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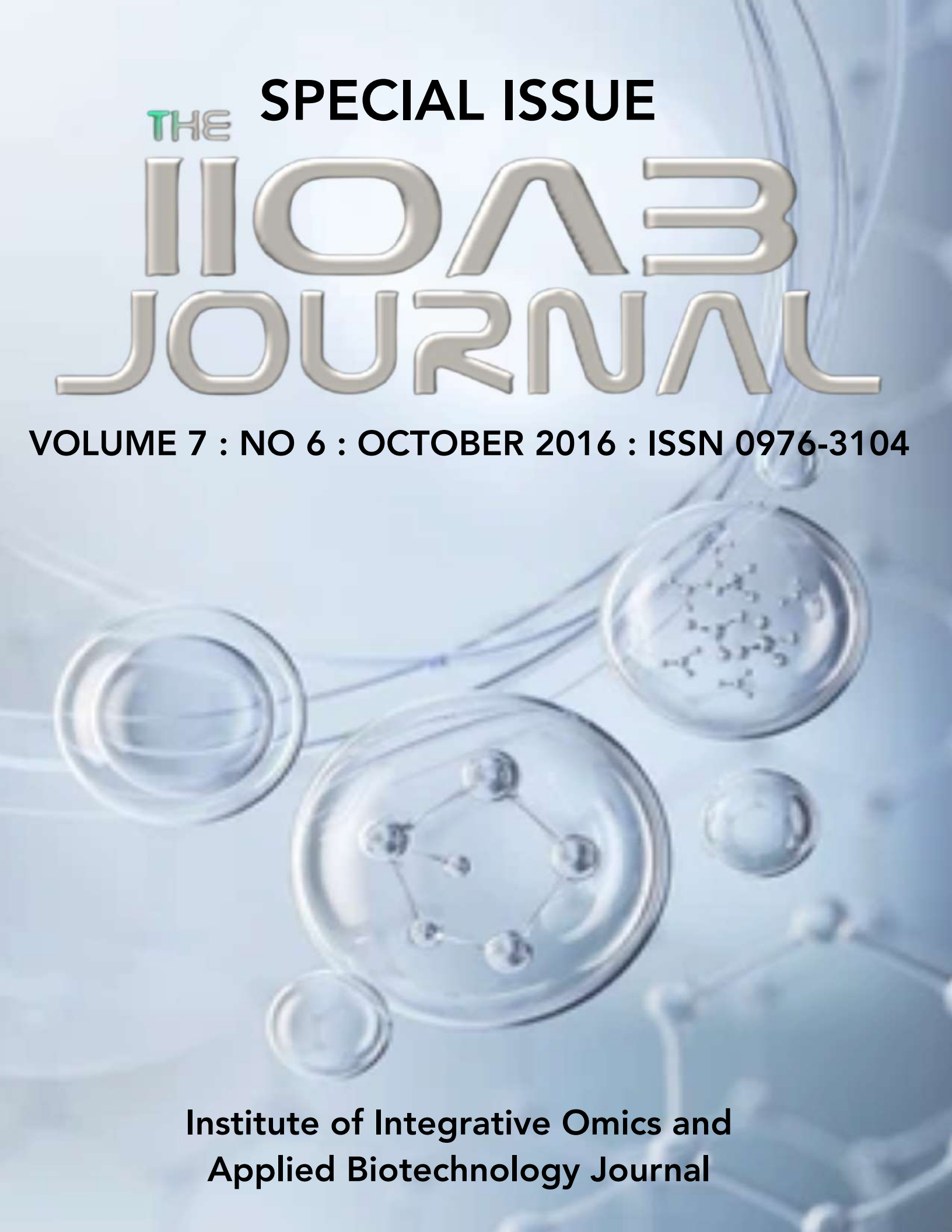

Institute of Integrative Omics and
Applied Biotechnology Journal

Dear Readers,

I extend a warm welcome to each of you to our distinguished scientific journal, dedicated to exploring the recent advancements in the field of Endodontics.

As the Editor of this special IIOAB Journal issue, it brings me great pleasure to witness the strides being made in the ever-evolving realm of Endodontics. Our journal serves as a conduit for disseminating cutting-edge research, innovative techniques, and transformative applications that redefine the landscape of endodontic practice.

Your expertise, dedication, and contributions are pivotal in shaping the future of endodontic care. Your groundbreaking research, novel methodologies, and clinical insights play an indispensable role in improving treatment outcomes, refining diagnostic approaches, and enhancing patient care in this specialized field.

The dynamic nature of Endodontics continually presents new challenges and opportunities. Your commitment to innovation not only drives progress within the discipline but also holds the promise of improving oral health and overall patient well-being.

I encourage each of you to share your pioneering insights, submit your impactful research, and engage in stimulating discussions within the pages of our journal. Let us collaboratively foster an environment where ideas flourish, collaborations thrive, and knowledge propels us toward achieving new milestones in Endodontics.

Thank you for your unwavering dedication to advancing the field of Endodontics through recent discoveries and innovations. I eagerly anticipate the wealth of transformative insights that will emerge from your invaluable contributions.

Warm regards,

Dr. J. Mandlik
Editor-in-Chief

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ARTICLE OPEN ACCESS

MICROBIAL CONTAMINATION OF LAB COATS WHILE PERFORMING ENDODONTIC TREATMENT

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ABSTRACT

Aims: *Lab coats are known to act as vectors in transmitting the potentially pathogenic multi-drug resistant type microorganisms. This study was conducted to determine the site, type and antibiotic susceptibility of microbial flora present on lab coats of health care professionals engaged in doing endodontic treatment in order to assess the risk of transmission of pathogenic micro- organisms.* ***Materials and methods:*** *A total of 20 lab coats of clinicians were included in the study. Swabs were taken from 3 different sites of the lab coat – collar, pocket and cuff, on the 1st and 3rd day. The swab samples were processed and the biochemical characterization of the isolates was done using standard microbiology protocols.* ***Results:*** *Of the three predetermined sites, the pocket was more contaminated than the chest and cuff. Coagulase negative Staphylococci was the most common isolate followed by Staphylococcus aureus and Gram negative non fermenters. All other isolates were either environmental microorganisms or skin commensals.* ***Conclusion:*** *In order to prevent transmission of infection, a strict protocol should be set into play in order to prevent cross contamination between doctor and patient.*

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KEY WORDS

Bacterial contamination, Coagulase negative Staphylococci, Lab coat, Nosocomial infection

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INTRODUCTION

Infection is a dynamic process involving invasion of body tissues by pathogenic micro-organisms and their toxins. Nosocomial/ hospital/ acquired infections are those which are not present or incubated before admission of patient to the hospital but obtained during the patient's stay in hospital. Lab coats, nurses' uniforms and other hospital garments, materials and articles may play an important part in transmitting pathogenic bacteria in a hospital setting .The hands of healthcare personnel are most commonly implicated in transmitting the pathogens [1]. Various nosocomial pathogens, such as methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and gram negative organisms is well documented [2]. Specifically in the area of dentistry, health care professionals are routinely exposed to potentially pathogenic microorganisms which are present in the surrounding environment. Most of them originate from the mouths of patients [3]. Contamination may occur from instruments through contamination vectors. These contaminated object infections may be transferred from patient to patient or from patient to professionals [4]. Methicillin resisitant *Staphylococcus aureus* which is the most pathogenic microorganism, comes in contact with health care professionals via direct hand contact with contaminated body fluids, devices, items or environmental surfaces [5].

There are very few studies regarding the wearing and laundering of lab coats in hospitals and medical practice. This study highlights the role of lab coats acting as vector for transmitting health care infections to the patients and the common areas where contamination occurs.

MATERIALS AND METHODS

The study was conducted in the Department of Conservative Dentistry and Endodontics, Sinhgad Dental College and Hospital, Pune with technical aid from the Department of Microbiology, SKN Medical College and Hospital, Pune. All the participants were informed about the study and necessary informed consent was taken. Ethical clearance was obtained from the ethical committee of college. Total 20 aprons of dental healthcare professionals (interns, PG students, faculty members) were included in the study.

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Inclusion criteria

- Postgraduate students, Interns and Faculty members of Department of Endodontics willing to participate in the study.
- Half sleeved aprons worn for 3 consecutive days.
- Aprons which are not exchanged with other colleagues.
- Aprons worn inside the department.
- Working for minimum 3 hours on patients.

Exclusion criteria

- Aprons worn outside the department.
- Aprons exchanged with other colleagues.
- Full sleeved aprons.

Sample collection technique

- The collection of microbiological samples from dental lab coats was performed by the technique of rolling a sterile swab moistened in glucose broth on the target site.
- Samples from each lab coat was taken from the three predetermined areas i.e. chest area, upper part of pocket and sleeve ends.
- The samples were appropriately labeled and then transported in glucose broth media to the laboratory for microbial analysis. Twenty clean, washed lab coats were used as controls.
- On the first day, 60 swabs were taken of contaminated lab coats after minimum three hours of working. At the same time, 60 swabs were taken from the lab coats used as controls.
- On the third day, 60 swabs were collected from the same contaminated lab coats from the target sites **[Figure- 1]**.

Collection of samples

Fig: 1. Pocket

Chest

Cuff

Laboratory procedure

The collected swabs were cultured on blood agar and Mac Conkey's agar **[Figure–2]**.The agar plates were incubated at 37^{0}cfor 24 hours. Gram staining was used to examine the morphology and staining reaction of the organisms.Biochemical evaluation included testing for catalase, coagulase, bile, oxidase, triple sugar iron, indole and citrate using standard prescribed protocols for identification and characterization of microorganisms. Methicillin resistance in *Staphylococcus* species were tested with the help of cefoxitin disc and oxacillin disk (Hi-Media Ltd, Mumbai, India) on Mueller Hinton agar by using Central Laboratory Standard Institutional (CLSI) guidelines. Antibiotic sensitivity testing was done by using Kirby Bauer's disc diffusion method as has been described in the CLSI guidelines 2011[6].

Statistical analysis

Statistical analysis was done using SPSS 20.0 v. Descriptive analysis was done to estimate the percentage of microorganisms and Chi Square test was done to assess the difference in proportions. Level of significance was taken at $p<0.05$.

