cellular toxicity. [12]
Cytototoxicity studies commonly use cell line for evaluation [13] and different methods of approach. [14] Simultaneous application of permanent/non target cell line (HeLa) and primary/target cell line (HGF) to the test irrigants was done to evaluate their susceptibility, while keeping all other factors constant.

The trypan blue assay can be used to measure cytotoxicity; where dead cells appear blue, whereas the living ones have a clear cytoplasm. Evaluation under microscope enables us to assess the cellular viability immediately after a particular period of time. [15] A contact time of 5minutes was chosen for the irrigants with the cell lines because MTAD (as per manufacturer's instruction) requires the above time as a final rinse in clinical endodontics. [16]

Surfactants 0.5% CTR and 1% SDS were chosen based on our previous reports on antibacterial efficacy. [17-18] The current study results reveal that all test irrigants (surfactants/ commercial) were cytotoxic on both cell lines. The use of target cell line (HGF) appears to be far more sensitive than permanent cell line (HeLa) thus suggesting cellular membrane being the target for cell viability. 0.9% normal saline had reduced scores on HGF than HeLa, thus suggesting difference in the structure of the cytoplasmic membrane and their altered permeability play a key role in cytotoxicity. [19] This has potential implications since irrigants contact their target tissues directly in clinical conditions such as in open apex and perforation sites and complicate tissue repair. [20]

Our study results are similar with Oncag et al who reported cetrexidin (0.2% CHX+0.2% CTR) had lower toxicity than 5.25% Na OCl. [21] Estrela et al used cetylpyridinium chloride (CPC) in concentrations of 0.1% and 0.2% as an endodontic irrigant for management of infected canals. [22] Dong et al reported that SDS is cytotoxic when used alone. [23] Barbosa et al reported that calcium hydroxide combination with SDS is not cytotoxic and hence may find its application as endodontic irrigant. [24] Fiume et al stated SDS in products applicable in contact with the skin, concentrations should not exceed 1%. [25]

The positively charged CTR molecule enhances linking with anionic phospholipid bilayer on the cell membrane of bacteria and is capable of altering the cytoplasmic membrane integrity. Loss of cytoplasmic membrane integrity leads to alteration of the functions involving membrane permeability and inactivation of the enzymes of cytoplasmic membrane; thus leading to protein denaturation and cell death. [26]

SDS is an anionic alkyl sulfate; has the properties of low surface tension, lubricant ability and increase lipopolysaccharide (LPS) disaggregation. Tertiary structure unfolding in the submicellar and chain expansion in the micellar range of SDS concentrations are the two major and discrete events reported leading to protein structure damage. [27] However SDS has the ability to solubilise proteins, thus targeting the cellular membrane and cytoplasmic contents leading to cell death. [28]

Marins et al found MTAD to be genotoxic on murine fibroblast cell and did not cause cell death. The lower concentrations of MTAD used (in contrast to manufacturers instruction) could have accounted for higher cell viability scores. [29] Zhang et al stated that MTAD was not cytotoxic when assessed by MTT method .[30] As stated by Eisenbrand et al single cell comet assays which are an integral part of cytotoxicity are not recommended on samples exhibiting cytotoxicity more than 30%; and thus was not performed in our study. [31]

MTAD contains 0.5% polysorbate (tween 80); a non-ionic surfactant which is water soluble thus increasing the wettability of the irrigant. [3] Lower concentration of citric

acid (4.25%) present in the irrigant could have contributed to cytotoxicity of the irrigant as well. [32] Tetracycline analogues (doxycycline in MTAD) inhibit matrix metalloproteinases and thus induce cell death in several cancer cell types. Further studies also reported to be cytotoxic, cause DNA damage as well; thus resulting in non-viability of HeLa cells and limited viability on HGF. [33]However the underlying mechanism may be presumed principally due to the acidic nature of MTAD (pH 4) that could have caused cellular lysis in addition to the above factors. [34]

CONCLUSION

To conclude all test irrigants were found to be cytotoxic on both cell lines at varying degrees. This study evaluated cytotoxicity based on cell membrane as the target factor. Each irrigant exhibit different mechanism of action and intended contact time may thus vary. Further studies should be designed using target tissue cell lines at multiple time intervals to evaluate cytotoxicity. Studies that evaluate metabolic activity (MTT assay) and genotoxicity (comet cell assay) should be considered before a final conclusion can be drawn.

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ORIGINAL RESEARCH

Oral hygiene practices and oral health status in southwest coastal population of India: a cross-sectional study

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ABSTRACT:

Oral hygiene is of paramount importance for upkeeping overall oral health. Many oral diseases can be prevented by maintenance of proper oral hygiene. The aim of this study was to evaluate the epidemiological co-relation between oral hygiene practices and oral health status in South West Coastal Population of India. This cross-sectional study was conducted on 2000 subjects selected by random sampling method, using a questionnaire. Oral hygiene index- simplified (OHI-S), community periodontal index (CPI), decay status (DMFT) and mucosal lesions were recorded according to WHO oral health assessment form for adults, 2013. The findings were statistically analyzed using Chi-Square test, Mann Whitney test and Kruskal Wallis test.

Population having OHI-S score 1 had significantly low decay status and better periodontal status, People who brushed twice daily had low DMFT status. Mouth rinsing was most common adjunct, use of mouth wash showed better overall oral health status. Removal of negative oral health beliefs and attitudes, reinforcement of regular brushing of teeth employing proper technique and using fluoridated toothpaste, periodic visits to dentist is imperative especially in rural populations and young adult**s.**

KEYWORDS: Oral Hygiene Status, Oral Health Status, Epidemiological Survey

INTRODUCTION:

The indicators of oral health burden include two major oral health problems- Dental caries and Periodontal diseases. Their higher prevalence and the effects on an individual's quality of life make them a major public health concern. Dental caries is seen across all age groups and in all populations. Though the diseases have been significantly controlled in developed countries, epidemiological studies have consistently shown the burden of such diseases in the developing countries. [1]

Major etiopathological agent for dental caries and periodontal diseases is bacterial plaque. It is believed that removal of plaque by brushing teeth and interproximal cleaning prevents demineralization of teeth due to a reduction of the concentration of caries causing pathogens. [2]

In developing countries, the increasing trend in dental caries can be attributed to nutrition changeover with effortless accessibility to refined carbohydrates, lesser utilization of fluoridated toothpaste and inconsistent tooth cleaning practices...[3] It is observed that there is a widespread and almost uniform neglect of tooth cleaning, which becomes more pronounced with age. Although people clean their teeth with toothpaste and toothbrush, their oral hygiene status is poor-indicating faulty brushing techniques. [4]
The start off for analyzing oral health status is an assessment of oral hygiene practices. There is a significant association between periodontal status and the material used for cleaning teeth; as well as between mouth rinsing with water and periodontal health. [5]
The National Oral Health Survey, conducted in 2005, by the Indian Dental Association (IDA), featured that 95% of Indian population endures gum disease, mere 50% uses a toothbrush, and barely 2% consults a dentist. [6]
The most commonly used method of teeth cleaning is brushing with toothbrush and toothpaste, whereas the use of mouthwash as an oral hygiene aid is comparatively low [7]

Several socioeconomic and cultural characteristics can also affect oral health status. Molars have an inescapably elevated risk for being decayed, missing and filled than the incisors, cupids, and bicuspids. Females usually have a high probability of decayed and filled teeth as compared to males, but a slim chance for missing teeth. The urban population shows a diminished risk for decay, but nearly 4 times pronounced tendency for having fillings compared to the rural population. Proletarians have increased plausibility for missing anterior and molar teeth, subjects with high socio-economic status often have more fillings. [8]
The changing lifestyle, use of fluorides, chiefly in toothpastes, improvement in oral hygiene, metamorphosis in sugar consumption, the revolution in diagnostic criteria, increased awareness and patient responsiveness, and the pre-emptive and restorative endeavors by dental health services are some of the pivotal facets for the abatement of dental caries and periodontal problems. [9]

As stated by the World Health Organization (WHO), "Promotion of oral health is a cost-effective strategy to reduce the burden of oral disease and maintain oral health and quality of life." [10]

Epidemiological data is most necessary for the effective formulation of strategies for oral health care and disease preclusion. This study intends to assess the interdependence between oral hygiene practices and the oral health status in the South West Coastal Population

AIM:

To study the epidemiological co-relation between oral hygiene practices and oral health status of in South West Coastal Population

OBJECTIVES:

1. To ascertain the prevalence and severity of oral diseases across gender, age, educational levels
2. To assess oral hygiene behaviors and awareness
3. To gauge the association of oral health status with oral hygiene habits/behaviors.

METHODOLOGY:

An observational, descriptive, hospital-based cross-sectional epidemiological analysis was carried out among patients attending the Department of Endodontics and Conservative Dentistry at A.B. Shetty Memorial Institute of Dental Sciences, NITTE University, Mangalore and its satellite Rural Centers. Information was collected on sociodemographics like age, gender, residence; dietary habits- frequency and form of sugar intake, oral hygiene behaviors -method, frequency, timing of cleaning teeth, use of mouthwash or floss, frequency and visit to dentist as response to a structured pro forma consisting of questionnaire and by intraoral clinical examination. Study technique was by an exit interview with the patient. Approval to effectuate the study was sought from relevant authorities. Informed verbal consents were obtained. Failure to obtain consent did not affect patients' treatment and confidentiality of the information given was assured.

The survey was conducted from May 2018- June 2018. 1000 patients were examined at the outpatient department of the Department of Endodontics and Conservative Dentistry at A.B. Shetty Memorial Institute of Dental Sciences and 1000 were examined at in the Rural Health Centres. Thus, 2000 patients were studied. Patient selection was by a random basis, and patients were interviewed one after another.

Dental caries status was appraised employing the World Health Organization (WHO) caries diagnostic criteria for decayed, missing, and filled teeth (DMFT index); Oral hygiene status was evaluated using the OHI-S index. Periodontal status was evaluated as per the guidelines of the World Health Organisation Oral Health Assessment Form for adults. [11]

Inclusion criteria:

Patients included in the study belonged to one of the age groups hereinafter: 15-30 years, 30-45 years, 45-60 years and >60 years, willing to participate, who gave verbal consent and who were able to understand and answer the questions.

Exclusion criteria:

Patients excluded from the study were the edentulous patients, those undergoing orthodontic treatment, those suffering from debilitating diseases or presenting with intellectual disability.

STATISTICAL ANALYSIS:

Data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) software for Windows (IBM Corp. Released 2013, Version 22.0. Armonk, NY, USA. Chi-Square, Mann Whitney U and Kruskal-Wallis tests were performed.

RESULTS:

Out of the 2000 patients examined, 1100 were males and 900 were females. 528 (47.3%) of the males had OHI-S score 1, whereas 580 (52.7%) had OHI-S score 2 or 3. 458 (50.9%) females had OHI-S score 1 and 442 (49.1%) women had OHI-s score 2 or 3. [Table 1].

Mean decayed teeth found in males was 3.46 whereas that in females was 3.39. mean missing teeth found in males was 1.03 whereas that in females was 1.1. mean filled teeth found in males was 1.92 and that in females was 1.97. the mean DMFT found in males was 6.42 whereas in females was 6.46. [Table 1]

48.9% of the population belonging to age group 15-30 years had OHI-S score 1, whereas the same score was seen in 49.3%, 48.8% and 47.8% in age groups 30-45 years, 45-60 years and above 60 years respectively. OHI-S score 2 or 3 in the four ascending age groups was seen in 51.1%, 50.7%, 51,2%, and 52.2% respectively. [Table 2]

Maximum decayed teeth were found in the age group 15-30 years with a mean of 3.55 whereas minimum decayed teeth were found in the age group > 60 years with a mean of 3.31. Maximum missing teeth were found in age group >60 years with a mean of 1.71and minimum missing teeth were found in the age group 15-30 years with mean 0.31 ($p<0.001$). Maximum filled teeth were found in the age group of 15-30 years with mean of 2.11 and minimum filled teeth were found in age group >60 years with mean of 1.58 ($p<0.001$) Mean DMFT of the four age groups were 5.96, 6.53, 6.56 and 6.58 respectively ($p<0.001$) [Table 2]

51.2% of the urban population had OHI-S score 1, whereas 47.4% and 49.0% population of periurban and rural populations had OHI-S score 1. [Table 3]

The mean decayed teeth in urban as well as rural areas were 3.44. The highest missing teeth were found in rural areas with a mean of 1.15 and the least number of missing teeth were found in urban areas- mean (sd)= 0.89(1.29). ($p=0.007$). The highest value of filled teeth was found in urban areas with mean 2.24 and lowest in rural areas with mean 1.83($p<0.001$) The mean DMFT in urban areas was 6.57, periurban areas were 6.37 and rural areas was 6.43 [Table 3]

About 63.4% of the total population are non-vegetarians and 51.6% of them has OHI-S score 1 whereas 29.3% of the total population were vegetarians and 44.7% of them had OHI-S score1.