RESULTS

Of the study participants, 60%were post graduate students, 25% were faculty and 15% were interns. Gram positive cocci dominated the colonization, followed by Gram negative cocci with the difference being statistically significant ($p< 0.05$).51%cultures showed Gram positive cocci, making it the major microbial group contaminating the lab coats in the dental operatory **[Table–1]**.Among the Gram positive cocci, coagulase negative *Staphylococcus* was the dominant microbe and 10% were gram negative bacilli. The microorganisms obtained in the study were Methicillin Resistant Coagulase negative *Staphylococci* (MR CONS), Methicillin sensitive Coagulase negative *Staphylococci* (MS CONS), Actinobacter, Methicillin sensitive *Staphylococcus aureus*

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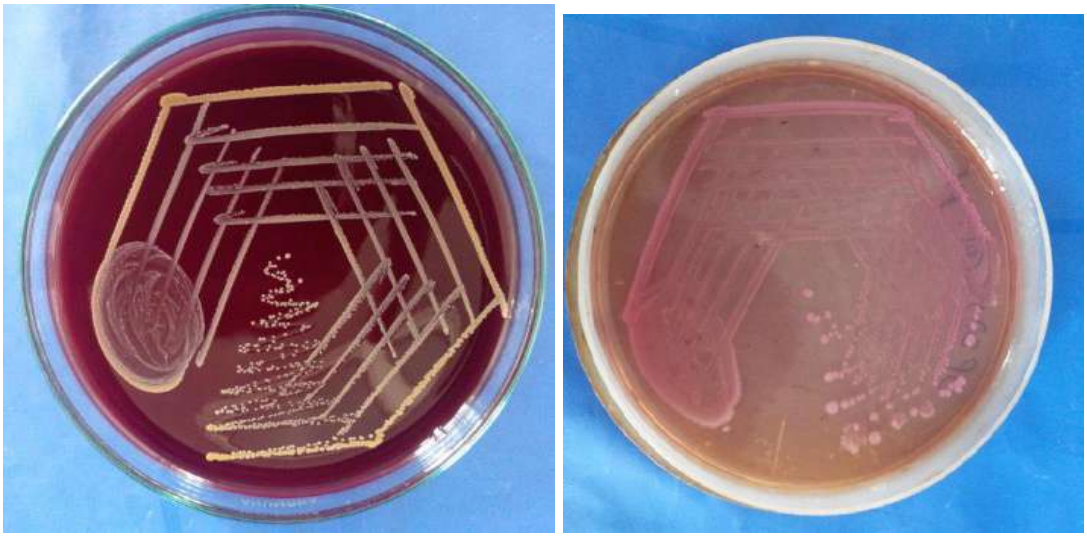
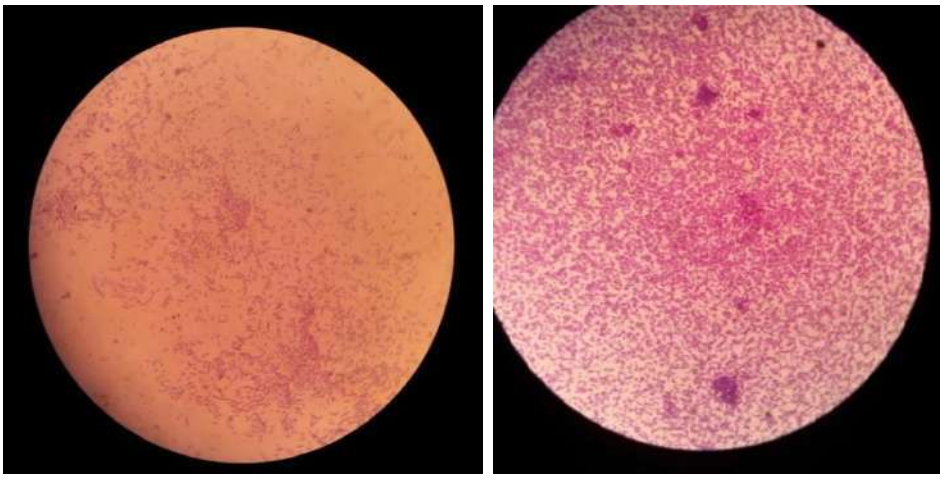


(A) (B)

Fig: 1. (A) Blood agar showing white beta hemolytic colony of *Staphylococci*, (B) Mac Conkey Agar with lactose fermenting colony of *Staphylococci*



(A) (B)

Fig: 2. Light microscopic screening of study slides. (A) Gram negative *Bacilli*, (B) Gram positive *Cocci*

Table: 1. Organisms isolated based on the morphology of study sample

Morphology	Number of organisms isolated in lab coats N=40 (%)	Number of organisms isolated N=120 (%)
Cocci	27(67.5%)	62(51%)
Bacilli	7(17.5%)	13(10%)
Total	34(85%)	75(62%)

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Table: 2. Types of various microorganisms isolated in 120 swabs (1st day and 3rd day)

Organisms	Total	1st day	3rd day
MR CONS	9	2	7
MSCONS	28	8	20
ACTINOBACTER SPECIES	3	1	2
MRSA	5	1	4
MSSA	14	3	11
MICROCOCCI	6	2	4
GRAM NEGATIVE BACILLI	10	3	7

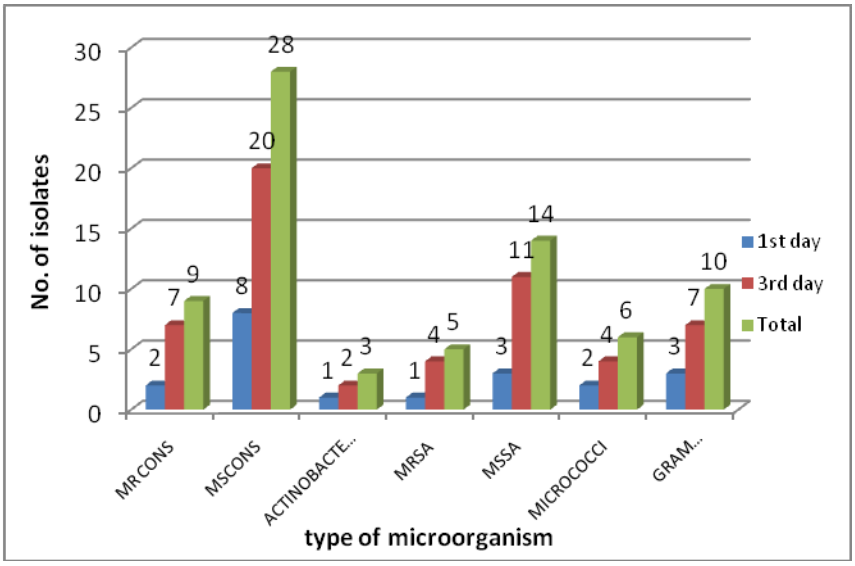

Fig: 3.Number of isolates and type of microorganism on 1st and 3rd day

Table: 3. Antibiotic sensitivity test results

Antibiotics	1st day		3rd day	
	Sensitive	Resistant	Sensitive	Resistant
AMIKACIN	90%	10%	80%	20%
AMOXYCILLIN	95%	5%	10%	90%
AMOX CLAV	75%	25%	50%	50%
AMPICILLIN	75%	25%	40%	60%
CEFTRIAXONE	50%	50%	50%	50%
CIPIROFLOXACIN	95%	5%	85%	15%
COTRIMOXAZOLE	35%	65%	20%	80%
ERYTHROMYCIN	30%	70%	10%	90%
GENTAMYCIN	20%	80%	15%	85%
PENICILLIN G	80%	20%	15%	85%
VANCOMYCIN	100%	0%	100%	0%
TETRACYCLIN	70%	30%	70%	30%

(MSSA), Methicillin resistant *Staphylococcus aureus* (MRSA), *Micrococci*, Gram negative *Bacilli* **[Table– 2]**. Microbial count was less on first day as compared to that of 3rd day **[Figure– 3]**. This difference was found to be statistically significant (p = 0.03). Antibiotic sensitivity showed that the microorganisms got more resistant to antibiotics Amoxicillin, PenicillinG, Cotrimoxazole, Erythromicin, Gentamicin at third day as shown in **[Table– 3]**.

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DISCUSSION

The lab coat can get contaminated by microorganisms due to improper handling practices. They get easily contaminated because patients continuously shed infectious microorganisms in the hospital environment, and the health care providers are in constant contact with these patients. *Staphylococci* are the pathogens belonging to the group of Enterobacter bacteria, which cause several infections to humans. They are facultative anaerobic gram-negative cocci mainly found in the skin and mucosa and are of three types *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus haemolyticus* [7]. Health care professionals are most susceptible to colonization, and the main form of transmission is through temporarily colonized hands. Importantly, treatment of infections caused by *S. aureus* has become difficult because of their higher resistance to various drugs [8].

S aureus is part of the normal human microbial flora and it is found in the nasal passages, throat, gastrointestinal tract and skin. It is considered as one of the most important pathogenic bacteria, causing series of infections [9, 10] leading to the formation of abscesses. It causes infections such as furuncles, folliculitis, scalded skin syndrome, meningitis, and pneumonia. Coagulase-negative *Staphylococci* (CONS) which is a skin commensal has recently got attention as a potential pathogen, specifically for nosocomial infections [11,12,13]. CONS are a major cause of nosocomial infection and septicemia, especially in cases of immune-compromised patients [12].

This study evaluated the type of microbial flora present on the lab coats of the clinicians working in the Dept of Endodontics and their antibiotic sensitivity. Three sites were chosen i.e chest, pocket, cuff for determining the type of microbial flora. Microbial contamination was thought to be highest as these sites most commonly comes in contact with the patients [14,15].This study showed that the numbers of gram positive cocci was the same as that of other studies and maximum of them were potentially pathogenic [15,16].This is consistent with other studies that showed contamination of lab coats ranging from 23% to 95% [17]. They possess a risk of cross contamination if the host is immune compromised. *Micrococci* may act as an opportunistic pathogen in patients with compromised immune systems and they most commonly cause blood stream infection. Gram negative *Bacilli* were also isolated, but these were significantly lesser in number and they may be potentially infectious, as was reported by Zachary and Grabsch. They have shown that bacterial survival rate is of longer period of time on hospital fabrics[18,19].Chacko et al have shown that on lab coat fabrics made up of either cotton polyester or polyester material, bacteria can survive between 10-98 days [20]. Hence the lab coats should be washed daily or at least once in 3 days [20]. Of the two predetermined sites selected for examination on the lab coat, the mouth of the dominant pocket was more contaminated than the chest and cuffs of the sleeve. This is similar to the study of Nelly and contrary to that of Uneke and Ijeocoma which indicated that cuff has more bacterial load than the pocket [21, 22]. Pocket is the highly contaminated area because it frequently comes in contact with the hands of the health care professionals harboring bacterial contaminants.

Antibiotic sensitivity testing showed resistant species of microorganisms on the lab coats against Amoxicillin, Penicillin G, Gentamycin, and Cotrimoxazole. Antibiotic sensitivity results showed the organisms which were sensitive to most common antibiotics on 1st day got resistant on 3rd day **[Figure-4]**.Of the *S. aureus* isolated, 10% were MRSA. The MRSA has emerged as significant bacteria in hospital acquired infections. According to the Centre for Disease Control and Prevention, more than 60% of all hospital infections are caused by MRSA in United States. Because of frequent dermal contact, lab coats can harbor these resistant bacteria. In orders to prevent cross infection, guidelines should be followed for handling and washing procedures of lab coats.

This is a uni-centric study, done to create awareness among our dental colleagues. This study reflects center-specific microbial contamination in a dental operatory. To reach to a more generalized conclusion, the study requires a multi-centric evaluation with a larger sample size.

CONCLUSION

The present study highlights the fact that the lab coats may act as a vector for transmission of cross infection. In order to prevent transmission of cross infection, a strict protocol should be set in order to prevent cross contamination between doctor and patient. Efforts should be made to limit the use of coats outside the working area and they should be laundered every day .Wearing of plastic aprons or altering lab coat material to plastic-laminated clothing or closely woven waterproof cotton can reduce the bacterial transfer rate and cross-contamination

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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FINANCIAL DISCLOSURE

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CASE REPORT OPEN ACCESS

A 4 YEAR CLINICAL, RADIOGRAPHIC AND CBCT EVALUATION OF REPLANTED AVULSED MAXILLARY CENTRAL INCISOR WITH EXTRA ORAL DRY TIME OF 4 DAYS: A CASE REPORT

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ABSTRACT

Replantation of avulsed permanent mature teeth presents a unique challenge. The dilemma to replant or not to replant continues. The decision is based on many factors such as the extra-oral time lapsed, age, root apex, patient's expectations, available resources, type of storage media etc. however replantation makes excellent provisional esthetic restoration in short term and maintains the arch integrity when outcome is successful in long.
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KEY WORDS

avulsion; re-plantation; delayed extra-oral time, CBCT

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INTRODUCTION

Trauma to the oral region occurs frequently. Among all facial injuries, dental injuries are most common of which avulsion occurs in 1-16%.of all dental injuries. It is the serious of all dental injuries [1]. Dental avulsion is consequences of injury that results in the complete displacement of a tooth from its alveolar socket and may affect multiple tissues .Trauma to the anterior teeth is the most prevalent with sports and automobile accidents the most frequent cause. Avulsion of permanent teeth occurs at any age, but is most common in young permanent dentition with higher prevalence in males [2]. This is because the root is still not completely formed and the periodontium and bone are very resilient [3]. DTI is an injury that results from an external force, involving the teeth, the alveolar portion of the maxilla or mandible, and the adjacent soft tissues [1 - 5]. When dental avulsion occurs, immediate replantation at the trauma site is the ideal procedure for maintaining the viability of PDL cells. However, immediate replantation is rarely achieved [6]. It presents a unique challenge to the clinician, it is the treatment of choice but it cannot always be carried out immediately [2]. The delayed replant is characterized by the absence of minimum conditions for survival of the cells, with the maintenance of the tooth in ways of storage that does not take care of to the cellular necessities of nutrition, osmolality, pH and temperature [7,8]. As a treatment of the avulsed tooth, replantation is the method which can restore occlusal function and esthetic appearance shortly after injury [9, 10]. Hence this paper presents a case report where the tooth was replanted after 4 days.

CASE REPORT

A 17-year-old male patient, reported to the Department of Conservative Dentistry & Endodontics, Modern Dental College and Research Centre Indore for treatment of avulsed tooth. He had sport injury four day prior to his visit

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to the department and his maxillary right permanent central incisor tooth had been avulsed. Patient came with the avulsed tooth kept dry in a polythene bag. Examination of the avulsed teeth revealed that the roots had closed apices and tooth crown was intact. The patient had no relevant medical history. No apparent bleeding was seen from the avulsed tooth socket Left maxillary central incisor and right maxillary lateral incisor showed Ellis class III fractures **[Figures–1 and -2]**. Periapical radiograph was obtained; no periapical changes were seen with 12 and 21 with no apparent root fracture .Empty 11 socket was seen **[Figure–3]**. Patient was explained about all the treatment options and their respective pros and cons. Treatment options included replantation, fixed partial denture, and implant for the replacement of missing teeth and restoration of 12 and 21.Patient agreed to treatment. Written consent was taken from the patient's guardian. Endodontic therapy with 11 was done extraorally the tooth was held with gauge piece soaked with 0.2% Chlorhexidine **[Figure–4]**. Local anesthetic was administered and the blood clot was removed from the socket. Tooth was then replanted into the socket with the help of finger pressure .occlusion was checked with adjacent teeth and also radiographically. The fractured fragment of 12, 22 was removed and pulp was extirpated. The teeth were splinted with a semi rigid arch wire and bonded with composite **[Figure–5]**. Anti-tetanus toxoid (ATT) was given prophylactically. Antibiotics were prescribed for 7 days and the patient was encouraged to maintain good oral hygiene. The patient was recalled next day for treatment of remaining teeth. After two weeks, the tooth were found to be stabilized and the splint was removed .Endodontic therapy of both the teeth was planned and accomplished. The patient was kept under follow – up for 3 years. No signs of root resorption were seen radiographically during the follow up period when examined clinically, radiographically with CBCT imaging **[Figures–6 and -8].**

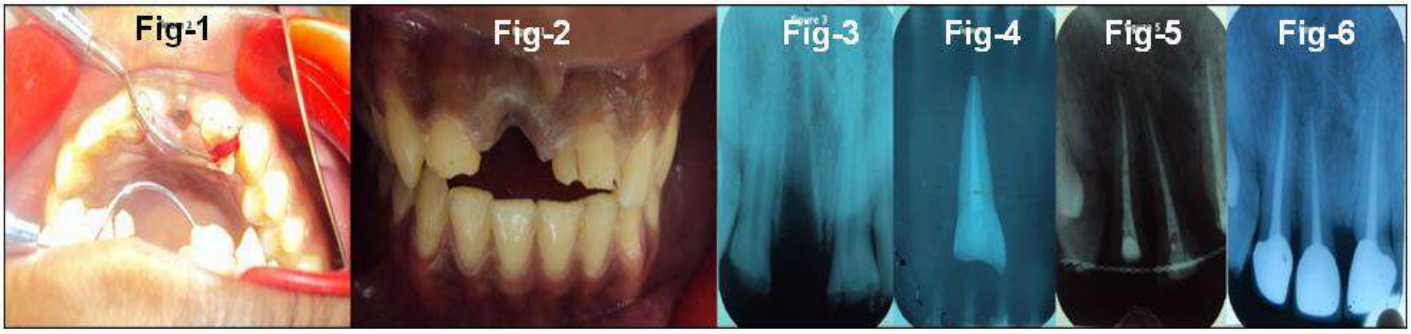

Fig: 1. Avulsed maxillary right central incisor, **Fig: 2.** Ellis class III fracture 12, 21, missing 11, **Fig: 3.**Radiograph showing empty 11 socket, **Fig: 4.** RCT with 11(extra-oral), **Fig: 5.** radiograph showing Replanted 11 RCT of 12 21 and splint, **Fig: 6. P**eriodic clinical and radiographic evaluation

DISCUSSION

Evidence strongly emphasizes need for minimizing extra-oral time of avulsed tooth; use of physiologic storage medium with impetus on maintaining vitality of periodontal ligament cells and initiating early endodontic treatment as being key factors in increasing success of replantation procedures [11- 15]. Treatment is directed at avoiding or minimizing the resultant inflammation which occurs as a direct result of two consequences of avulsed tooth namely attachment damage and pulpal infection.

In the present case the extra oral time was 4 days without placement in any storage medium. When a tooth has an extra-oral dry time of more than 60mins, the periodontal ligament is not expected to survive. Pre-treatment of such a tooth, prior to its replanting, will render it more resistant to resorption .If the tooth remained dry for more than 60 minutes with no consideration for preserving the periodontal ligament, the endodontic therapy could be performed extraorally.

In our case patient came four days after the injury with the tooth kept in a polyethene bag due to unawareness. When such events do occur, even if the treatment is delayed, considering the benefits of function, esthetics and physiological impact on patient that might result from the therapy replantation should be attempted .hence, we decide to replant the avulsed tooth in spite of extremely unfavorable conditions. Preparation of the socket was done which consists of removal of obstruction, blood clot and bone fragments if any in order to facilitate the replantation, preparation of socket was performed with the use of curette and irrigation with saline while the assistant was holding the tooth with gauge dipped in 0.2 percent CHX. Root canal treatment was done in conventional manner, there is

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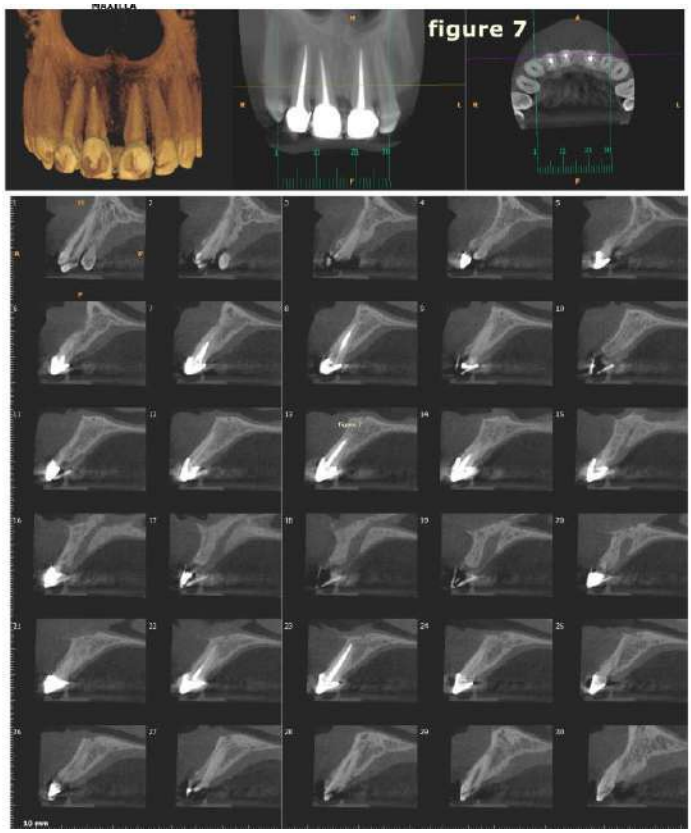
consensus in literature that replanted tooth should be endodontically treated because the necrotic pulp and endotoxins affect the PDL through the dentinal tubules and play a decisive role to the resorption process. However, when endodontic treatment is carried out on avulsed teeth, it improves the chances of retention and prevention of replacement resorption[16,17].

Splinting was done with composite and ligature wire as it allows physiologic movement of tooth during healing. It should allow movement of tooth and should have more memory so that the tooth is not moved during healing. Splinting was kept for 4 weeks .the objective of avulse, loose or displaced tooth is to protect the attached apparatus and allow repair and regeneration of periodontal fibers. After splinting the radiograph was taken to verify the position of tooth as post-operative reference in the occlusion was checked. The follow-up was done at regular interval. Resorption (inflammatory, replacement) are usually observed after 1-2 months and surface resorption is observed after 12 months [18, 19].



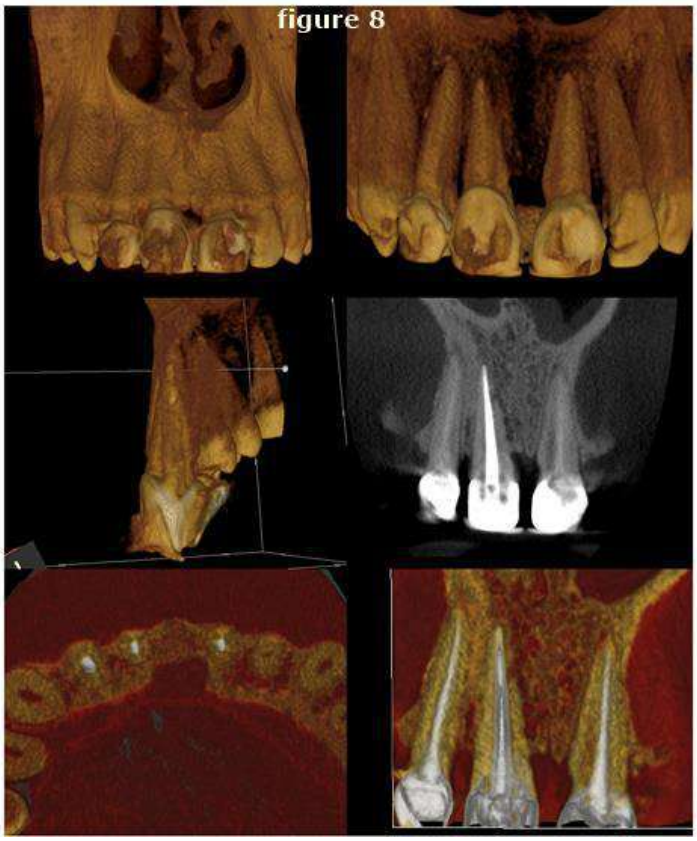


FIG: 7. CBCT section evaluation showing no signs of resorption, **FIG: 8.** CBCT 3D reconstruction

An ankylosed tooth can be diagnosed clinically within 1 month by its percussion sound (high, metallic) and radiographically within 2 months (disappearance of PDL space and invasion of bone into the root).The presence of a high-pitched percussion tone that differs from that from uninjured control teeth has been used as a criterion for the clinical diagnosis of ankylosis [21,22]. However, irrespective of the state of the tooth or time spent out of the mouth, the avulsed tooth that is reimplanted remains the best implant [23].