Only 7.3% of people were pescetarians and 42.5% of them had OHI-S score 1 (p=0.006)[Table 4].The most commonly used cleaning aid was toothbrush (95.6%) where the 48.6% toothbrush users have OHI-S score 1 and 51.4% users had OHI-s score 2 or 3. The most common type of toothbrush used was soft bristled toothbrush and the highest cleaning efficacy was of medium bristled toothbrush. (p=0.02) [Table 5]

The mean DMFT found in the population using a toothbrush as cleaning aid was 6.44, whereas in those using finger was 6.58 and in those using other aids was 4.67(p=0.004). The highest mean of decayed teeth was found in people using hard-bristled toothbrushes, the mean being 3.53 whereas the lowest was in people using a medium bristled toothbrush, mean of 3.33 (p=0.009). The highest mean of missing teeth was found in people using hard-bristled toothbrushes, the mean being 1.27 whereas the lowest was in people using soft bristled toothbrush, mean of 1.01. The highest mean of filled teeth was found in people using medium bristled toothbrushes, the mean is 1.99 whereas the lowest was in people using hard-bristled toothbrush, mean of 1.53. (p=0.001)[Table 5]

Most common brushing technique being horizontal scrub with 49.7% population having OHI-s score 1 and least common technique was vertical scrub with 45.5% population using this technique having OHI-S score 1. No significant association between caries status, periodontal status, oral hygiene status, and brushing technique was found.

About 54.4% study population brushed their teeth at least once daily and 50.2% of them had OHI-S score 1. 50.1% population brushed their teeth for more than 2 minutes and had OHI-S score 1. [Table 6]

The mean decayed status in people brushing occasionally, once per day and twice per day was 3.34, 3.47 and 3.41 respectively. The mean missing status was 1.27, 1.06 and 0.89 respectively ($p<0.001$).The mean filled status was 1.76, 1.85and 2.3respectively ($p<0.001$). The mean DMFT was 6.36, 6.39 and 6.59 respectively. [Table 6]

50% of the study population who brushed at least once daily had CPI score 0.50.0% of population who brushed their teeth occasionally, 37.5% of people who brushed at least once daily and 12.5% of those who brushed twice daily had presence of periodontal pocket of 6 mm or more (CPI score 4) ($p<0.001$) [Table7]

48.7% population used toothpaste as an adjunct where 75.9% people used fluoridated toothpaste and 48.7% population has OHI-s score 1. (p=0.03) [Table 8]

The mean decayed and missing status in people using fluoridated toothpaste was 3.42 and 1.04 respectively. Whereas in people using non-fluoridated toothpaste was 3.47 and 1.12 respectively. The mean filled status in fluoridated toothpaste users and non-fluoridated toothpaste users were 1.99 (1.41) and 1.79 (1.47) respectively. (p=0.005) [Table 8]

86.9% population was aware of dental flossing and 83% population practiced oral rinsing. 7.4% population practiced dental flossing (p=0.03) and 9.8% population used mouthwash for rinsing daily. (p=0.02) [Table 9] The highest DMFT score was found in

people not using any oral hygiene aid with the mean being 6.46 and the lowest score was found in people practicing flossing on regular basis, the mean was 6.16 [Table 9]

About 39.5% population conferred to a dentist within the last 6 months and 52.2% of them had OHI-S score 1whereas 16.5% population never consulted a dentist before and 57.1% of them had OHI-S score 1. (p=0.03) [Table 9]

3.9% of the total population had CPI score 0 and OHI-s score 1. 27.2% of the total population had both CPI and OHI-S score 1. 35.7% of the total population had CPI and OHI-S score 2. (p=0.001)[Table 10]

The mean decayed status in the population having OHI-S score 1 and OHI-S score 2/3 was 3.17 and 3.68 respectively. (p<0.001). The mean missing status in the population having OHI-S score 1 and OHI-S score 2/3 was 0.92 and 1.19 respectively. (p<0.001). The mean DMFT status in the population having OHI-S score 1 and OHI-S score 2/3 was 6.09 and 6.77 respectively. (p<0.001)[Table 10]

2.4% of the total population had presence of a precancerous lesion and 1.9% of the total population had presence of the precancerous condition. No significant association was found with oral hygiene. 6.1% of the study population had presence of aphthous ulcers whereas 12.7% had traumatic ulcers and 1% had herpetic ulcers. Of the 10.2% had OHI-S score 1 (p=0.01) [Table 11]

Table 1:

Gender	OHI-S 1	OHI-S 2/3	Mean Decayed	Mean Missing	Mean Filled	Mean DMFT
Male (n=1100)	520 (47.3%)	580 (52.7%)	3.41	1.03	1.92	6.42
Female (n=900)	458 (50.9%)	442 (49.1%)	3.39	1.1	1.97	6.46

Table 2

Age (years)	OHI-S 1	OHI-S 2/3	Mean Decayed	Mean Missing p<0.001	Mean Filled P<0.001	Mean DMFT P<0.001
15-30 (n=376)	184 (48.9%)	192 (51.1%)	3.55	0.31	2.11	5.96
30-45 (n=750)	370 (49.3%)	380 (50.7%)	3.44	1.08	2	6.53
45-60 (n=598)	292 (48.8%)	306 (51.2%)	3.39	1.21	1.94	6.56
>60 (n=276)	132 (47.8%)	144 (52.2%)	3.31	1.7	1.58	6.58

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 3

Location	OHI-S 1	OHI-S 2/3	Mean Decayed	Mean Missing P<0.007	Mean Filled P<0.001	Mean DMFT
Urban (n=430)	220 (51.2%)	210 (48.8%)	3.44	0.89	2.24	6.57
Periurban (n=692)	328 (47.4%)	364 (52.6%)	3.4	1.04	1.9	6.37
Rural (n=878)	430 (49.0%)	448 (51%)	3.44	1.15	1.83	6.43

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 4

OHI score	Diet type (p=0.006)			Deleterious habit (p=0.003)					
	Vegetarian (n=586)	Non-vegetarian (n=1268)	Pescetarian (n=146)	None (n=958)	Smoking (n=400)	Pan (n=308)	Tobacco (n=140)	Alcohol (n=174)	Others (n=20)
1	262	654	62	486	196	132	84	74	6
2/3	324	614	84	472	204	176	56	100	14

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 5

Cleaning aid	OHI-S 1	OHI-S 2/3	Mean Decayed	Mean Missing	Mean Filled (p<0.001)	Mean DMFT
Toothbrush (n=1912)	930 (48.6%)	982 (51.4%)	3.44	1.07	1.93	6.44
Finger (n=76)	38 (50%)	38 (50%)	3.18	0.89	2.5	6.58
Stick/others (n=12)	10 (83.3%)	2 (16.7)	3	1	0.67	4.67
Bristle type	OHI-S (p=0.02)		Mean decayed (p=0.009)	Mean missing	Mean filled (p<0.001)	Mean DMFT
	1	2/3				
Soft (n=992)	460 (46.4%)	532 (53.6%)	3.49	1.01	1.98	6.48
Medium (n=842)	442 (52.5%)	400 (47.5%)	3.33	1.08	1.99	6.39
Hard (n=12)	76 (45.8%)	90 (54.2%)	3.53	1.27	1.53	6.39

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 6

Frequency of brushing	OHI-S 1	OHI-S 2/3	Mean Decayed	Mean Missing (p<0.001)	Mean Filled (p<0.001)	Mean DMFT
Occasionally (n=402)	198 (49.3%)	204 (50.7%)	3.34	1.27	1.76	6.36
Once daily (n=1088)	546 (50.2%)	542 (49.8%)	3.47	1.06	1.85	6.39
Twice daily (n=510)	234 (45.9%)	276 (54.1%)	3.41	0.89	2.3	6.59

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 7

Frequency of brushing	Periodontal status (p<0.001)				
	0 (n=56)	1 (n=544)	2 (n=1004)	3 (n= 380)	4 (n=16)
Occasionally (n=402)	20 (35.7%)	92 (16.9%)	190 (18.9%)	92 (24.2%)	8 (50.0%)
Once daily (n=1088)	28 (50.0%)	308 (56.6%)	558 (55.6%)	188 (49.5%)	6 (37.5%)
Twice daily (n=510)	8 (14.3%)	144 (26.5%)	256 (25.5%)	100 (26.3%)	2 (12.5%)

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 8

Type of toothpaste	OHI-S (p=0.03)		Mean Decayed	Mean Missing	Mean Filled (p<0.005)	Mean DMFT
	1	2/3				
Fluoridated (n=1518)	722 (47.6)	796 (52.4%)	3.42	1.04	1.99	6.45
Non-fluoridated (n=482)	256 (53.1%)	226 (46.9%)	3.47	1.12	1.79	6.38
Oral hygiene aids	OHI-S (p=0.02)		Mean Decayed	Mean Missing	Mean Filled (p=0.002)	Mean DMFT
	1	2/3				
None (n=1554)	754 (48.5%)	800 (51.5%)	3.44	1.08	1.93	6.46
Floss (n=196)	108 (55.1%)	88 (44.9%)	3.41	0.96	1.8	6.16
Interdental aid(n=102)	38 (37.3%)	64 (62.7%)	3.39	1.27	1.84	6.51
Mouth rinse(n=148)	78 (52.7%)	70 (47.3%)	3.34	0.85	2.32	6.51

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 9

OHI-S score	Dental flossing (p<0.03)		Last visit to dentist (p<0.03)			
	Yes (n=262)	No (n=1738)	Never (n=790)	<6 months (n=502)	6-12 months (n=378)	>1 year (n=330)
1	112 (42.7%)	866 (48.8%)	412 (52.2%)	244 (48.6%)	162 (42.9%)	160 (48.5%)
2/3	150 (57.3%)	872 (50.2%)	378 (47.8%)	258 (51.4%)	216 (57.1%)	170 (51.5%)

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 10

OHI-S score	Mean Decayed (p<0.001)	Mean Missing (p<0.001)	Mean Filled	Mean DMFT (p<0.001)	Periodontal status				
					0 (n=56)	1 (n=544)	2 (n=1004)	3 (n= 380)	4 (n=16)
1	3.17	0.92	2	6.09	38 (3.9%)	544 (55.6%)	290 (29.7%)	106 (10.8%)	0
2/3	3.68	1.19	1.89	6.77	18 (1.8%)	0	714 (69.9%)	274 (26.8%)	16 (0.8%)

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 11

OHI-S score	Precancerous lesion		Precancerous condition		Ulcer (p=0.01)			
	Present n=48	Absent n=1952	Present n=37	Absent n=1963	Absent N=1604	Aphthous N=122	Herpetic N=20	Traumatic N=254
1	26 (2.7%)	952 (97.3%)	15 (1.5%)	963 (98.5%)	776 (79.3%)	52 (5.3%)	16 (1.6%)	134 (13.7%)
2/3	22 (2.2%	1000 (97.8%)	22 (2.2%)	1000 (97.8%)	828 (81%)	70 (6.8%)	4 (0.4)	120 (11.7%)

*p<0.05 statistically significant, p>0.05 Non Significant, NS

DISCUSSION:

The oral cavity plays multiple important functions including mastication, bolus formation, taste sensation, deglutition. It also serves as a portal to the gastrointestinal tract and respiratory system along with acting as the main site for verbal communication. Poor oral hygiene and consequent oral diseases may disrupt the integrity of this area.[12]

The present study was undertaken on two thousand people, with a purpose to determine the ubiquity of dental caries, periodontitis, oral mucosal lesions along with oral hygiene status in relation to the oral hygiene practices.

The findings of the present study showed that there was no statistical distinction between the oral hygiene status of males and females, which is in dissimilitude with a study in Nigeria by Olabisi A et al. [1] The mean decay rate in males was more, however, this was statistically insignificant. This was contrasting to studies by Olabisi A et al. and Umesi- Koleoso et al. where females had more caries but concluded of having no significant statistical differentiation in caries prevalence betwixt the two genders. [1,13] The population having OHI-S score 1 had significantly low decayed, missing and DMFT status.