The outcome of replantation treatment though unpredictable, can be categorized as [24]:
Favorable outcome: Closed apex:

- Asymptomatic,
- Normal mobility,
- Normal percussion sound.

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- No radiographic evidence of resorption or peri-radicular osteitis: the lamina dura should appear normal.

Unfavorable outcome: Closed apex:

- Symptomatic,
- Excessive mobility or no mobility (ankylosis) with high-pitched percussion sound,
- Radiographic evidence of resorption (inflammatory, infection-related resorption, or ankylosis-related replacement resorption).
- When ankylosis occurs in a growing patient, infra-position of the tooth is highly likely leading to disturbance in alveolar and facial growth over the short, medium and long term.

Regular follow-up was done and the tooth was checked clinically and radiographically. Conventional radiographs don't provide a true and full representation of the lesion, especially in the buccal lingual direction. They are unable to identify the true extent, location or the portal of entry of a resorptive lesion [25]. Advanced imaging assists in diagnosis the outcomes whether favorable or unfavorable and thereby further modifying the treatment plan. Hence CBCT was taken as it is a reliable and valid method of detecting external inflammatory root resorption and performs significantly better than intraoral peri-apical radiography [26]. Because treatment of resorption can be very complex and unpredictable, accurate imaging is important to the diagnosis and treatment plan. The diagnosis of resorption is usually based upon the radiographic examination [27].

CONCLUSION

With the huge amount of information available in literature, the media and internet, dentists should utilize these resources to make the patients aware that if the avulsed tooth cannot be immediately reimplanted, it should be kept in a proper storage medium [28].

CONFLICT OF INTEREST

There is no conflict of interest amongst the authors.

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ARTICLE OPEN ACCESS

THE EFFECTIVENESS OF TWO APEX LOCATORS IN DETECTING SIMULATED HORIZONTAL ROOT FRACTURES: AN INVITRO STUDY

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ABSTRACT

The aim of this in vitro study was to evaluate the accuracy of two electronic apex locators (EALs): Root ZX and Propex II, in the detection of fractures in teeth having simulated horizontal root fractures (HRF). A sample size of 40 recently extracted, single rooted, permanent human teeth with sound roots and with no evidence of root resorption or fracture was selected for this study. Post decoronation of the tooth at the CEJ, endodontic access was prepared and patency of the canal was checked using 15 K files (Dentsply, Tulsa, Okla) along with use of EDTA as the chelating agent. Horizontal root fractures were simulated using a 0.2mm thick disc horizontally until half of the canal was exposed circumferentially, in the horizontal plane, either at coronal, middle or apical third of the tooth by operator No.1. The teeth along with the Lip clip of the Apex Locator were embedded in an alginate model and the alginate was continuously bathed with water to maintain the conductivity of the medium. Once the respective EAL read "APEX" on gradual advancement of a 25 K file through the canal, readings were noted using a digital vernier caliper as suggestive Horizontal fracture length by Operator No. 2 . The Real Fracture lengths (RFL) were then measured using a size 40 K file (Dentsply, Tulsa, Okla) under 3x magnification and with these actual length readings the readings of the two EALs were compared, allowing a tolerance of 0.5mm and 1.0mm .Results were analyzed using analysis of variance and measurements recorded were analyzed with the Mc Nemer's chi-square ($\chi 2$) test. ($p\_0.05$) Both the EALs used in our study detected fracture location with approximately 50% accuracy at 0.5mm tolerance level but at 1.0 mm tolerance level Root ZX showed 90% accuracy and Propex II showed 70% accuracy. Thus Root ZX showed a higher accuracy rate in detection of simulated horizontal root fractures. Results concluded that the investigated EALs are capable of determining the working length of the HRF and that Root ZX showed a higher accuracy rate in detection of simulated horizontal root fractures. It should be emphasised that the results obtained in this in vitro study cannot be directly extrapolated to the clinical situation, but can provide an objective examination of a number of variables that are not practical to test clinically.

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KEY WORDS

Electronic Apex Locator (EAL); Root ZX; Propex II; Horizontal Root Fracture (HRF)

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INTRODUCTION

Root Fractures have been marked as one of the most notorious injuries for a tooth, as they exhibit inadequate discernable signs and symptoms and are often difficult to diagnose and hence treat, along with having an unprecedented prognosis. Incomplete root fractures whether vertical, horizontal, or oblique in nature are often among the most difficult cases to diagnose clinically and radiographically in clinical endodontic practice [1]. Horizontal root fractures maybe located either in the apical third, middle third, or the cervical third of the root, and primarily dictate the treatment protocol and prognosis of the tooth. Andreason found that in teeth with root fractures with displaced apical segments, the coronal segment can lose its vitality while the apical segment remains vital, under such circumstances an endodontic treatment is performed only on the coronal root canal segment, considering the fracture plane as the limit of the working length [2]. Although the outcome of a horizontal root fracture is generally favorable (60%-80% cases), complications such as pulpal necrosis, radicular resorption, and pulpal canal obliteration can arise [2]. Thus a unitary diagnosis followed by a correct treatment protocol, vastly dictates a favorable or an unfavorable outcome.

Foundation of a sound treatment protocol for a supposed root fracture depends majorly on a complete history of possible trauma and thorough diagnosis. Although Clinical diagnosis and Radiographic examinations are vital pillars of good diagnosis, they fall short in truly detecting presence or absence of root fractures, because of the varied directions and angulations of propagated fractures [1, 3].

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Electronic Apex Locators as an adjunctive diagnostic tool for detection of root fractures have proven to be of significant importance as it uses the electrical resistance principle. Any fracture site that communicates with the periodontium, on file introduction within the canal, will be detected as a new connection between the canal and periodontium and be read as "apex" on the digital display monitor [1, 4].

The accuracy of EALs was poor because of the influence of fluids or pulp tissue in the canal. Advances in EALs technology have led to the development of EALs that make accurate readings in the presence of electrolytes. In vivo studies show the Root ZX to be accurate in locating the minor diameter to within 1mm. Recently, a new fifth generation EAL, the Propex II, has been developed (Dentsply Maillefer). It measures with multi-signal frequencies the energy of the signal, not the amplitude as for all EALs [5]. All modern EALs are able to detect root perforations and lateral canals within a clinically acceptable limit [6, 4]. Any connection between the root canal and the periodontal membrane, such as root fracture, cracking and internal or external root resorption will be recognized by the EAL, which serves as an excellent diagnostic tool in these circumstances [7].

The aim of this study was to evaluate in vitro the effectiveness of two apex locators in detecting simulated horizontal root fractures and to prove apex locators as an additional clinical diagnostic aid in detecting fractures and their exact location so as to help the clinician to decide on the best treatment option for the particular tooth.

MATERIALS AND METHODS

Forty single rooted, permanent human teeth, extracted for periodontal reasons, were used in this study. Teeth were preserved in 10% formalin for 24 hrs and then in 0.9N saline till further use. Teeth with sound roots and no evidence of root resorption or fractures were used. The teeth were decoronated at Cemento-Enamel Junction so as to prepare flat uniform horizontal surfaces and endodontic access cavity was prepared using Endo access bur (Dentsply). The canals were located and the patency of the canals was checked using 15 K files (Dentsply, Tulsa, Okla) along with EDTA as the chelating agent. Horizontal root fractures were simulated using a 0.2mm thick disc until half of the canal was exposed circumferentially, in the horizontal plane, at coronal, middle or apical third of the tooth by operator No.1. After fracture simulation, access cavities were dried with cotton pellets and all root canals were dried with absorbant paper points. The teeth along with the Lip clip of the Apex Locator were embedded in an alginate model as suggested by Kaufman et al [8]. The alginate was continuously bathed with water to maintain the conductivity of the medium. First the fractures in all the samples were detected using the third generation apex locater (Root ZX) with its lip clip embedded in the alginate model. The 25 K file was gradually advanced down in the canal without excessive force until the liquid crystal display of Root ZX indicated the flashing word "APEX". Once the beeping sound was heard the file stopper was placed adjacent to the flat coronal surface. The file was removed, and the distance between the stopper and the file tip was measured with a digital vernier calliper to 0.01 mm accuracy. Similarly the readings were taken with the fifth generation apex locater Propex II. All the readings were carried out by a second operator, who was kept blind about the fracture locations. This was done to avoid biased readings and to ensure standardization of the experimental technique.

After recording the lengths of the simulated fractures, using both apex locaters, the samples were removed from the Alginate model and the fractures were completed with the safe sided diamond disc to obtain the actual fracture length measurements. The Real fracture lengths [RFL] were then measured using a size 40 K file (Dentsply, Tulsa, Okla) under 3X magnification and with these actual length readings, the readings of the two EALs were compared, allowing a tolerance of 0.5mm and 1.0mm. Measurements were obtained and those not within these limits were considered as unacceptable.

RESULTS

The length measurements obtained with the two apex locators were analyzed and the accuracy was compared with actual fracture lengths observed under 3X magnification, with the Mc Nemer's chi-square (χ^2) test ($p<0.05$). **Table-1** shows that at 0.5 mm tolerance the p value for Propex II and Root ZX ($p<0.001$) is highly significant, whereas on comparing the accuracy between Propex II and Root ZX the p value is not significant. **Table-2** shows that at 1.0 mm tolerance the p value for Propex II ($p<0.001$) is highly significant, p value for Root ZX ($p>0.05$) is not significant whereas on comparing the accuracy between Propex II and Root ZX the p value is significant.

In this study at $\pm$0.5 mm tolerance both the EALs showed statistically significant results when compared with real fracture length (RFL) and at $\pm$1.0 mm tolerance Propex II showed statistically significant result but Root ZX showed statistically insignificant result. Also at $\pm$1.0 mm tolerance level a statistically significant difference was found between the two EALs, with Root ZX being more accurate than Propex II **[Figure-1]**.

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Table-1: Percentage Accuracy of fracture location between the two apex locaters with a 0.5 mm tolerance

Tolerance	Accuracy	Propex II (n%)	Root ZX (n%)
0.5 mm	Accurate	17 (42.5%)	19 (47.5%)
	Non accurate	23(57.5%)	21(52.5%)

Non accurate	Propex II (n%)	Root ZX (n%)
Long	1(2.5%)	3(7.5%)
Short	22(55%)	18(45%)

Accuracy at 0.5 mm tolerance	Mc Nemer's Chi-square (χ^2) Test	P value
Propex II vs RFL	21.04	p<0.001 **HS**
Root ZX vs RFL	19.04	p<0.001 **HS**
Propex II vs Root ZX	0.1	p>0.05 NS

n- number of samples (40) , %- percentage in each group, RFL- Real fracture length

Table: 2. Percentage Accuracy of detecting location of fracture between the two apex locaters with a 1.0 mm tolerance

Tolerance	Accuracy	Propex II (n%)	Root ZX (n%)
1 mm	Accurate	28 (70%)	36(90%)
	Non accurate	12(30%)	4(10%)

Non accurate	Propex II (n%)	Root ZX (n%)
Long	1(2.5%)	
Short	11(27.5%)	4(10%)

Accuracy at 1.0 mm tolerance	Mc Nemer's Chi-square (χ^2) Test	P value
Propex II vs RFL	10.08	P<0.001 **HS**
Root ZX vs RFL	2.25	P>0.05 NS
Propex II vs Root ZX	6.12	P<0.01 **Sig**

n- number of samples (40), %- percentage in each group. RFL- Real fracture length

DISCUSSION

Root Fractures are traumatic injuries of teeth involving dentin, pulp and cementum. Horizontal root fractures that comprise 0.2%-7% of all traumatic injuries commonly occur in the anterior maxillary region [9], and incisors with complete root formation are the most affected teeth because of the decreased elasticity of the alveolar bone cavity [10]. The type and location of fracture depends on age of patient, amount of force, and direction of blow [11]. Diagnosis of root fractures cannot be done by means of radiographs within the first hours after the dental trauma incident; thus the necessity of periodical clinical and radiographic controls becomes necessary [3]. Clinically, one must carefully evaluate mobility, the displacement of the coronal segment, the presence or absence of tenderness on palpation of the soft tissues, and percussion of the teeth in question [9]. Accurate radiographic diagnosis poses challenges if the root fracture plane is beveled buccopalatally, making its interpretation on the radiograph difficult. Also the radiographic accuracy is influenced by a number of factors such as tooth inclination, position and angulation of x-ray beam, and superimposition of the anatomical structures among others [2].

One of the perplexing problems in endodontic therapy is unforeseen horizontal or vertical fractures of the root canal wall which are often difficult to diagnose and to treat. It has been postulated that apex locaters could be used to determine the position of a fracture if it communicates with the periodontal membrane. Nahmias et al [12] and Chong and Pittford et al [13] reported that if there is any connection between the root canal and the periodontal

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membrane such as root fracture and cracks it would be recognized by the EALs. Ebrahim et al evaluated in vitro the accuracy of three different apex locaters in detecting simulated horizontal and vertical root fractures, they found that the three EALs tested were accurate and acceptable clinical tools in the detection of horizontal root fractures [7]. From a theoretical point of view, EALs would mark the first zone having a periodontal communication as the apex rather than marking the true foramen. This communication could be a fracture, a fissure, a perforation or a lateral canal [4].The accuracy of EALs is influenced by many factors for example electrolytes, foramen size, resorption, pulp vitality and instrument size. There are still concerns as to whether high electro conductive irrigants such as blood, irrigant fluids can affect the accuracy of EAL performance [14]. Clinically these factors must be considered while using EAL to detect the root fracture. Clinically it is more important to be able to diagnose the exact location of a fracture rather than its mere presence as this can influence the treatment options and eventual fate of the teeth [15].

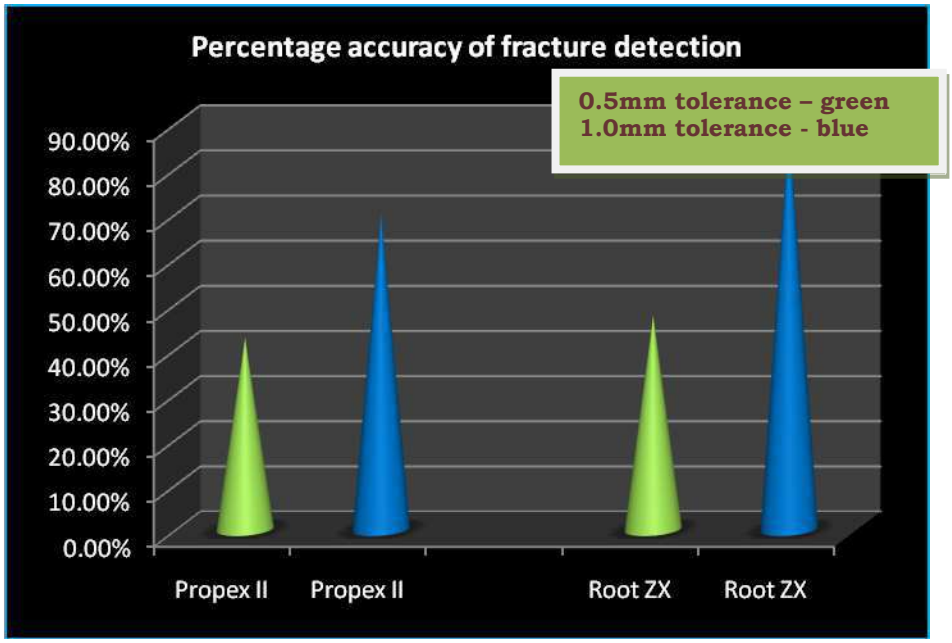

Fig: 1. The percentage accuracy of fracture detection of both the apex locaters (third – Root ZX and fifth – Propex II) at 0.5 mm and 1.0 tolerance

In our study we compared third generation apex locater (Root ZX) and fifth generation apex locater (Propex II) for their accuracy in detecting the simulated horizontal root fractures. The Root ZX, third generation apex locater that uses dual frequency and comparative impedance principles, was described by Kobayashi and Suda. The Root ZX simultaneously measures two impedances at two frequencies by ratio method [12]. A number of in vitro and in vivo studies on the accuracy and reliability of Root ZX to locate apical constriction have been reported but there are very few documented studies on accuracy of Root ZX to detect the horizontal root fracture. In a study conducted by Ebrahim et al the Root ZX was more accurate in detection of horizontal root fractures than the other apex locaters [7]. Ozgur Topuz et al compared the accuracy of two apex locating hand pieces in detecting simulated horizontal root fractures. In this study Tri Auto ZX was more successful than TCM Endo V in detecting the simulated horizontal fractures [1]. Fernando Goldberg et al evaluated the ability of four EALs to determine the location of simulated horizontal oblique root fractures. The results of this study showed that Root ZX showed unacceptable measurements as compared to Propex and NovApex [3]. Dr Lotika Beri and Dr. Gaurang compared three EALs for detecting the location of simulated oblique fractures and found that Root ZX showed 86.6% accuracy at 0.5mm tolerance [2]. Hua Xi, Kou Qiang, Yi Xue, Za Zhi conducted a study to evaluate the accuracy of Root ZX in detecting simulated horizontal root fractures. They concluded Root ZX lacks diagnostic value for horizontal root fractures without soft tissue ingrowth, but provides preferable veracity for horizontal root fractures with soft tissue in growth [16].

The Propex II is a ratio type apex locater that determines the impedances at two frequencies like the third

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generation apex locaters but unlike the third generation apex locaters it only uses one frequency at a time that eliminates the need for filters that separates the different frequencies which helps eliminate the noise inherent in the filters, and increases the accuracy [17]. Very few studies have been conducted to evaluate the accuracy of Propex II, since the time it has been developed and all of them relate its accuracy with respect to working length. An ex vivo studies conducted by Luigi Ciancoi et al [5] and Manuele Mancini et al [18], showed that the Propex II gave more accurate results as compared to other two EALs in determining working length. Contrary to these results, in an In- vitro study conducted by Mahima Tilakchan et al [19] and Kenner Bruno Miguita et al [20]; the Propex II was not as accurate as other EALs tested. However, the literature review reveals that there are no studies evaluating the accuracy of Propex II to detect root fractures and in this respect ours maybe the first study.

The results of our study show that both the EALs are able to detect the fracture location. These results are in agreement with those of Azbal et al [4], Ozgur Topez et al [1], Fernando Goldberg et al [3], who found that the EALs were able to detect simulated horizontal root fractures. Measurements attained with the ± 0.5mm tolerance range are considered highly accurate. Shabahang et al [21] and Fernando Goldberg et al [3] suggested that 1.0 mm tolerance be considered as clinically acceptable, especially when the determination of the apical limit becomes more difficult because of the fracture plane inclination with respect to the root axis. Also, in contrast to the apical terminus, no constriction can be felt at the site of fracture during treatment [3]. Moreover, another source of error that may arise in the direct determination of the fracture length might be the difficulty in the visual control of the relation between the rubber stopper/reference point, rubber stopper/ digital vernier calliper scale, and file tip/ digital vernier calliper scale. In addition, sometimes it is challenging to visualize the exact point where the tip of the file reached the cervical border of the foramen, even with magnification [17]. Therefore in our study ± 0.5 mm and ± 1.0 mm tolerance levels from real fracture location (RFL) were used to test the accuracy of the two EALs. Both the EALs used in our study detected fracture location with approximately 50% accuracy at 0.5mm tolerance level but at 1.0 mm tolerance level Root ZX showed 90% accuracy and Propex II showed 70% accuracy. Thus Root ZX showed a higher accuracy rate in detection of simulated horizontal root fractures. These results are in agreement with those obtained by Ebrahim et al [7] and Dr. Lotika Beri [2].

Literature review reveals that the EALs show tendency to make shorter measurements than the longer ones [4, 22]. Our study is in accordance to these findings. The results of our investigation show that both the EALs gave a far greater number of shorter measurements than longer ones, as compared to RFL. Research has yet to support a significant increase in accuracy or precision of 5th generation Propex II over the 3rd generation Root ZX. Recent studies have not shown improvement in 4th and 5th generation EALs when compared to the 3rd generation Root ZX in terms of accuracy in locating the minor constriction. The use of the descriptor "fourth and fifth generation" appears to be an attempt to imply product superiority rather than to describe a factual improvement in technology [13]. Although a few studies [2,3,4,7] have examined the ability of EALs in detecting the root fractures, no studies are present in current literature on the accuracy of the Propex II device in fracture detection. The manufacturer does not specify any technical characteristics of Propex II and hence it is unclear as to why Propex II is not as accurate as Root ZX in detecting the fracture.

CONCLUSION

Under the experimental conditions of this study, it can be concluded that the investigated EALs are capable of detecting horizontal root fractures. They also determine the working length of the coronal root segment in a high percentage of teeth with horizontal root fractures at a ±1.0mm tolerance. Nevertheless, further research needs to be conducted to study the role of EALs in cases with root fractures. Understanding and overcoming the minor discrepancies between EAL readings and the Real Fracture length may make EALs an important diagnostic tool in accurately detecting horizontal root fractures.

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CONFLICT OF INTERESTS

There is no conflict of interest amongst the authors.

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CASE REPORT OPEN ACCESS

MAXILLARY FIRST MOLAR': AN ENIGMA FOR ENDODONTISTS

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ABSTRACT

Morphological variations found in the root canal system are not the exception but the rule. These variations occur with great frequency in the maxillary 1st molar. The identification, thorough debridement and obturation of the complex anatomy leads to successful endodontic therapy. The use of adjuncts such as CBCT imaging and operating microscope greatly aid in visualization and precision in treatment.