Umesi-Koleoso*et al*. documented a mean DMFT of 0.72 ± 1.67, whilst a mean DMFT of 0.85 ± 1.50 was accounted for by Okoye and Ekwueme amid younger age groups. This study exhibited mean DMFT of 5.96± 1.57 in the age group 15-30 years and 6.53± 1.79 in the age group 30-45 years. The higher DMFT score may perhaps be since the study was conducted among those having oral symptoms, which called for their soliciting oral health care services. [13,14]

The OHI-S score was relatively high throughout all age groups signifying uniform neglect of oral hygiene. Similar findings were found in a study in Vellore by Athuluru et al [4] The oral hygiene status in urban population was slightly better than periurban and rural populations, but with no significant statistical distinction. The mean decay rate in urban and rural population was similar, but the mean missing rate was lower in the urban population.The average number of filled teeth was significantly greater in the urban population. This could be attributed to a higher level of awareness and availability of dental care.

It was seen that non-vegetarians had significantly better oral hygiene and pescetarians had relatively poor oral hygiene as compared to vegetarians.In all age groups, around 95.6% of this study population exercised tooth brushing for cleaning their teeth, which is higher compared to the data in the National Oral Health Survey and Fluoride Mapping 2002–2003 (46.37% and 51.9%) [15] and prior studies by Batra et al and Singh et al. [16,17] Though the majority of the population used toothbrush as cleaning aid, their oral hygiene was not good, suggestive of improper brushing techniques. Use of soft-bristled toothbrush showed better missing, filled and DMFT status.

People who cleaned their teeth twice per day showed a significantly low mean of missing teeth and a high mean of filled teeth. The number of missing teeth was higher in people

who cleaned their teeth occasionally. The periodontal health was significantly better in people who brushed their teeth twice a day.

Fluoridated toothpaste was the most common adjunct used along with a toothbrush to clean teeth. People using fluoridated toothpaste showed low decay and missing status and their filled status was significantly higher than those using non-fluoridated toothpaste.

In our study, mouth rinsing (83%) was the most adopted other oral hygiene aid by many people which is similar to the data of the National Oral Health Survey and Fluoride Mapping 2002–2003[15] and a study conducted by Singh SV in 2013. [17] People using no oral hygiene aid (other than brushing teeth) showed greater decay status. Those using mouthwash for oral rinsing had low decay, low missing, and high filled status. The DMFT status was comparatively lower in those practicing dental flossing. The overall oral health of those using mouthwash was significantly better.

Marinho VC [18] and Zeger SL [19] associated the wielding of dental floss and other interproximal cleaning aids with a decline in periodontal diseases such as gingivitis, but their studies did not demonstrate their association with a decrease in caries. The population that visited the dentist in the last 6 months showed better oral health status which could be attributed to oral prophylaxis, professional dental care, and procedures. This survey reported high levels of gingival bleeding and an even higher number of people with calculus but low levels of advanced periodontal problems. This was in accordance with other studies by Athuluru D [4] and Varenne B. [20]

About 2.4% population had the presence of oral precancerous lesion and 1.9% had the presence of oral precancerous condition. In their study, Gonzalez-Henandez et al. concluded that daily tooth brushing is a preventive factor antagonistic to oral cancer. [21] Deficient dentition, measured by the number of missing teeth and amount of cariosity, was incidental with the risk of oral cancer as observed by Graham et al. [22]

In contrast, poor oral hygiene had only a trivial bearing on the risk of oral cancer as observed by Marshall et al. [23]. and Talamini et al. [24]

We did not observe a significant correlation between oral hygiene and oral precancerous lesions or conditions.

In their case-control studies comprising of thousands of cases, Guha et al. described in their results that missing teeth and periodontal diseases due to inadequate and infrequent tooth brushing, indicated by the poor oral condition of the mouth may be unassociated causes of oral and esophageal cancers. [25] About 12.7% of the population had traumatic ulcers which could be attributed to faulty rigorous brushing or use of hard-bristled toothbrush or alternates to a toothbrush, presence of sharp tooth, etc. Substandard oral hygiene due to fitful tooth brushing, improper brushing techniques and inadequate use of oral hygiene aids is a risk factor for multiple oral diseases. These risk factors are modifiable and require patient compliance; hence, these emphasize the exigency to elucidate the public and policymakers for disease prevention.

CONCLUSION:

Overall this survey has dispensed a critique of the oral health status of the South West Coastal population in India. As ever, primary prevention remains ideal for disease control. By establishing a significant relationship between caries status, periodontal status, mucosal lesions, oral hygiene status, and oral hygiene practices, further policies and plans can be implemented to achieve behavioral changes to bring about an amelioration in oral health status. Additional research to scrutinize the oral health of the various rural populations and augmentation of constructive dental conventions are entailed.

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REVIEW ARTICLE

3 D printing and Endodontics: Reviewing this eternal connection.

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ABSTRACT:
Dentistry being a composite of art, skill, biology and technology has been advantageous on the front of accepting digitalization with ease. Major breakthrough in the field of diagnosis came with Cone Beam Computed Tomography. However, few could imagine that the real game changer was yet to come. 3 D printing stands tall today on the platform raised by CBCT. 3 D printing is new to dentistry but roots down to 3 decades to its inception. Its use is well known in automobile, aerospace and other industries. Endodontics is one field which has harnessed all the potential of 3 D printing technology. Right from diagnosis to making clear models, surgical stents and templates, Endodontics has found the application of this technology in all its versatility. The review throws light on the basics of 3-D printing technology, different types of printing machines and its indication in the field of Endodontics along with documented cases and literature.
KEY WORDS:3 D printing ,CBCT, endodontics, Rapid prototypes.

INTRODUCTION

3D printing describes a manufacturing approach that builds objects one layer at a time. Multiple layers are added in a similar way to form an object. Common synonyms used to describe this technology are additive manufacturing and rapid prototyping.[1,2]

The inception of this technology can be dated back to 1990s. It is a preferred mode of manufacturing in the architectural, automobile and aerospace industry.[3,4,5] Vintage cars parts and requirement of customized parts like in satellites and aerospace, orthopaedic parts, architectural models had increased its use to many other fields. Today it is being used in medicine, dentistry, sports industry, Jigs and Fixtures, forensic medicine etc.[6,7]

Dentistry has a long time association with Computer Aided Design Computer Aided Milling (CAD CAM) It is a Subtractive manufacturing process in which removal of material from a block is done to get desired model. CAD CAM is commonly used for the milling of crown copings and bridge frameworks in modern dentistry.[8] It had allowed us to use more recent and aesthetic materials over traditional casting alloys. This has given the liberty to use different type of materials to create models, identical anatomical structures, make customized objects while saving labour and time.[9] The dental technicians get more time for precise layering and characterization thereby improving the overall esthetics and functionality of the prosthesis.

CAD CAM has its own set of limitations. Process is slow and leads to wastage of material. Accuracy is governed by the properties of the material, size of the cutting tool, the directions in which cutting tool can move and the complexity of the end product. It also demands the dentist to communicate the surrounding structures, occlusion records etc.[10 11] 3D printing, however, comes into its own for the accurate one-off fabrication of complex structures in a variety of materials with properties that are highly desirable in dentistry and in surgery. It is classified in to 4 types based on manufacturing methods Here is a chart explaining you about the division and subdivision of 3 D printing technologies.[12]

1. **Liquid-based processes**
   In this technology, the model is created by selectively solidifying liquid resin by application of laser or other light source such as UV light. Photo polymerized resins are used in this technology. Stereolithography, inject printing and poly jet printing are the 3 D printing technologies which works on liquid based process.
2. **Powder-based processes**
   In this broad category of 3 D printing object is prepared by adhering fine powder into increments with the help of adhesive spray or fusing powder granules with heat/laser**.** Selective laser sintering and Direct material laser sintering are produced by this method.
3. **Solid-based processes**
   These printers extrude molten or semiliquid material from a nozzle to create objects. Commonly used are molten thermoplastic materials that set very fast after leaving the nozzle head. Fused Deposition modelling (FDM) are solid based technology.
4. **Paper/ Plastic based process**
   These 3D printers are based on principles of lamination. Cutpaper, metal or plastic are successively layered and glued together to form a solid object. The sheets of paper are cut by blade or laser before being glued together. Laminated object layering in one such example.

Stereolithography, FDM and Polyjet printing are the most commonly used 3 D printing Technology in dentistry. In 1983, Charles Hull printed a three-dimensional object by Stereolithography for the first time.

Principles of 3 D printing[7,13]:

The process by which 3 D printed models are created is divided into following steps

1. Acquisition of 3 D patient model which could be physical or digital models.
2. Creating STL file
3. Preparing a model for printing
4. Printing of a model
5. Post processing of a model

1. **Acquisition of 3 D patient model which could be physical or digital models:**
   In medicine/ dentistry, the data we have an access to is a volumetric data in the form of Computed Tomography (CT) data, Cone Beam Computed Tomography (CBCT) data and Intraoral Or Laboratory Optical Surface Scan data.. All the volumetric data from CT/CBCT is in DICOM (Digital image for communication in Medicine) format. CAD software of 3 D printers is not compatible with DICOM, so it is to be converted into Standardized format(STL) which is compatible with CAD.
   Normally, Optical surface scan data is compatible with the CAD software of 3 D printer. Most of the 3 D printers are having in built CAD software which allows acquisition of Digital image directly. Impressions of Crown, bridge, inlay etc. are acquired in the similar way. The digital images obtained are directly sent to 3 D printer for preparation of model.

2. **Creating STL file**
   STL stands for is Standard Tessellation Language (STL). 3D printers understand individual objects (or "parts") defined by surfaces that enclose a region of space. The STL format defines surfaces as a collection of triangles (called *facets*) that fit together like a jigsaw puzzle. DICOM files has to be converted into STL format to be compatible with 3 D printer. This work normally requires skilled radiologist to minimize the error and for production of accurate model. Additive Manufacturing File Format (AMF) was introduced in June 2011 to overcome the limitations of the simple STL format, such as enabling the user to incorporate features including surface texture, color, and material properties into each part.

   High contrast images are preferred for accurate reproduction of 3D model. CT images are having higher contrast than CBCT images. If we are receiving volumetric data in the form of CBCT then Large FOV CBCT are preferred over Low FOV. This however requires more radiation exposure for the patient.

3. **Preparing a model for printing**
   Radiologists define objects of interest by separating structures on DICOM images on the basis of tissues and pathophysiology. From the entire scan, the area of interest is identified which is later converted into STL format. In Dentistry, as major data is in the form of CBCT, the soft tissue details are recorded with rubber base impressions and sent to the laboratory. In the lab, the superimposition of both the images is done with help of specialized software.

4. **3 D printing**
   Depending upon the type of manufacturing technology we use, printing is done on the platform by adding one layer over the other. The thickness of each layer is determined by the distance the platform is lowered after completion of one layer (typically 0.003-0.002. A subsequent layer is formed on top of the previously completed layers. Each succeeding layer bonds to the previous one because of self-adhesive property and thus form a complete, three dimensional object.

5. **Post processing of a model**

This includes removal of support, Sandblasting/Jet-washing/Grinding, heat treatment and sterilization of models.

USE OF 3 D PRINTING IN ENDODONTICS

1. Guided Access Cavity Preparation for calcified tooth

Pulp canal obliteration is the most common finding in the routine endodontic practice.It is one of the chief reasons for the root or crown perforation. Mostly in Endodontics, now a days calcified canal is treated with help of Dental Operating Microscope but sometimes when the calcification is extended beyond middle third of root 3 D guides are of a great help. These guides will help you to do accurate entry into the pulp canal space where canal is present. To prepare the guide, CBCT of the tooth is evaluated in all the sections like sagittal, axial and coronal. Later with the help of CAD software the actual canal location is determined. Rubber base impressions of the arch are taken to get the soft tissue details whereas CBCT will provide you hard tissue details. Scanning of Rubber base impression will be done to convert it into digital format. Later both the digital soft and hard tissue images are overlapped and used to prepare the guide. Here it is very important for the operator to select and communicate the bur dimensions which will be used while preparing the access cavity before printing the guide. Clinical utility of this technique is well documented in multiple case reports involving anatomic abnormalities and obliterated canals.[14-17]Buchgreitz et al. (2016) found the mean deviation of the access cavities to be lower than the 0.7-mm threshold and Zehnder et al. (2016) and Connert et al. (2017) also found small deviations from the intended access (0.12–0.34 mm at the tip of the bur).Guided access has been found to be very effective overall.[18-21]

2. Patient Education

Dentists have relied heavily on radiographs or photographs for patient education and motivation. However, a layman finds it difficult to comprehend the radiographs especially the minute details an Endodontist can perceive. Hence there is difficulty in obtaining consents and make the patients believe in formulated treatment plans. But due to the availability of 3D printed models, patient education has been enhanced significantly.[7,22,23]. It has become convenient for the patients to observe and understand the nature of the pathology and the endodontic therapy required for the same. This in turn has increased the awareness and even the compliance of patients for non surgical and surgical endodontics. Right from the curvatures of the root, to anatomical abnormalities, size of the pathologies, everything can be explained to the patient with ease using colour, large sized models[24].

3. To Understand Internal Complex Anatomy Of The Root Canal/ Surrounding Area

Encountering complex root canal anatomy is a common phenomenon. The inherent risks or procedural errors like perforations can happen in the event of minor negligence or failure to understand the anatomy[25]. Such an incident can compromise the treatment

outcome or negatively affect the prognosis. Double curvatures, multiplanar curvatures, dilacerated roots, fused roots, pulp canal obliteration, anatomic abnormalities like dens invaginatus, premolars with enigmatic anatomies, all can be visualized for mental imaging through3D printed models before performing the procedure to avoid any mishaps.[22,24,26,27] Sectional 3D printed models have proven to be of prime importance in determining the apical morphology of root canals allowing view of laterals, accessory canals and apical ramifications. In cases of C shaped canals, radix entomolaris and paramolaris the 3D observations provide the operator with a complete panorama of the canal system for efficient chemicomechanical preparation and three dimensional obturation.[28]It also makes the study of adjoining vital structures as well as pathological lesions convenient and convincing. This further aids in precise diagnosis and predictable treatment[22].

4. For Research Purpose

The exponential advancement in endodontics is the result of an enormous amount of research work being done in the field. In vitro studies form the base of the research which is further confirmed by in vivo studies and clinical trials. However it is difficult to establish a certain criteria for a large sample size in a clinical trial. Many in vitro studies require standardized teeth samples in terms of anatomy. This could be root anatomy, its curvature, root canal shape or its configuration. These studies demand exhaustive effort in sample collection as the inclusion and exclusion criteria's lead to loss of sample. Even after best of efforts, it is an impossible task to select a particular type of anatomy with all similar features in large numbers. This daunting task has been nullified because of 3D printing technology. A typical anatomy or a clinical scenario which needs anatomic reproduction can be easily replicated and duplicated in any number. All the samples are true representatives or identical prototypes and the study can be conducted without any unknown errors or bias. This ability of 3D printing has recently been employed in pre-clinical research. Factors such as the shaping ability[29] and stress values [30]of different rotary file systems, centering ability of access preparations [31]and different obturation techniques for C- shaped canals[32] have been investigated with uniformly controlled canal configurations. Mohmmed et al. (2017a)[33] demonstrated growth of E. faecalis biofilms on SLA materials comparable to dentine and subsequently applied this novel in vitro model to evaluate irrigation techniques [34,35].It has been used very extensively in medical and surgical field such as orthopaedics, neurosurgery, cardiovascular & respiratory & thorax. However the use in dentistry is scarce but is surely taking up pace with time. Use of 3D printing in endodontic research will help in recreating difficult and scarce clinical scenarios which will help in redefining the arcs of endodontic treatment.

5.As surgical guide/ as reflector

Endodontic microsurgery surgery requires meticulous planning and subsequent careful handling of soft and hard tissues. Improvements in magnification, armamentarium and materials have established EMS as a predictable procedure [36,37,38].Tissue preservation during surgery is of prime

importance which defines the success and failure of surgical treatment and duration of healingoutcomes[35]. Human errors and its consequences cannot be ruled out from this field. 3 D printing has found its application in this discipline and has proved its worth. Stents have been printed which directly lead to osteotomy perforation sites preventing unnecessary bone loss and saving precious surgical time. Free hand technique has been found to be inferior to this technology in an in vitro model [39]. Cases presenting with intact Buccal cortical plate but with large periapical lesions have been treated using this technology. Stents defining the outer dimension of osteotomy are prepared. The bony cuts made allow the bony plate to be removed in toto and replaced to the original site at the end of the procedure. Targeted stents also prevent any neurovascular bundle damage. A case report[40] described the use of a 3D printed guide for traditional root-end surgery. Strbac et al. (2016)[41] designed a stent defining the upper and lower margins of the osteotomy, as well as the root resection site and angulation, resulting in increased clinical efficiency and precision, minimizing risk of sinus perforation. Soft tissue reflection has been made possible using stents during the surgery. This prevents soft tissue injury as well as allows adequate reflection and convenience during the surgery. Patel et al. (2017)[42]demonstrated the use of a 3D printed custom tissue retractor to enhance visualization and soft tissue handling during EMS on a maxillary incisor.

6. For Autotransplantation of tooth

Auto transplantation is a viable treatment option in young patient with early loss of 1st or 2nd molar, in cases of impacted canine, avulsion of anterior teeth or when we want to save need natural tooth for strategic location. Success of auto transplantation is largely depends upon the viability and minimal damage to PDL cells. Extra oral time of the transplanted tooth is crucial for the long term success .In conventional methods, after extraction, the extracted tooth is used as template for the recipient site and it has to bear all the insults made during fitting in the bony socket .This leads to the increased extra oral time and damage to the PDL cells.[41,43,44,45]. 3 D printing model of the tooth which is going to be transplanted, can be a great help in this situation. Manipulation of the recipient bone sites can be completed with 3 D printed tooth. After checking proper fitting of the 3 D model, the actual tooth is extracted and placed in the socket. This reduces extra oral time and prevents damage to the PDL cells from repeated insertion and removal. Studies, case reports and in vitro models supports this method.[46-54]Strbac et al. (2016)[41] used the autotransplantation of immature premolars in a maxillary incisor avulsion using a 3 D model. The CAD was used for the selection of donor teeth based on the dimension and stage of root development. Prototype tooth was modified to accommodate the dimensions of Hertwig's epithelial root sheath and to minimize damage to the apical papilla. A systematic review by Verweij et al. (2017)[45] found an overall success rate of 80–91% when 3 D printing was used for auto transplantation and the extra oral time was reduced to 1 minute.

7. Using clear tooth technology for preclinical work and simulation

Clear teeth allow visualisation at every step and real time feedback of the procedure being performed right from access cavity, working length determination, biomechanical preparation to obturation of canals. Freshly extracted teeth or human cadaver teeth do provide a clinical simulation but they are difficult to obtain and do not allow to visualise the procedure being done inside the canal. Their storage, disinfection and the ethical concerns associated are always a hindrance. Simple resin blocks can be obtained at a cost but do not give natural tooth type tissues to work upon. 3 D printed tooth models are the best alternative to all as they overcome the problems of both. 3 D tooth models which are fabricated of porous hydroxyapatite based matrix with hardness similar to dentin have been made [55]. Specialised models for practicing regenerative endodontics have also been developed. As per Marending et al. (2016)[56], 3 D printed models (RepliDens, Zurich, Switzerland) were well accepted with the students as there was no variation in different models.

8.Simulation/mock procedure model

Simulation exercises on tooth replicas are common and advantages over extracted teeth [55-58]. It has been demonstrated earlier for cases like extra- canal invasive resorption [28] and molar with three distal roots [28]. Kfir et al. (2013)[27] used a clear tooth replica to simulate ideal access, instrumentation and obturation preoperatively in a complex type 3 dens invaginatus scenario, before treating the clinical case. There is a huge scope of this indication in training residents for endodontic surgery in complicated case scenarios. Hence 3D printed models provide for preparations, assessments and trials before the actual treatment.

CONCLUSION

3 D printing has lit up the link between conventional blind endodontics and the real hidden world of endodontics. It's upto the doctor now to "See it to believe it" and perform the procedures in most predictable manner utilizing this technology. Be it preclinical exercises, research work or handling a complicated case, 3 D printing has it all covered.

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REVIEW ARTICLE

The Caries Continuum: The Fluoride Shibboleth

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ABSTRACT

Continuum is per se a constant cycle in which adjoining elements are not discernible different from one another, but the extremes are quite distinct. Dental caries is also a disease continuum and over the centuries there has been a dramatic perception in the understanding of the disease process, the dynamics of demineralization and remineralization mechanism, the microbial dysbiosis of the biofilm, the caries detection and diagnosis methods and evolution of population-specific management strategies. This paper gives a structured overview of the current concepts of cariology and the available therapeutic options, beyond the traditional use of fluorides, available to prevent, intercept, combat and even reverse the disease process.

Keywords: Dental caries, demineralization, remineralization, microbial dysbiosis.

INTRODUCTION

Dental caries is a multifactorial microbial oral malady leading to dissolution and destruction of calcified tissues of the teeth. Till date, it stands to be an omnipresent, widely prevalent, most common global health care concern. In the event of continuous, generous intake of fermentable carbohydrate compounded by factors like lack of salivary buffering clearance, poor oral hygiene practice it results in microbial dysbiosis due to increase in acidogenic, aciduric bacteria like streptococci mutans. This ecological shift will tip the balance in favour of demineralization of the dental hard tissues which if not limited will over a period of time lead to frank cavitation of enamel.[1-3] As per the Global Oral Health Data Bank, the prevalence of dental caries ranges from 49% to 83% across the globe. [4]

FLUORIDATION:

There has been a steady regression in the incidence of caries in developed countries owing to their well-rounded and structured preventive programs. The health care program of America is a fluoride centric one with the thrust both on topical fluoride application and fluoridation of water, 70 % USA has fluoridated supply of water.[5] Centres for Disease Control and Prevention has heralded the fluoridation of drinking water as one of the 10 monumental public health landmarks of last century. But the rest of the world is divided on the implementation of water fluoridation. Most of the countries of Europe do not implement fluoridation of drinking water, except Ireland, Poland, Serbia, Spain, and the UK. Salt fluoridation is practised in Germany, France, Switzerland, and Austria. Barring these countries, the rest of Europe has refrained from water as well as salt fluoridation [6]

In the lower and middle-income group or developing countries, the prevalence of dental caries is much higher. In India, water fluoridation still remains an unsurmountable oral health issue due to the diversity in ethnicity, food habits, socioeconomic strata, cultural beliefs and habits. The immensely diverse variables are further impacted by a serpentine oral health care policy with a meagre on the ground dedicated workforce. Thus, dental caries continues to be a menace which is the prime cause of loss of teeth accompanied by the psychological trauma it entails. Water fluoridation as a viable caries preventive modality has been widely studied but the inherent challenge is in its implementation and it has been documented that only 10 % or lesser, of the population of world, has direct approach to this preventive modality. [5-6]

Safe and clean water to drink is a constitutionally guaranteed right in India and millions of rupees are invested in water sanitization however centralised systems of water supply and monitoring have been found to be ineffective. Inappropriate levels of fluoride in the drinking water lines have led to skeletal and dental abnormalities. Thus, adherence to the recommendation of WHO for the upper limit of water fluoridation is 1.5mg of fluoride per, litre, depending upon the climatic conditions, is absolutely essential. This is also the protocol followed by most countries including Canada, China, Australia and some European nation.USA had a higher upper limit of water fluoridation but have now reduced it from the earlier practiced 4mg/l.[7]

Increased concentration of fluoride in groundwater occurs in the majority of the dry and arid landmass of India. According to the Geological Survey of India (GSI), there are certain regions in India which should be considered as fluoride qui vive. Fazilka and Jalalabad in Punjab, in the frontier district of Ferozepur; Region of Haryana like Hisar, Faridabad, Gurgaon, Fatehabad, Rewari and Mahendergarh ; parts of Uttar Pradesh like Rae Bareilly and Unnao; in Madhya Pradesh it is commonly seen in Sidhi; in some district of Tamil Nadu and in parts of district of Beed of Maharashtra; and in parts of Andhra Pradesh of in district of Nalgonda.[8]

Not only in India, but also in countries being traversed by a natural fluoride belt, like parts of Japan, America, Algeria, Afghanistan, China, Libya, Syria, Jordan, Egypt, Sudan, Kenya, Turkey, Thailand, Iraq, Iran, and the natural fluoride levels is a matter of grave concern.[9] It could be the reason for the upsurge of the anti-fluoride campaign. The world is divided over the fruitfulness of fluoridation of water in prevention of carious lesions. Most of the civic health state machinery globally is of consensus that optimal water fluoridation, commensurating with the climatic conditions is an effective method of combating initiation of dental caries.[9]

In 2015 the Cochrane Review published a critique on fluoridating public water supply for preventing and inhibiting carious afflictions. It was in consonance with other studies that water fluoridation brings down the prevalence of dental caries in primary dentition and young permanent teeth. The authors did not find sufficient evidence to determine the utility of water fluoridation in adults. Likewise, this review did not find sufficient data to stop water fluoridation program thus squashing the demands of the anti-fluoride campaign. However, they found sufficient evidence attributing the incidence of dental fluorosis with increased fluoride levels up to 5mg/L in public water supplies.[10]

The anti-fluoride group have challenged the role of water fluoridation in preventing dental caries. The group is divided and some are of the opinion that topical fluoride application is a more effective preventive strategy. Rationality of why western regions of Europe have not embraced fluoridation is that fluoride is a chemical/ medicine, thus to administer any medicine one has to take informed consent. Further, it is a community-based programme and thus it cannot be individualized for every individuals‘ specific need. Be it a child, an adult, a medically compromised person with liver dysfunction or renal failure each individual will get the same amount. The 2000 Nobel Laureate of year 2000 for in the field of medicine and the science of physiology, Dr Arvid Carlsson was instrumental in, Sweden exercising a veto against Fluoridation. According to him drugs and its dosage should be given as per the nature of the ailment but water fluoridation is not individualistic. Further, there are other sources of fluorides in food and beverages and this may add to the total fluoride load.[11] According to Cheng et al, ‒although water fluoridation has been practised for nearly 70-80 years, there still hasn‘t been a randomized controlled trial.‖[12] Thus evidence-based research on water fluoridation is few and more trials need to be undertaken.