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KEY WORDS

Morphological variation; CBCT; Maxillary first molar; Endodontic therapy

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INTRODUCTION

Sound knowledge of the root canal system and its frequently encountered variations help clinicians in achieving successful endodontics [1] .Anticipating the presence of variations should be a routine practice while performing endodontic therapy. Maxillary first molars have the most complicated root canal morphology amongst the permanent dentition; therefore, their anatomy has been evaluated extensively in various studies.

Maxillary first molars usually present with three roots and three canals, with a second mesiobuccal canal (MB2) canal seen in 18- 96.1% of the cases [2]. Nevertheless, it can present with more or less number of roots or root canals than the accepted norm. Other variations for maxillary first molars include one [2], four [3], and five [4] roots, which may include unusual morphologies of root canal system within individual roots. Cases with five [5], six [6], seven [7], eight root canals [8] or a C-shaped canal configuration [9] have also been reported earlier. Two-rooted maxillary first molar with two canals have rarely been reported. Such anatomic variations have been reported in limited number of studies for maxillary second molar.

This case series presents maxillary first molars with unusual morphologies of patients who reported to the Department of Conservative Dentistry and Endodontics: 1. two roots and two root canals, 2. three roots with five canals (2 Distobuccal canals), 3.three roots with five canals (2 palatal canals) and 4. three roots with 6 canals (3 mesiobuccal and 2 distobuccal canals). The use of adjuncts such as the operating microscope and Cone Beam Computed Tomography (CBCT) are imperative in the identification and location of additional canals and therefore must be used during treatment.

CASE DESCRIPTION

Case 1

A 38-year-old female patient reported to the postgraduate clinic with the chief complaint of spontaneous pain in the upper right posterior tooth for the past 15 days. The patient reported symptoms of prolonged sensitivity to hot and cold beverages. On clinical examination, the right maxillary first molar (tooth #16) presented with features of hypoplasia with attrition. The tooth was tender on vertical percussion. The tooth was hyper-responsive to thermal testing (cold and heat test). Preoperative intra oral peri-apical radiographs (IOPAR) revealed an occlusal radiolucency involving the enamel, dentin and approaching the pulp space [**Figure– 1a**]. On close examination of

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the IOPAR only two root outlines were evident. A diagnosis of symptomatic apical periodontitis was made and root canal therapy was decided on as the definitive treatment option.

Local anesthesia was administered and rubber dam was placed. A conventional endodontic access opening was performed. On viewing the access cavity only two canal orifices were located, and thus the search for the other canals was undertaken using the dental operating microscope. The shape of the access cavity was dictated by the location of orifices. Root canals were explored with ISO #10 K-files. However, the attempt to locate another canal was unsuccessful. CBCT was advised in relation to tooth #16 to confirm the varied root canal morphology **[Figure– 1b]**.

CBCT confirmed the presence of two roots (buccal and palatal) with two canals. At the next appointment, working length was determined and a hybrid technique was used for chemo-mechanical preparation using standard irrigation protocol **[Figure– 1c]**. Buccal and palatal canals were instrumented with hand K files up to 70 and WaveOne primary file respectively. Obturation was performed using cold lateral compaction of gutta-percha (Dentsply Maillefer) for the buccal canal and WaveOne GP cones for the palatal canal using AH Plus resin sealer (Maillefer Dentsply, Konstanz, Germany) **(Figure 1d, 1e, 1f)**.

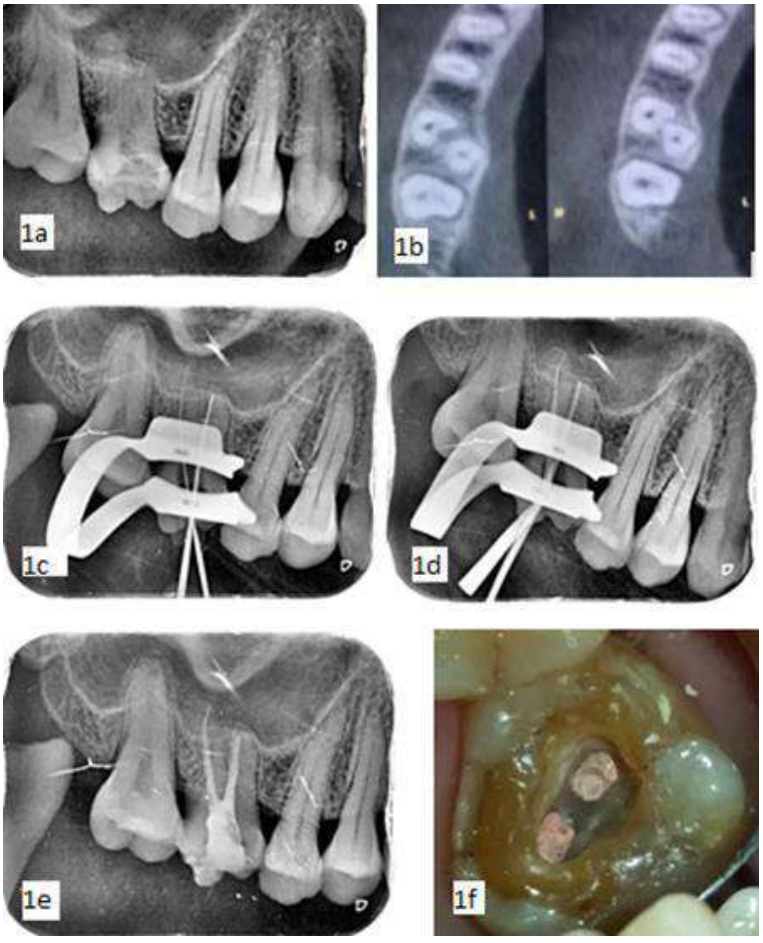

Fig: 1a. Preoperative periapical radiograph, **1b.** CBCT axial plane,1c. Working length determination, **1d**. Master cone selection,**1e.** Post-obturation radiograph, **1f.** Clinical image showing two canal orifices

Case 2

A 29-year-old male patient presented with pain in the right upper back tooth region since 2 days. The pain was spontaneous and severe in nature. On clinical examination deep dental caries was observed with tooth # 16. The tooth was also found to be tender on vertical percussion. An IOPAR revealed a radiolucency involving enamel,

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dentin and approximating the pulp on the mesial aspect. Widening of the periodontal ligament space was observed. As the patient was experiencing excruciating pain, emergency access opening was performed.

On performing the access opening 5 canal orifices were observed **[Figure– 2a].** The canals were scouted with standardized ISO #10 K files. An informed consent was obtained from the patient and a CBCT scan was advised to investigate the presence of any additional canals. The scan revealed the presence of 5 separate canals **[Figure– 2b]**.

Tooth #16 was isolated using rubber dam and coronal pre-flaring was done with a Protaper SX Rotary NiTi file. The working length was determined using an apex locator (Apex ID, Sybron Endo) and verified radiographically **[Figure– 2c]**. Chemo-mechanical preparation was done using RaCe rotary Ni-Ti files following standard irrigation protocol. The MB1, MB2, DB1 and DB2 canals were instrumented upto #25/0.06. The palatal canal was prepared till #30/0.06. The 5 roots were obturated with the corresponding size gutta percha cones using AH plus sealer **[Figure– 2d]**.

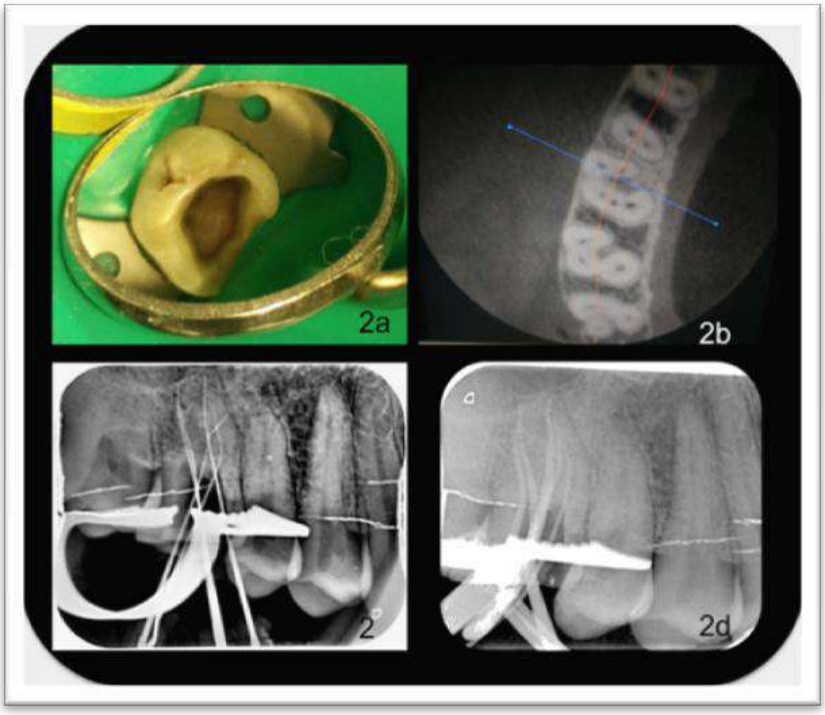

Fig: 2a. Clinical image showing 5 canal orifices, **2b.** CBCT image, **2c.** Working length determination, **2d.** Master cone selection and obturation

Case 3

A 27-year old male patient reported to the Department with the chief complaint of pain in the right upper back tooth region since a month. The pain was intermittent in nature and increased on mastication. The patient's medical history was non-contributory.

On clinical examination deep dental caries was observed with tooth # 16. The tooth was also found to be tender on vertical percussion. The IOPAR revealed a radiolucency involving enamel, dentin, extending to the pulp on the mesio-proximal aspect with periapical widening.

In the pulp chamber floor, the 3 root canal orifices were identified: MB, DB, and palatal. The pulp chamber floor was then scouted to locate the fourth canal in the MB root. After probing with a DG 16 (Dentsply) endodontic explorer and scraping calcifications with a spoon excavator, a small hemorrhagic point was noted in a groove

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approximately 2 mm from the MB orifice in a palatal direction. At the same time a similar hemorrhagic point was noted near the orifice of the main palatal canal.

To verify the presence of extra canals CBCT was taken w.r.t 16. The CBCT clearly showed the presence of 2 mesial orifices, 2 palatal orifices and a single distal orifice **[Figure– 3a]**. Protaper SX (Dentsply) was used for coronal pre-flaring, following which K files were used to clean and shape the canal system. The working length was determined using an apex locator (Apex ID, Sybron Endo) and verified radiographically (**Figure-3b**). This was followed by rotary instrumentation of all the canals up to 25/0.04 with Hyflex CM rotary NiTi files. The canals were obturated using AH plus sealer and 25/0.04 gutta-percha **[Figure– 3c & 3d]**.