Fluoride is a known cumulative poison which even in low concentration is biologically active and inhibits various enzymes as it interferes with bonding of hydrogen ions.[13] Only 50 percent of ingested fluoride is excreted and remaining has been reported to be deposited in human pineal gland.[14,15] It is an established hepatotoxic and nephrotoxic in children[16]. Studies have shown correlation between fluoridation and chromosomal aberration, mutagenicity and malignancy. [17] A 14-year Harvard study has shown fluoride to be the potential causative agent for osteosarcoma.[18] Many studies have also proved it to be a neurotoxin.[19-21] High degree of fluoride ppm in water was stated to affect the IQ

of children.[22,23] When fluoride is added to water in which there is aluminium, it will lead to formation of complexes of aluminium fluoride which can interfere with neurochemical and hormonal signally mechanism.[24] Thus, water fluoridation has been shrouded in controversy and its implementation has both the public health authorities and medical fraternity divided.

TOPICAL FLUORIDE APPLICATION

Along with other treatment modalities, topical fluoride application has been widely practised to diminish the risk of caries developing. Randomized clinical trials have provided the cariostatic benefit of topical fluoride application. The evidence-based studies prove not only are topical fluorides successful in preventing dental caries but also are safe especially in individuals who are at high caries risk.[25]

Hence postulation is that fluoride‘s preventive effect is attributed to its propensity to reduce the decalcification of calcified tissues and inhibit growth of bacteria and interfere with its metabolism. Demineralization takes place at a critical pH when the oral habitat is has a low concentration of ions, in comparison to the tooth‘s ionic content. The enamel crystal or lattice of enamel is constituted primarily of carbonated apatite and in presence of refined carbohydrate, the dissolution of it takes place by the organic acid which are the produce of metabolites of cariogenic biofilm. Remineralization is the process by which the weak link i.e carbonated apatite is replaced by acid-resistant crystals of fluoro-apatite by ingress of ions of calcium and phosphate. Caries thus is a dynamic process with interlaced spell of demineralization and remineralization and fluoride therapy has been the most widely used and accepted means of caries prevention. [26-28]

However, current studies indicate that repeated application of fluoride leads to development of fluoride resistant strains of Streptococci mutans. The cariogenic microbes have gradually progressed and metamorphosed to strategize means and methods to counteract and withstand certain ppm of fluoride. A strain resistant to fluoride can grow and survive in an environment which is of 3 to 4 times the level which a fluoride sensitive strain can tolerate. This acquired resistance may be transient or permanent. Thus, the need of the hour is to look beyond the scope of action of fluoride and search for an alternative regime for caries prevention and inhibiton.[28-31]

ALTERNATIVE THERAPEUTIC STRATEGIES

Fluoride antimicrobial action is targeted towards three main characteristics of oral cariogenic microflora i.e adherence of microorganism to tooth, the acidogenicity and the acidurance or aciduric property. Thus, the game plan would to develop a therapeutic strategy which targets the biofilm, either the integrity of the biofilm or its adherence to the tooth.[31]

Streptococci Mutans partake in genesis of cariogenic biofilm on ecologically viable niches of the tooth. Cariogenic microbiosis are essentially well-assembled communities of microbes housed in an extracellular gelatinous array. Sucrose is essential for the adhesion and coaggregation of streptococci. Another significant role player are the enzymes known as glucosyltransferases (GTFs), they are intrinsically integrated by strains or serotypes of streptococci. The initial fitment paves way for formation and growth of microbial film. The adhesin - antigen I/II of streptococcal mutans coapt with galactosides of the saliva acquired glycoprotein which in turn is the epicarp or pellicle deposited on the tooth. Alternative moieties of surface adhesion are glucan-binding protein (GBP), GTFs, and serotype carbohydrate. When sucrose is present, then GTFs generate extracellular glucans from glucose. The GBP is a receptor agonist protein which is distinguished and markedly different from GTFs, and categorically tether to the glucans. GTFs, they themselves have a definitive glucan-receiving dominion and hence can serves as site for receptor- adhesion for glucans. Thus, mutans streptococci conjoin precast glucans with help of GBP and GTFs, and this eventually results in accumulation and aggregation of cariogenic microflora.[32] The extracellular matrix provides a protective and conducive low pH ecological niche for the bacteria
to flourish and express its virulence, it also provides biological properties which enhances adhesion and coaggregation of microorganisms. The unique characteristics of the extracellular matrix-mould compounds and complexes the challenge for the establishment of effective antibiofilm regime to eradicate or inhibit caries. [33]

Customary perspective generally focuses at achieving remineralization or focus on the antimicrobial activity but keeping in mind the complex nature of biofilm, may not be a 100 percent foolproof or highly efficacious method. Hence, the new approaches have shifted on disrupting the cariogenic biofilm or modifying it, some newer trends include specific targeting the cariogenic bio-organisms, modulating the biofilm pH and synergistic technique of killing the cariogenic microorganisms and degrading the extracellular matrix. The evolution of newer smart nanotechnology methods that rely on programmed drug release or activation or eliciting a response which is dependent on critical pH. These new techniques are being developed with a view to change the ecological niche of the bacteria so that the bacteria cannot survive and also at the same time create a more conducive microenvironment which facilitates remineralization. [33]

1. IMMUNOTHERAPY IN DENTAL CARIES

a) ACTIVE IMMUNIZATION

The concept of immune protection has generated a lot of interest over the years. Bowen was the first who discussed the contribution or status of immunization in caries prevention.[34] Though he had not standardized the parameters of immunization, he observed that monkeys which were intravenously immunized with Streptococci mutans developed resistant to caries. In a study done by Mestecky et al ingestion of gelatin pilules filled with specific sub particle moieties of Streptococci mutans gave rise to generation of discrete salivary secretory IgA.[35,] This was one of the first studies which

introduced the concept and role of mucosal immune complex in dental caries. Subsequent studies reiterated that in Streptococci mutans induced dental caries under controlled conditions, immunization could alter the response to Streptococci mutans and thus prevent caries. Further, studies by Hajishengallis,[36] Gregory,[37] Luo[38] all established that mucosal immunization had a substantial role to play in caries prevention. Experimentally both active and passive immunization seem like the perfect solution. This seems like a fallacious claim if one takes into consideration the uncontrolled variables like diet, fluoride levels, patients immune status, and length of presence of cariogenic biofilm. Understanding the adhesion of Streptococci mutans to the tooth surface may be the key to deciphering the mutans code. Targeting the specific moieties and phases of adhesion, aggregation coaggregation is what is desired and should be practised. [36]

In theory, many stages of Streptococcal mutans contagion are liable to immune mediation. Microbiota teeming in the oral salivary fluids are removed from the oral cavity by antibody orchestrated aggregation even before the colonization of the said organisms. Antibody may block the bacterial-surface receptors that are required for adherence of caries superbug or deactivate the enzymes which are liable for glucan genesis or they can alter metabolic relevant assignment script of the enzymes. The inference drawn from all these studies is that adherence of the biofilm and or the metabolic cycle of the Streptococci mutans will be targeted. Deliberating on the molecular pathogenesis of dental caries, it can inferred that purified antigens of streptococci mutans can be isolated and used to kindle immunity in laboratory caries model.[33] Subcutaneous or subdermal inoculation at the vicinity of major sialaden glands of purified fraction of surface adhesin Antigen I or II or intranasal inhalation or passive administration of the antibody will prevent adhesion of the microorganism to the pellicle and aggregation of the mutan streptococci.[33]

The intact GTF might be injected hypodermally in proximity to significant sialaden glands, else intrarectal administration may also be explored. Another route which can be studied is oral or buccal mucosal instillation. Antibody designated for that particular GTF operation can disable GTFs and hamper with glucan integration, thus preventing adhesion and coaggragation of streptococci mutans. Glucan binding protein has also the same route of administration along with passive administration of the antibodies. The mechanism of action is same that it will interfere and prevent coherence of the microbes to the hard tissue of the tooth and also prevent aggregation of the microorganism.[33-38]

b) PASSIVE IMMUNIZATION

To avoid any inherent complications arising from administration of active immunization led to the development of oral application. Passive immunity is conferring active humoral protection with preformed agglutinin. Passive immunity is shown to emerge naturally in the event of maternal agglutinins being relayed to the in-utero foetus across the placenta. Another means of artificial induction is when high dose of attenuated

specific antibodies to a specific pathogen are introduced with antibody laden products in non-immune individuals. Compared to active vaccination, passive immunization has well-defined advantages such as high specific activity, devoid of toxicity and lack of reactivity with the human complement system thus prevents development of nonspecific inflammation and has a rapid onset. Conversely, according to Mai et al, in the event of lack or deficit of expected active reciprocation on the part of the recipient, there is absolutely zero ordination of immunological recognisance, and thus the inoculated agglutinins will linger for limited time in oral cavity, ranging from few hours to 3 days in dental plaque. Hence repeated applications are required. Passive immunization for dental caries includes transgenic plant antibodies, bovine milk antibodies, monoclonal antibodies and use of egg yolk based antibodies.[33]

Monoclonal antibodies to AgI/II topically applied to enamel surface after oral prophylaxis adheres to the salivary pellicle. Streptococci mutans itself binds to the antibody and this may directly lead to bacterial liquidation caused by activation of the complement pathway and opsonization. The use of Guy‘s 13 monoclonal antibodies has been hypothesized to directly impede with the adherence mechanism of mutans. Studies by Michalek, Lehner have shown that after cessation of antibody treatment the newly developed plaque will be resistant to new colonization of streptococci mutans.[40-43]

Eggs have been deliberated upon as an easily available source for the production of polyclonal lectin which is unique and distinctive to a variety of agglutinogen such as bacteria, hormones, enzymes or virus. Evidence-based studies have shown that yolks from the eggs of S.mutans inoculated chickens provide agglutins which are known as Immunoglobulin Y (IgY). It confers passive immunity against caries by immobilizing S.mutans and disabling S mutans ability to convert refined sugar into acid. The IgY immune counter induced by inoculation with serotypes of S.mutans, MT8148, exhibited ELISA receptivity with several, at times 7 serotypes of mutans when they were cultivated in a sucrose mimicking, environment. A study was conducted by Kruger et al to ascertain the virtue of chicken egg yolk lectins against GTF’s dispensed as a drinking infusion experimented in a desalivated animal archetypal model. Evaluating the inhibition of assays, it was observed that the chicken anti-GTF antibodies diminished the activity of both GtfC and GtfB factions in mixture. IgY antibodies against Cell associated Glucosyl transferase (CA-GTF) of S mutans are obtained by immunizing hens with CA-GTF antigen, causing them to produce antibodies as part of their normal immune response. The antibodies thus produced are transferred to the egg yolk through maternal transfer. These IgY antibodies which are proteins are then extricated from the yolk of eggs laid by the hens, extracted and purified by the classic process of protein purification. Once formulated it is given orally to humans, where they are reported to diminish the quantity of habitant S mutans colonies by forming antigen-antibody complexes of mature dental plaque. Several mechanisms have been postulated for the anticaries mechanism of IgY;The most accepted is the prevention of bacterial adherence, agglutination, opsonization followed by phagocytosis, neutralization of toxins and inactivation of enzymes.[43-47]