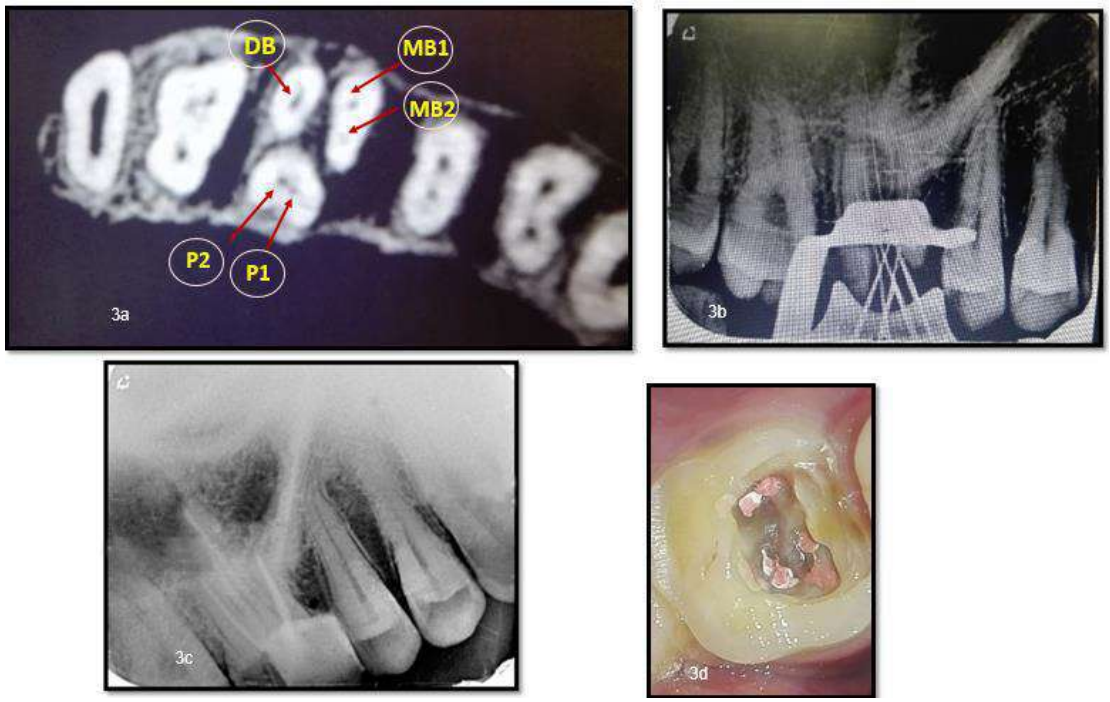

Fig: 3a. Working length determination, **3b.** Post-obturation radiograph, **3c**. Clinical image showing 5 canal orifices, **3d.** CBCT image

Case 4

A 28-year old female patient reported to the Department of Conservative Dentistry and Endodontics, with the chief complaint of severe pain in the left upper back region of the jaw. The patient was experiencing severe discomfort, hence emergency access opening was undertaken after administering an infiltration of local anaesthesia. On visualizing the access cavity, 7 canal orifices were visible- 3 mesio-buccal, 2 disto-buccal and 2 palatal [**Figure– 4a**]. To verify the number of canals present, a CBCT scan was advised.

The CBCT scan was viewed and 6 canals were found to be present: 3 mesio-buccal, 2 disto-buccal and one large palatal canal [**Figure– 4b**]. Rubber dam was placed and all the 6 canals were negotiated using ISO #10 K files. Coronal pre-flaring was done using Protaper Sx rotary Ni-Ti file. The working length was determined [**Figure– 4c**]and chemo-mechanical preparation was completed using Hyflex CM and RaCe rotary NiTi files. The MB1, MB2, MB3, DB1 and DB2 canals were enlarged to #25/0.04 using Hyflex CM rotary NiTi files. The palatal canal was enlarged to #30/0.06 using RaCe rotary NiTi files. Irrigation between files was done using 3% Sodium Hypochlorite solution. Final rinse was done with 1 ml of 17% EDTA followed by 2% Chlorhexidine gluconate solution with an intermediate rinse of distilled water. The 6 canals were obturated using gutta percha of corresponding sizes along with AH plus sealer [**Figure– 4d**]. A post-obturation CBCT scan was taken to re-confirm the obturation of all the canals [**Figure– 4e**].

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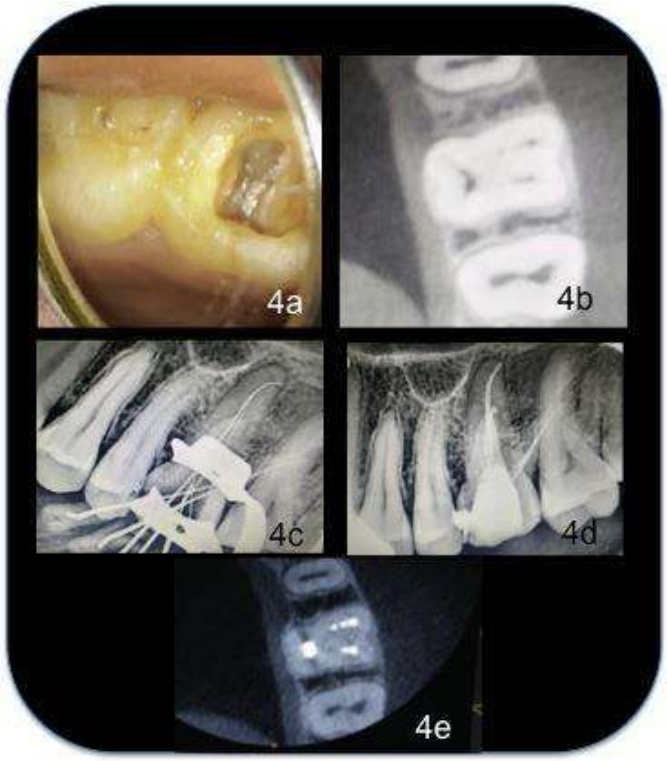

Fig: 4a. Clinical image showing 6 canal orifices, **4b**.CBCT image, **4c.** working length determination, **4d.** Post-obturation radiograph, **4e.** Post obturation CBCT image

DISCUSSION

Due to the varied morphology, endodontic treatment in multi-rooted teeth becomes a challenging task. The occurrence of a root with a tapering canal and a single foramen is the exception rather than the rule. A wide range of variations and complexities with regard to maxillary molar have been reported in literature [10]. It is not unusual to see one or more additional canals in a maxillary first molar and on the other hand finding canals less than the normal expected number is also plausible. An examination of the floor of the pulp chamber offers clues to the type of canal configuration present.

The development of roots involves formation of an epithelial diaphragm. In multi-rooted teeth, the epithelial diaphragm undergoes differential growth, which causes the division of the root trunk into two or three roots. Depending upon the number of divisions that occur, subsequent numbers of roots are formed.

In multi-rooted teeth the epithelial diaphragm is genetically programmed to undergo differential growth but under very rare condition this differential growth may fail to take place. And this may give rise to the formation of single or bi-rooted maxillary first molar [11]. Fusion of two buccal roots is one of the most common aberrations of maxillary molars. A total of 0.4% of first maxillary molars and 2.2% of second maxillary molars have been reported to have this variation [12].

Root canal morphology should be comprehensively examined on preoperative radiographs from different horizontal angles. The use of additional radiographic views with 20-degrees mesial or distal angulations is a good practice for the assessment of the root canal morphology and anatomy [9]. Nevertheless, it is not completely reliable because of its inherent limitation of it being a two-dimensional representation of a three-dimensional object.

The use of advanced diagnostic tools such as CBCT, aids in the accurate and conclusive assessment of cases with unusual canal morphology [8]. Reconstructing CBCT images require significantly lower radiation dosage as compared to alternative conventional computed tomographic scans. This is because in CBCT imaging, the raw

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data is acquired in the course of a single sweep of a cone-shaped x-ray source and a reciprocal detector around the patient's head. Efficient use of the radiation beam and elimination of a conventional image intensification system (used in conventional computed tomography scanners) has resulted in a huge reduction in the radiation exposure to the patient.

Carr et al affirms that the operating microscope has greatly improved the ability of the endodontist to visualize the internal anatomy of the root canal with greater clarity [13]. Magnification and imaging tools should be employed to confirm the root canal morphology and avoid procedural mishaps like gouging, perforation, missed canals etc. which may comprise the prognosis.

CONCLUSION

Clinicians must have adequate knowledge of the morphological variations that can occur in the root canals. It is important that the various canal morphologies be evaluated prior to and during endodontic treatment. Advanced diagnostic aids like CBCT and dental operating microscope can aid in achieving predictable treatment outcomes.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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REVIEW OPEN ACCESS

CBCT: A VALUABLE IMAGING TECHNIQUE IN ENDODONTICS

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ABSTRACT

Radiographic examination represents essential part of contemporary management of endodontic problems, from diagnosis & treatment planning to outcome evaluation. Intra-oral &panoramic radiographic assessment have inherent limitations that 3-diamensional anatomy (3D) is compressed in 2 dimensional (2D) image, superimpositions of anatomic structures resulting in geometric distortion of area and anatomic noise that can hide region of interest.Cone beam computed tomography (CBCT) is relatively new method that produces three dimensional (3D) information of maxillofacial skeleton including teeth & their surrounding tissue with lower effective radiation dose than traditional CT scan. CBCT imaging can be used in all phases of treatment including diagnosis, treatment planning, during treatment phase & through post treatment assessment & follow up. CBCT has great potential in managing endodontic problems, as well as for assessing root fractures, apical periodontitis, resorptions, perforations, canal anatomy & nature of alveolar bone topography around teeth. The purpose of this article is to review the use of CBCT imaging in the diagnosis & treatment planning & assessing the outcome of endodontic complications & advantages of CBCT over conventional radiography.

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KEY WORDS

Periapical Radiograph (PR); Cone beam computed tomography (CBCT); Apical periodontitis; Periapical lesions; Root canal treatment; Vertical Root fractures.

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INTRODUCTION

Imaging is an important diagnostic adjunct to the clinical assessment of the dental patient. The introduction of panoramic radiography in the 1960s and its widespread adoption throughout the 1970s and 1980s heralded major progress in dental radiology. However, intraoral and extraoral procedures, used individually or in combination, suffer from the same inherent limitations of all planar two-dimensional (2D) projections: magnification, distortion, superimposition and misrepresentation of structures. Computed tomography was available for 3-dimensional dental imaging in the 1980s, but due to the high cost, limited access, and radiation exposure, utilization was limited to management of craniofacial anomalies, complex surgeries, and other unique dental situations. In 1988, cone beam computerized tomography (CBCT) was introduced to dentistry. This technology offered 3-dimensional visualization and more complex and more accurate imaging compared to analog and digital radiographs [1,2]

CONE BEAM COMPUTED TOMOGRAPH

A CBCT scanner for dentomaxillofacial use was developed in the late 1990s and, since the very first report, [3] use of this technique has become widespread in dentistry. Using CBCT, a 3D volume of data is acquired in the course of a single sweep of the scanner. The technique is contingent upon a simple, direct relationship between the sensor and the source, which rotates synchronously 180—360° around the patient's head. The X ray beam, which is cone-shaped (hence the name of the technique), captures a cylindrical or spherical volume of data, described as the field of view (FOV). One of the major advantages of CBCT over computed tomography (CT) is the significantly lower effective radiation dose to which patients are exposed.[4] The dose depends on the region of the jaw to be scanned, the exposure settings of the CBCT scanner, the size of the FOV, the exposure time(s), the tube current (mA) and the energy/potential(kV) [5-7].