IgY has been observed to aggregate S mutan cellules into extensive assemblages, making them vulnerable to deracination by salivary clearance and tendering them incapable of anchoring on and eventually to colonize on enamel surface. It has also been noted that anti-CA-GTF moiety of IgY efficaciously abolishes the GTF catalytic locus, thereby inhibiting the ability of CA-GTF enzyme to produce water-insoluble glucan as well as impede various facets of protein glucan synergy. This results in ineptitude of streptococci mutans to affix to teeth enamel with support of glucan reliant cohesion and disrupts early stage of biofilm formation [43-47]

In an unpublished data by Sharma & Londhe et al, it was observed that Ig Y passive immunization was as effective as laser caries inhibition method in reducing adherence of streptococci mutans. (Fig 1) Further, both laser group and IgY group alone or in combination fared better than fluoride application group

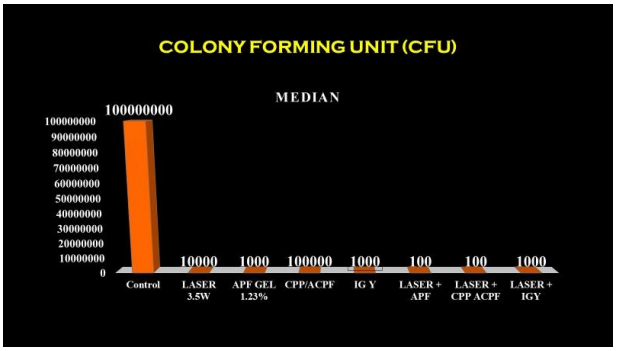

Fig 1:Comparison of effect of different surface treatment on adherence of streptococci mutans.

Systemic inoculation of cows with a vaccine from whole-cell of Streptococci mutans cells generated IgA lectins in serum as well as the milk which is incorporated in daily diet. In a animal study on rats, the use of immunized whey footprinted in undiminished S.mutans, minuscule biofilm formation and truncated caries activity. Another development in field of passive inoculation documented was the trial of transgenic plants to yield the indispensable agglutinin. Transgenic plants proposes countless merits as compared to immunogens procured from zoological sources, the construction of multimeric forms of immunoglobulins and indeed, a secretory IgA has in recent times been fabricated from tobacco plants by cross-hybridization of 4 chains of plants. This immunoglobulin exhibits anti-S.mutans properties and prevents the agglutination of the cell .[45-47]

2. LASER CARIES INHIBITION

In the past, lasers have been investigated for increasing the aid resistance of the tooth, but most of the studies were in-vitro and no parameters were decided upon. The hypothesized mechanisms of actions have ranged from reduced permeability of enamel to chemicals agents caused by melting of hydroxyapatite crystals to changed enamel crystal lattice orientation. The lasers which have been investigated in the past for caries

inhibition are carbon dioxide lasers, Nd: YAG, and erbium family lasers.[48-52] Aluminium gallium arsenide laser was investigated as an alternative to high powered lasers to prevent and inhibit dental caries by Sonali Sharma & Mithra Hegde et al. The hypothesized mechanism of action is that these wavelengths selectively target and remove carbonate ions from hydroxyapatite crystals which results in increased acid resistance of enamel. Furthermore, the modified constituent has exponentially enhanced ingress of topical fluoride application, which in turn decreases the dissolubility and leads to remineralisation of noncavitated lesions. Till date, there were no set parameters which could define means and ways to bring out caries inhibition.[48-52] The authors formulated a standardized protocol for caries inhibition and verified the results in clinical studies. To corroborate the clinical findings, in-vitro studies like Vickers hardness, FTIR, SEM EDAX, XRD were conducted. Based on results of clinical and in-vitro studies it was concluded by Sharma and Hegde et al that irradiation by AlGaAs Laser has a caries inhibitory role and maintained the structural integrity of the tooth. Unpublished data by the authors also reiterates that aluminium gallium arsenide lasers interfere with the adhesion of the streptococci mutans to the enamel.[53-59]

NANOTECHNOLOGY FOR CARIES PREVENTION

The most important aspect to be considered in the demineralization of tooth structure is that a substantial amount of ions are depleted from lattice of apatite network adversely affecting the integrity of the tooth. Since enamel is acellular, devoid of supply of nutrients, the mature enamel once formed cannot repair itself. Subsurface incipient lesions can be remineralized from the nucleation centres of remaining crystals by epitaxial growth, the source of phosphate groups and calcium ions could be from saliva when it is concentrated with regard to hydroxyapatite. The natural biomineralization process has inspired researchers to harness or mimic natural remineralization of enamel and of collagen in demineralized dentin. This concept is known as biomimetic mineralization.[60, 61]

The main protein in development of enamel is amelogenin. It is recommended that de novo interaction between nanospheres and nanofibers of self-assembly of amelogenin be brought about to regulate the seeding, nucleation and exponential expansion of apatite crystals in enamel.[60-62] One method is by utilizing the structured dimensionally stable framework provided by a self-assembling molecular system of amelogenin. Other techniques is to use judiciously constructed beta-lamina-forming peptides that extemporaneously form three-dimensional well rounded fibrillar scaffolds as counter to individual natural trigger-points which set in motion a series of events of mineralization with surface characteristics mimicking that of enamel matrix.[60] P11 4 is one such bioactive peptide which will induce denovo nucleation of hydroxyapatite crystals by assembling into a three-dimensional scaffold in reciprocation to environmental signals of falling pH.[62]

Dentin when reviewed on a structural level it appears as a fibre reinforced composite. The intertubular dentin which is low in mineralized content serves as the matrix and it is fibre reinforced by the highly mineralized peritubular dentin. When comparing remineralization of enamel and dentin it was noted that fluorides have limited role, this could be attributed to the concept that dentin has a more organic matrix and lacks residual crystals which could act as sites for nucleation. This organic matrix is constituted by Type 1 collagen and non-collagenous proteins (NCP). The inorganic constituent is carbonated calcium deficient hydroxyapatite which is embedded in an organic matrix. It has been postulated that NCPs inhibit mineralization and dentin remineralization can be brought about only by their removal. Hence, compared to enamel remineralization that of dentin is a greater challenge. The NCP commonly encountered are dentin matrix protein- DMP1 and DMP2 also known as dentin phosphoprotein. Thus the remineralization of enamel can be brought about by stabilized complexes of amorphous calcium phosphate. For dentin, these nanocomplexes in conjunction with phosphorylated collagen can bring about remineralization of dentin collagen.[60]

Current literature discloses that nanotechnology promises neoteric blueprint in prevention, inhibition and management of caries process, by individually targeting microbial biofilms and enhance remineralization of incipient carious lesions. Nanoparticles consist of a group of substances with dimensions limiting between 1 and 100nm and also possessing exhibiting physiological characteristic with a expansive spectrum of research utilization. The nanoparticles are associated with altered physiological, mechanical biological and chemical properties such as microhardness, physiochemical reactivity and its accentuated surface energy reactivity. Nanomaterials in the group of nanocarriers are also utilized to transport the desired drug or gene from one site to another or serve as target delivery system. Commonly used nanocarriers include micelles and liposomes and they can be used in immunization modality and for transporting antimicrobials to target the cariogenic microbiota. Nanocarriers are thus currently garnering a lot of consideration for its variegated aptitude in futuristic clinical utility. [61]

Knowing the complexities of tooth morphology, and targeting the specific causative microbiota or the ecological niches with specifically functionalized nanomaterials and which in turn get activated at critical pH, would be a much-needed breakthrough. Horev et al has discussed the use of polymeric nanostructures that are constituted by diblock copolymers and 2-propylacrylic acid which self-assemble into cationic nanoparticles in tandem to the fluctuation of pH. They have affinity to bind to the adhesins of streptococci mutans and they can envelop the nonionic zeta potential antimicrobials like farnesol. On fall of pH, these smart nanomaterials can release the antimicrobial.[61] Another technique is use of catalytic inorganic nanoparticles containing biologically acceptable ions like iron oxide with peroxidase-like activity which bind conversely to the cariogenic biofilm. In acidulous conditions, the catalytic nanoaggregates will liberate from H_2O_2 free radicals which will concurrently debase the defensive, shielding EPS

barricading matrix and kill the cariogenic microbiota.[63-65] Other emerging techniques include coating the tooth with a nanocoatings containing chitosan or nanocomposite. Nano remineralizing techniques use remineralizing functional groups such as CaF_2, Beta TCP, hydroxyapatite crystals. The enamel is made up of hydroxyapatite crystals as building blocks. Thus, nanotechnology relies on the fact that nanosized hydroxyapatite crystals can easily integrate into the exposed dentinal tubules and further they have greater surface area and thus bind well with protein. Their high biological reactivity makes them an ideal material to repair dentin and enamel. Current research has shown that to synthesize biomimetic dental enamel, calcium phosphate nanoparticles can be used. Casein phosphopeptide amorphous calcium phosphate nanocomplexes (CPP-ACP), amelogenin chelate with ions of calcium and thus are known to be biomineralizers.[63-66] Zhang et al. have mentioned the concocting of a sound and balanced phosphorylated chitosan-ACP nanocomplexes with the potential to remineralize the subsurface enamel lesions lesion via simulating the biomineralization approach by phosphorylating chitosan with amorphous calcium phosphate (Pchi–ACP), which had the similar remineralization pattern as that of enamel. In fact, it was observed that Pchi–ACP remineralization rate was higher than that of fluorides. [67]

FUTURE PERSPECTIVE

A vaccine to combat dental caries offers a great window of opportunity for a well-rounded public health care policy, especially for high-risk groups. Currently, the endeavour for forging a caries vaccine may be thwarted, as most of the financial resources are directed to address life-threatening issues. Active or passive immunogen stratagem, which attack essential links in the molecular pathogenesis of streptococci mutans hold immense potential . New intriguing strategies targeting the cariogenic microflora and challenging the microbial dysbiosis, which includes mutans -specific antimicrobial peptides, regulation of the pH of the biofilm via arginine metabolism, are still in different phases of research. Smart nanomaterial responding to the dynamic fluctuant pH has the potential to be the mainstay in prevention and management of dental caries. Biomodification of tooth surface by lasers is another potential area of research. The huge gap between experimental lab-based research and clinical trials needs to be bridged.

CONCLUSION

Complex immunogenetic pathways intricated in carious process are very pivotal to deciphering the radical nature of this miasmic malady. It also is pertinent in stemming algorithms of caries detection and diagnosis and thus may pave the way for new therapeutic innovations. Amalgamating these stratagem into broad-based civic health initiative might lead to prevention, inhibition and even eradication of dental caries. Till date fluoride was used to control dental caries not eradicate it. Cariology research in our country is still in the infancy stage and our state legislative health policies should prioritize this aspect of oral health care to enable a reduction in the caries burden.

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REVIEW ARTICLE

Optimizing preclinical training and experience

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Technology and globalization are a wonderful combination. There is rapid scientific, material and clinical development in dentistry[1]. With globalization, the wonders are available for all of us to see, learn and experience. Can dental school education keep up with this pace? There is a recent trend for recent graduates to rely on "practical training" crash courses for developing their clinical skills to tackle real-world challenges in dentistry. With the advent of social media often dental training is sought from "experts" on social media. While any effort to improve as a clinician is appreciated and encouraged the only drawback is sometimes the source of this education lacks credibility or these courses are expensive and time-consuming.

How can the dental fraternity be prepared for the change in trends?

Preclinical training for dental students is a crucial aspect of dental education that if properly utilized can be a key solution to the above challenge. Since this training occurs anywhere between the first and third year of dental school curriculum there is ample time and scope to utilize this training period. The objective of preclinical training is for students to not only develop manual dexterity but to expose them to all kinds of clinical scenarios they may encounter in clinics in and outside of dental school[2]. Optimizing preclinical training and experience can be one way to fill the gap between theoretical learning and practical training.

Following are a few dimensions of preclinical training that can be explored -

A **structured all-inclusive curriculum** can be designed to consider every procedure a recent dental graduate is expected to perform. This is crucial since it is almost practically impossible for all dental students to do all procedures on patients in the dental school setting. For e.g.; while preclinical training focuses on major areas like cavity preparation or crown preparation secondary procedures are often overlooked – eg, gingival retraction cord placement, composite manipulation, utilization of matrices, rubber dam application, deep caries management, bonding protocols for different materials, temporary restoration fabrication, post and core techniques or even treatment planning of cases comprehensively etc. Preclinical training on a mannequin, typhodont or extracted teeth for all the procedures instills a familiarity and foundation for these concepts that students can build upon subsequently.

Competency-based approach – Outcome-based training is being implemented in most western countries. This is outlining the "learning objectives" for training and evaluating periodically if the objectives are achieved. For instance, students can be tested periodically on their manual dexterity by conducting “timed competency exams” on typhodonts or extracted teeth. These examinations should be held on time with resources for the students to be able to practice the skill set [3]

Calibrated scoring criteria- Scoring criteria can be designed objectively so that inter and intra grader variability among the tutors can be minimized. E.g. criteria for different procedures can be explicitly designed as Clinically optimal (A grade), Clinically acceptable(B) and Clinically unacceptable (C grade).Frequent focus groups can be held for tutors to become calibrated as close to one another so that grading is fair and neutral.[4] Studies have shown that unreliable evaluations lead to student frustration, which can manifest in disengagement from the learning process and result in poor performance outcomes.[5]

Clinical shadowing opportunities- Incorporating shadowing experiences for pre-clinical students in their curriculum allows them an opportunity to see their pre-clinical training being applied to patients. Innovative strategies like asking students to write/ discuss their observations can promote critical thinking.

Feedback – Regular healthy feedback from students for different preclinical classes allows room for improvement. This could be in the form of questionnaires with comments to allow for student input in the learning process.

The goal is to provide students with the skill set to serve the needs of society effectively. A society that is becoming increasingly aware thanks to globalization and technology.

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REVIEW ARTICLE

The Robust Journey of Endodontic Sealers- A Clinician's Perspective

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Successful endodontic treatment is primarily based on proper cleaning, shaping, and obturating the radicular space by establishing a fluid impervious seal. Although various root filling methods have been introduced, gutta-percha remains the main core filling material. But since it fails to bond to root dentin, a root canal sealer is used as an adjunct in the obturation of the root canals.[1]

Initially, sealers used were modified zinc oxide-eugenol (ZOE) cements grounded on Grossman or Rickerts's formula that were enormously utilized throughout the world. They provided an adequate disinfection of dentinal tubules in the pulp chamber to a depth of around 250 μm and an effective antimicrobial property when compared to other sealers.[2]

Sealers based on eugenol exhibited greater solubility which was then compensated by AH Plus sealer. It displayed considerably the lowest weight loss among the variety of root canal sealers in water and in artificial saliva with altered pH values, irrespective of the medium of solubility used. [3 When evaluated with other sealers, AH Plus showed the greatest stability in solution form. AH Plus is cogitated as a benchmark "Gold Standard" owing to its exceptional assets, such as minimal solubility, less expansion, dentin adhesion, and an effective sealing ability.[2]

Endodontic sealers based on calcium silicate hydraulic cements were developed since they set in presence of moisture which is regarded as a great clinical benefit. Mineral Trioxide Aggregate (MTA) when used as a root canal sealer compacted against dentin, forms an interfacial layer in the presence of phosphate. When examined under SEM analysis and X-ray diffraction this adherent layer resembles hydroxylapatite in composition and structure[4]

Similar to findings for calcium hydroxide, root dentin is also weakened by alkalinity of MTA. This has also been hypothesized due to the combination of reduced MTA tensile strength and inefficient bonding to dentin. Irrespective of the ideal biologic characteristics of MTA, thin dentinal walls still make these teeth more prone to fracture.[5]

Recently introduced bioceramic sealer, BioRoot RCS has displayed encouraging outcomes. In addition to its biocompatibility and hydroxapatite formation, various studies have reported an increase in the fracture resistance of teeth obturated with it in contast to MTA.[6] EndoSequence BC Sealer utilizes the moisture within the dentinal tubules after canal irrigation to start and finish the setting reaction. In extra dry canals, the setting time of EndoSequence BC Sealer may be extended from the usual 4 hours.The pH of EndoSequence BC Sealer during the setting process is higher than 12 (Material Safety Data Sheet information), which enhances its bactericidal properties.[7] The Ca^{2+}discharged from EndoSequence BC Sealer was way more (2.585 mg/l) in comparison to that released from AH Plus (0.797 mg/l), primarily after 7 days.[8]

Freshly mixed Endosequence BC Sealer displayed more cytocompatibility in comparison to MTA according to an in vitro study evaluating the toxic effect of these sealers on human gingival fibroblasts. [9] [10] In case if MTA based material is extruded out of the canal, severe pain is experienced by the patient which is comparatively little or completely absent, when bioceramic-based sealers Bio Aggregate or iRoot SP are extruded.This absence of pain may be described with the features of these new materials. Hydroxyapatite is produced during hardening and post the hardening completion, they display the same features as the hydroxyapatite based bioceramics which are non resorbable and utilized in bone replacement in oral and maxillofacial surgery. This renders the material as osseoinductive and better compared to its contemporary sealers.However, inconsistencies in the results of various studies disclose that these sealers do not meet all of the necessities required of the ideal root sealer. [7]

The evolution of sealers from the conventional to the most recent bioceramics have the predilection to change the perception the way sealers have been used in the near future. With this new approach of root canal filling, these biomaterials have opened a new dimension on how a sealer can also have the propensity toward mineralization through the formation of hydroxyapatite crystals apart from merely filling voids and creating a hermetic seal.

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REVIEW ARTICLE

Holistic dentistry in a modern practice with Cocos nucifera.

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Abstract: This report highlights the benefits of virgin coconut oil which can be used as an effective preventive agent before onset of dental caries. Along with its antibacterial and antiseptic properties it also has a moisturizing effect on soft tissues, hydrates the oral cavity in cases of xerostomia and gingival inflammation. Hence, need of widespread clinical trials among diverse populations in the future, can promote these nutraceuticals as a topical applicator for patients with chronic systemic diseases and during therapy to patients with head and neck cancers.

Keywords: coconut oil, holistic dentistry, oil pulling, preventive dentistry

Introduction:

The word coconut is derived from coco which is taken from Portuguese and Spanish language during the 16th-century. It gets this name owing to the indentations on the coconut shell-like that on a "human skull" and Nucifera is a term derived from Latin meaning "nut-bearing". (1)

The properties of the coconut palm are so diverse and versatile hence it has several synonyms like "the tree which provides all the necessities of life" (*kalpavriksha*) in Sanskrit, "the tree of a thousand uses" (*pokokseribuguna*) in Malay and "tree of life" in the Philippines. (2) The common product of this plant is coconut water if fermented gives us vinegar. Followed by coconut milk, coconut oil, the husk, and shell are also used as a source of energy in the form of charcoal. A frayed twig of coconut root is used as toothbrushes, a remedy for dysentery and diarrhea in rural India even today. (3)

Discussion:

Of all its produce Virgin coconut oil (VCO) is in recent times gaining popularity globally due to its medicinal and holistic properties. It is obtained directly from fresh coconut meat by either drying the freshly grated coconut at a temperature less than 600 C, followed by pressing for oil or by adding an enzyme to milk from the coconut meat and aging for several hours.

Refined Coconut oil and VCO are almost the same in terms of chemical properties, both forms of oil have medium-chain fatty acid (MCFA) in their compositions. Commercially available oil is refined, bleached and deodorized and hence loses the biologically active substances. *Tocopherols, Tocotrienols, Phytosterols, Flavonoids*, and other *polyphenols* are all biologically active constituents of virgin oil with antioxidant activity. They may also have anti-atherogenic, anticarcinogenic and antiseptic actions. *Tocopherols* may also have a role in the prevention of coronary heart disease, gingival bleeding, and cancer. (4) Virgin oil is unprocessed at low temperatures and retains biological active nutrients which are responsible for the prevention of chronic diseases and may be used in therapeutic interventions for cancer patients to tolerate the side-effects of radiation in the oral cavity. (4,5)

Although the practice of oil pulling has its origin from Ayurveda and comprises of rinsing without ingesting edible oil to prevent and manage oral conditions such as tooth decay, trismus, halitosis, gingival inflammation, and dry mouth or xerostomia. A search of literature gives us several studies as early as 1992 by Rosenberg *et al* (6) who prove its anti-microbial activity and studies more recently done by Asokan*et al*(7) who have highlighted the anti-bacterial capability of coconut oil pulling effective on bacteria such as *S. mutans, S. mitis* and *S. viridians, L. acidophilus*.

The safety and effectiveness of oil pulling on hard tissues of a tooth has been assessed by Hegde MN and colleagues who have reported changes in the hydroxyapatite crystals when the pH of the oral cavity drops below the critical value. They performed a comparative study of virgin coconut oil with other natural therapeutics and concluded better enamel crystallinity post-radiation using oil due to the formation of a protective layer. (5)

Oil pulling has been stated as an effective preventive therapy in several microbiological studies but there is a lack of human studies to support its clinical effectiveness. Its saponification mechanism of action not only moisturizes the soft tissues but also forms a protective layer over the hard tissues as a mechanical barrier. (7,8) Further studies can bring to light other widespread benefits of coconut oil as a nutraceutical which remains an area of research yet to be discovered. No other commercially available over the counter oral rinse can compete with the benefits of coconut oil to be used daily. Oil pulling offers a less invasive and readily acceptable alternative to many patients as it is palatable, readily available and non-toxic even if accidentally consumed during the pulling process. (9,10) However, there is a need for clinical trials with diverse populations to gather scientific evidence for it to be prescribed for routine use to maintain oral hygiene among patients with long-standing systemic diseases, patients undergoing radiation or chemotherapy.

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CASE REPORT

Endodontic management of a mandibular first molar with six root canal systems : A Case Report

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ABSTRACT

Root canal therapy cannot proceed successfully without the precise knowledge of anatomy of root canal system. Mandibular first molar usually has two roots (mesial and distal) and three to four root canals. This is a case report of a 25-year-old male patient reported with intermittent pain in the lower right back region of jaw since 1-week. Refined access cavity revealed initially two canals in mesial and three canals in the distal root. Using magnifying dental loupes one additional canal (middle mesial) was identified in mesial root. Thus, this case reports the successful non-surgical endodontic management of a mandibular first molar with six root canal systems with three canals in the mesial root and three in the distal root. One-year follow-up showed the patient to be asymptomatic and complete healing of periapical radiolucency was appreciated radiographically.

Keywords : Endodontic Management, Mandibular First Molar, Six Root Canals, Magnification Using Loupes, Post Obturation CBCT, One Year Follow-Up.

INTRODUCTION

The success of endodontic treatment depends on several factors such as diagnosis, proper access and most importantly skills levels towards the thorough cleaning, shaping, and obturation of the root canal system.[1]

The incomplete debridement of the root canal system, inability to locate canals affects and compromises the treatment outcome. Thorough knowledge of the root canal anatomy is essential for a predictable outcome. The mandibular first molars normally have mesial and distal roots with two mesial canals and one or two distal canals, respectively.[2] Rarely, there is an accessory canal known as the middle mesial canal, located in the mesial root. It is located in the developmental groove present between the Mesio-Buccal (MB) and Mesio-Lingual (ML) canals, with a reported incidence of 1% to 15%.[3] Another rare occurrence is the presence of the third canal within the distal root which is located between the Disto-Buccal (DB) and Disto-Lingual (DL) canal and is referred to as the Middle-Distal (MD) canal .it has an incidence of 0.2–3% .[4]

A preoperative radiograph in different angulations, Cone Beam Computerised Tomography (CBCT), Ability of operator to externalize the internal anatomy of a tooth and abiding by Krasner and Rankow laws of access opening help to locate canal orifices on the pulp chamber floor in a systematic way. Visual and diagnostic aids also play an important role in detecting atypical root canal anatomy.

Studies on the internal anatomy of mandibular first molar have revealed type IV and type I root canal system configuration to be most prevalent in mesial and distal roots respectively. [5] The presence of six or more root canals is highly exceptional and such teeth are described in a small number of case reports only with their incidence being unknown. [6-10] (Table 1).

This case report describes successful non-surgical endodontic management of mandibular first molar with three Mesial canals (Mesio-Buccal [MB], Middle Mesial (MM), Mesio-Lingual [ML],) and three Distal canals (Disto-Buccal [DB], Middle Distal (MD), Disto-Lingual [DL]).

Table 1 : Reported cases with six or more canals in lower mandibular

Author name	Tooth and location	Number of canals in mesial root	Number of canals in distal root	Total number of canals	Patient age/sex
Ryan et al (2011) [4]	36 USA	3(MB, MM, ML)	3 (DB, MD, DL)	6	52/female
Martinez-Berna and Badanelli (1985) [6]	36 & 46 USA	3(MB, MM, ML)	3 (DB, MD, DL)	6	22/female
Reeh et al (1985) [7]	36 USA	4 (MB1, MB2, ML1, ML2)	3 (DB, MD, DL)	7	23/male
Ghoddusi et al(2007) [8]	36 USA	2(MB, ML)	3 (DB, MD, DL)	6	30/male
Gupta et al (2012) [9]	46 USA	3(MB, MM, ML)	3 (DB, MD, DL)	6	38/male
Baziar et al (2014) [10]	36 USA	2(MB, ML)	4(DB1,DB2,DL1,DL2)	6	42/male

Case Report

A 25-year-old male patient reported to the Department of Conservative Dentistry and Endodontics with the chief complaint of intermittent pain in the lower right mandibular region of jaw since one week. While the clinical examination revealed deep mesio-occlusal caries in 46 there was no tenderness on percussion or palpation nor any mobility and the electric pulp tester gave a delayed response. Radiography revealed mesio-occlusal radiolucency involving enamel dentine and pulp with peri-apical radiolucency (Figure 1) suggesting a differential diagnosis of Acute Irreversible pulpitis.

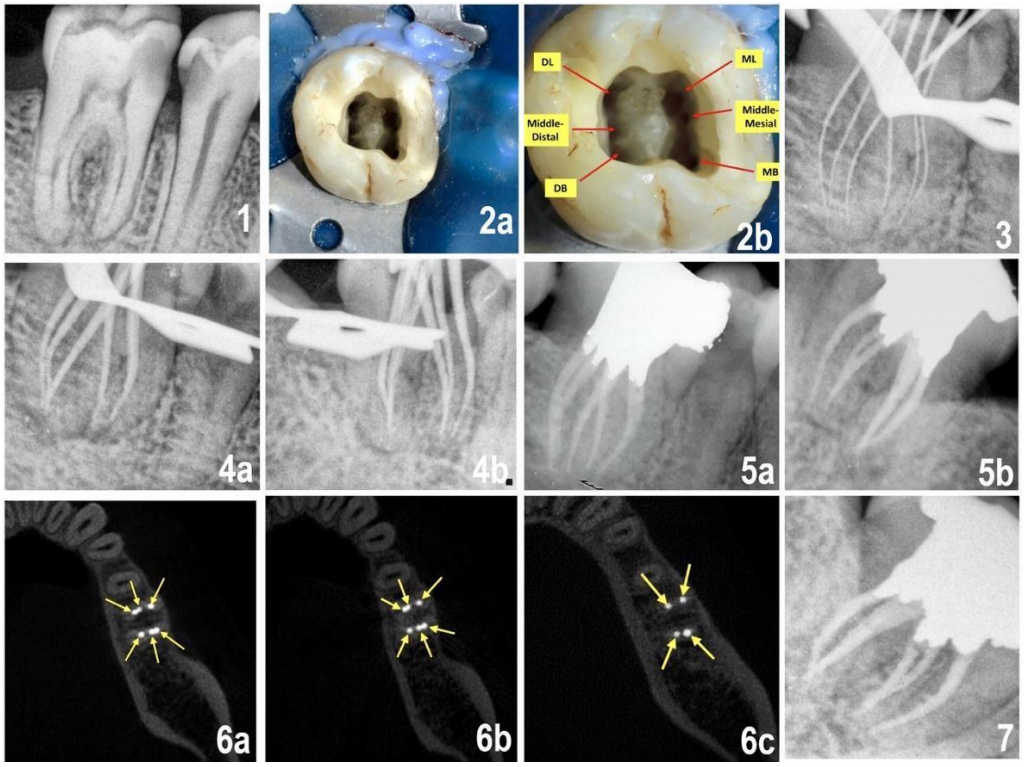

Fig.

TREATMENT:

Endodontic Treatment was initiated with the administration of local anesthesia (4.4 mL 2% lidocaine with 1:100 000 epinephrine) followed by rubber dam isolation. Caries removal revealed a pulpal exposure with a hyperaemic pulp. The pulp tissue was excavated from the pulp chamber and the orifices identified were mesiobuccal (MB), mesiolingual (ML), distolingual (DL) and distobuccal (DB) and middle distal (MD). Besides, the presence of the ML canal was found to be distant to the MB canal, which suggested that the mesial root is wider than normal (Figure 2a). The developmental groove between the ML and MB canals was further evaluated and an additional orifice was identified, present between the ML and MB canals, and located close to the ML canal under Magnifying loupes (2.5X, STAC dental equipment's Inc., Brampton, Canada). The canal was identified as the middle mesial (MM) canal (Figure 2b).

Working length was determined with the help of ISO 10 K-file (Mani Inc. Japan) and was confirmed with the help of an electronic apex locator (Propex Pixi, Dentsply Maillefer, Ballaigues, Switzerland) and periapical radiographs (Figure 3). The working lengths of MB, MM, ML, DB, MD, and DL were 22, 21, 21.5, 20, 20 and 20.5 mm, respectively. Canal preparation and shaping were done using Hero shaper rotary files 20/04 and 25/04 ((Micro-Mega, Besançon, France) and alternate irrigation with 2.5% sodium hypochlorite, EDTA and sterile saline. An Interappointment calcium hydroxide dressing was placed for a week. The patient was recalled after a period of 1-week. The MB, MM, ML, DB, MD and DL canals were finished with hero shapers 25/04 ((Micro-Mega, Besançon, France), and the MM and MD were prepared with hero shapers 20/04 ((Micro-Mega, Besançon, France). The Gutta-percha Cone fit was verified radiographically in mesial and distal angulation (Figure 4a and 4b). A final rinse of normal saline was given and the canals were dried with paper points. Following disinfection of the gutta-percha points for 1 min in 5.25% sodium hypochlorite, the canals were then obturated using Sealapex (Kerr Manufacturing Co.) as the root canal sealer. A Nayyar core coronal seal was given. Post-obturation radiographs were obtained at mesial and distal angulations to evaluate the three-dimensional pack in all six canals (Figure 5a,5b respectively). At one year follow-up, the patient was asymptomatic and wanted replacement of missing teeth in the lower arch for which he was advised CBCT. Examination of the scanned images in the run of the endodontically treated tooth showed independent 3 mesial and 3 distal obturated root canals in coronal third (Figure 6a and 6b) and with only two canals in the apical third (Figure 6c) indicating a Type II (3-2) configuration.

As, at 1year follow-up visit, the tooth was found to be asymptomatic with a normal radiographic periapical area (Figure 7), a full-coverage crown is planned for the treated tooth.

DISCUSSION

Permanent Mandibular first molar erupts at the age of 6 years. It is the most heavily restored tooth and most common tooth to require endodontic treatment in the adult dentition. Morphologically, mandibular first and second molars have a mesial and distal root. 65% of cases mesial root has two canals and distal root has one canal, however, 30% of cases distal root shows the presence of two canals. Literature has reported an incidence of accessory canals in the mesial root to be 2.07%-13.3%.11 The incidence of the middle mesial canal (MM) and the middle distal canal (MD) in mandibular first one molar are 1-15% and 0.2-3%, respectively.12

The mesial root of the mandibular first molar tooth has two canals with type IV configuration i.e (2–2) canal type in 52.3% of cases and type II configuration i.e (2–1) canal type in 35% of cases. While , in the distal root, the most common frequent configuration is Type I (62.7%), followed by Type II (14.5%), and Type IV (12.4%) according to the Vertucci's classification (Figure 8).5 However, Gulabivala et al (2001)13 reported additional root canal types (3–2) and (2–3) on Burmese population (Figure 9) and Sert S and Bayirli GS (2004)14 evaluated root canal configurations in the

Turkish population and added fourteen new root canal configurations to the Vertucci's classification, numbering from Type IX to Type XXIII (Figure 10)

This report highlights a rare case of a mandibular first molar with six root canals. Three canals were located in the mesial root, while three were found within the distal root. Both the roots had canals with type II and type XV (3-2) configuration according to classification proposed by Gulabivala K and Sert S respectively, that is, the middle mesial canal merged with the mesiolingual canal in its apical third and exited through a common foramen, and the middle distal canal joined the distobuccal canal in its apical third having a mutual exit (Figure 10). The mesiobuccal and distolingual canals were independent-type with separate orifices and apical portal of exits.

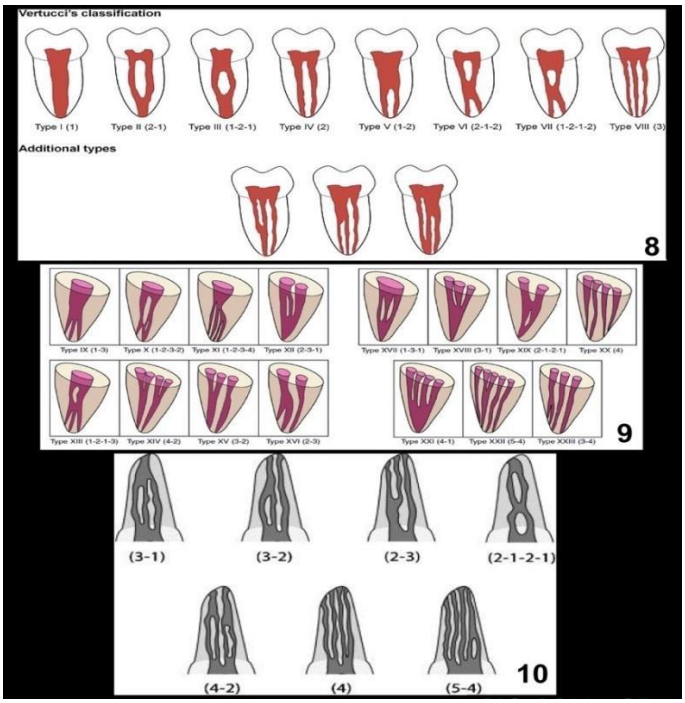

Fig.

It has been reported in the literature that secondary dentin deposition occurs with age due to which partitions develop leading to progressive differentiation and separation of canals as well as the formation of intercanal communications. The prevalence of such intercanal communications is usually high in the middle age group while being the lowest among young and old age groups.[15] In 1974, Barker et al. [16] reported a fifth canal with an independent MM canal in the mandibular first molar followed by a similar finding by Vertucci and Williams, while Martinez-Berná and Badanelli [6] stated the third canal in the distal root for the first time.

A case recently reported by Gupta et al [9] discussed the presence of six root canals in a mandibular right first molar. In their case report, it was found that the middle mesial was confluent in its apical third with the mesiobuccal canal, whereas the middle distal had a mutual exit with the distolingual canal.

Pomeranz et al [17] classified the MM canals into 3 types: Fin , Confluent and Independent type. When an instrument could pass freely between the Mesio-Buccal (MB) or Mesio-Lingual (ML) canal and the Middle Mesial (MM) canal it was classified as "Fin". The MM anal was classified as confluent when the canal originated as a separate orifice but joined the Mesio-Buccal (MB) or Mesio-Lingual (ML) canal in the apical third of the canal and, was termed independent if it remained separate from orifice to the apex. The MM canal in this case report was Confluent type.

Preoperative radiographs, even if taken at oblique angles, often are not sufficient to reveal unusual anatomical variations. For success in endodontic treatment, clinicians should also be aware of root canal anatomy and its associated variances. Various techniques such as radiographs at various angulations, appropriate access cavity, good lighting and magnification using a dental operating microscope, careful visualisation, inspection of the chamber floor with an endodontic explorer, use of dyes, transillumination, white line test, red line test, bubble test, ultrasonics, chamber floor troughing and advanced imaging techniques such as Cone Beam Computed Tomography (CBCT) prove to be beneficial in locating accessory canals. Examination of the floor of the pulp chamber offers clues to the location of orifices and the type of canal system present.
Thus, the clinician should be aware of the variations in the root canal anatomy that could be encountered during the root canal treatment of mandibular molars. Every attempt should be made to find and treat all root canals to ensure successful treatment outcome.

Conclusion

Mandibular molars have complex root canal morphology. The clinicians should be aware of the anatomical variations of the different human dentition and use all the available tools to detect and treat their clinical cases. With the advancements in operating aids, the future generation will experience root canal morphology variations in permanent mandibular molars.

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