The use of CBCT technology in clinical dental practice provides a number of potential advantages for maxillofacial imaging.[8]

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- X-ray Beam limitation:
  Reducing the size of the irradiated area by collimation of the primary X-ray beam to the area of interest minimizes the radiation dose. Most CBCT units can be adjusted to scan small regions for specific diagnostic tasks.
- Image accuracy:
  The volumetric data set comprises a 3D block of smaller cuboid structures, known as voxels, each representing a specific degree of X-ray absorption. The size of these voxels determines the resolution of the image. All CBCT units provide voxel resolutions that are isotropic- equal in all 3 dimensions. This produces sub-millimeter resolution ranging from 0.4 mm to as low as 0.076 mm. Because of this characteristic, subsequent secondary (axial, coronal, and sagittal) and multiplanar reformation (MPR) images achieve a level of spatial resolution accurate enough for endodontic measurements.
- Rapid scan time:
  Because CBCT acquires all projection images in a single rotation, scan time is rapid (10-70 seconds) and comparable to medical spiral MDCT systems and panoramic radiography, which is desirable because motion artifacts due to subject movement are reduced.
- Dose reduction:
  Published reports indicate that the effective dose of radiation (average range 36.9-50.3μSv) is significantly reduced by up to 98% compared with conventional fan beam CT systems. This reduces the effective patient dose to approximately that of a film based periapical survey of the dentition (13-100 μSv) or 4-15 times that of a single panoramic radiograph (2.9-11 μSv)

For most endodontic applications, limited volume CBCT is preferred over large volume CBCT for the following reasons:

- Increased spatial resolution to improve the accuracyof endodontic-specific tasks such as the visualization of small features including accessory canals, root fractures, apical deltas, calcifications, etc.
- Highest possible spatial resolution that provides a diagnostically acceptable signal-to-noise ratio for the task at hand.
- Decreased radiation exposure to the patient.
- Time savings due to smaller volume to be interpreted.

APPLICATION OF CBCT IMAGING IN ENDODONTICS

Assessment of Root Canal Anatomy

The success of endodontic treatment depends on the identification of all root canals so that they can be accessed, cleaned, shaped, and obturated [9]. The prevalence of a second mesiobuccal canal (MB2) in maxillary first molars has been reported to vary from 69% to 93% depending on the study method employed. Conventional radiographic techniques, at best, can only detect up to 55% of these configurations.Because of the 2D nature of conventional radiography, it does not consistently reveal the actual number of canals present in teeth. In several studies, CBCT imaging was superior in detecting the number of roots to PRs [10-12].CBCT reconstructions are somewhat important in assessing teeth with an unusual number of roots, dilacerated teeth, and dens in dente [13-15]. Root morphology (ie, the number of root canals and whether they merge or not) can be visualized 3-dimensionally.

Different studies have used CBCT to study the root canal morphology of maxillary molars. Blattner et al [12] assessed the prevalence of second MB canals in extracted maxillary first and second molars in vitro. The teeth were sectioned axially to confirm the true number of root canals. In total, an 80% correlation was reported between CBCT findings and the results obtained by tooth sectioning. Neelakantan et al.[13]compared the efficacy of six methods(modified canal staining and clearing, CBCT, peripheral quantitativeCT, spiral CT, digital radiography and contrast medium-enhanced digital radiography) in identifying the rootcanal systems of 95 teeth. Their results showed that CBCTwas as accurate as the gold standard (a modified canal staining and clearing technique). 3D reconstructions of CBCT images allow clinicians to fully appreciate the internal endodontic anatomy of the root canal system in each type of tooth. CBCT images is also helpful in finding extra canals (Fig 1)

Detection of Apical Periodontitis

CBCT scanning is a tomogram and eliminates anatomic noise, thus enabling the detection of radiolucent endodontic lesions before the buccal or lingual plate is demineralized [16,17]. Apical periodontitis(AP) is correctly identified with conventional radiographic methods when the disease is in an advanced stage according to the periapical index (40% demineralization). When lesions are small, CBCT imaging shows better diagnostic results [18-27]. CBCT software may be used to maximize the diagnostic yield of the captured data, as the reconstructed slices are geometrically accurate because pixels of CBCT images are isotropic. Therefore, periapical

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lesions will not show changes in size or disappear on reconstructed scans as can happen with intraoral radiography as a result of poor irradiation geometry [24].

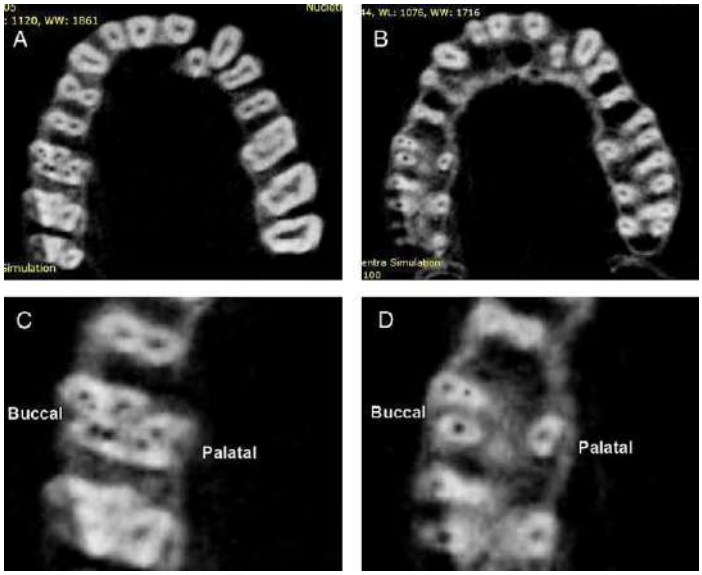

Fig:.1.(A and B) CBCT images of #3 showing axial sections at the (A) cervical and (B) apical level. (C and D) Enlarged axial section CBCT images at the (C) cervical and (D)apical level showing three roots and seven canals.

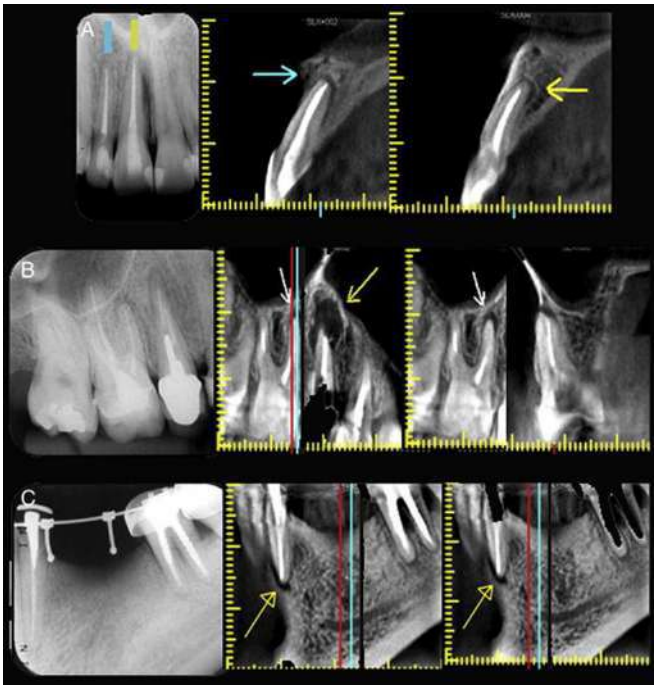

Fig:2. In x-ray periapical lesion is not seen in maxillary anterior but is clearly seen in CBCT image

Estrela et al.[16]compared the accuracy of CBCT, panoramic and periapical radiographs from a consecutive sample of 888 imaging exams of patients with endodontic infection (1,508 teeth) in the detection of apical periodontitis (AP). While a gold standard was not available, they found the detected prevalence of apical periodontitis to be significantly higher with CBCT. Bender and Seltzer [17,25] and Schwarz and Foster [26] showed that the size of the periapical lesion is often underestimated using periapical radiographs. CBCT

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enables the detection of radiolucent endodontic lesions before the lingual or buccal plate is demineralized [19, 27, 28] Use of CBCT eliminates the superimposition of anatomical structures and is useful in identifying processes occurring within the cancellous bone. Both in vivo [16, 20, 29] and in-vitro [19,30]studies have shown that CBCT detects periapical lesions more effectively than periapical radiographs.

Pre-surgicalAssessment

CBCT is particularly recommended for diagnosis and treatment planning before endodontic surgery.The benefits of the use of CBCT during endodontic surgery include elimination of the superimposition of anatomic structures, such as the zygomatic buttress, alveolar bone, maxillary sinus and other roots, and early detection of the presence and dimensions of apical lesions and changes in apical bone density[19,31] The axial, coronal and sagittal planes obtained with CBCT scans also provide clinicians with a clear view of the anatomical relationship between root apices and neighboring structures, such as the mandibular canal, mental foramen and maxillary sinus [31-33]. CBCT imaging may play an important role in microsurgery on the palatal root of maxillary molars; the distancebetween the cortical bone plate and the palatal apex can bemeasured, and the presence of the maxillary sinus between the roots can be assessed [34].

Root Canal Treatment Quality Assessment

The most important area in which CBCT can be applied in endodontics is in determining the outcome oftreatment.Conventional and digital PRs have been widely used for follow-up after root canal treatment. However, in teeth with apical periodontitis, microscopic findings and radiographic examinations are often divergent[35].Chronic periapicalinflammation often persists for years after root canal filling,even in the absence of clinical symptoms and radiographicalterations [36,37]. The most recent literature demonstrates that the detection of periapical lesions following root canal treatment using CBCT is more accurate than that using radiographic evaluation [29, 38-41]. In a retrospective longitudinal cohort study,Ferna´ndez et al.[40]evaluated the outcome of endodontic treatments as assessed by conventional and digital PRs andCBCT during a 5-year follow-up period. They suggested that CBCT was more sensitive than PRs for the visualization of periapical lesions in a long-term evaluation. Liang et al.[41]compared the quality of root canal treatment using PRs and CBCT in teeth with vital pulps. They found thatthe treatment outcome, length and density of root fillings andoutcome predictors as determined using CBCT differed fromthe corresponding values determined using PRs. CBCTdetected periapical lesions in 25.9% of the teeth, comparedwith 12.6% using PRs. Root fillings with voids and unsatisfactorycoronal restorations negatively influenced the outcome.

Assessment of vertical root fracture, resorption

While root fractures are less common than fractures of the crown and occur in only 7% or fewer of dental injuries, [42,43] they are difficult to diagnose accurately using conventional radiography. Numerous authors have illustrated the usefulness and importance of CBCT in the diagnosis and management in specific aspects of dento-alveolar trauma, especially root fractures [44-46], luxation and/or displacement, and alveolar fracture [47]. CBCT has found particular application for the diagnosis of root fractures (Fig.5.5). Identifying the presence of vertical root fracture (VRF) is often an endodontic challenge. Radiographic features suggestive of VRF such as J-shaped and halo-shaped radiolucencies do not appear until significant bone destruction has occurred and similarly shaped radiolucencies may manifest themselves in cases of apical periodontitis not associated with VRF. Four standard procedures have been described to allow a correct and definitive diagnosis [48]: a visualization during an exploratory surgery [48], a visualization after tooth extraction,[48] a radiographic visualization as long as there is a separation of fragments [48] and a Cone Beam Computer Tomography visualization of the fracture[49,50]. Ex vivo studies have demonstrated that CBCT is more sensitive than conventional radiography in the detection of vertical fractures in roots. However, care should be taken when assessing root filled teeth for VRF using CBCT as scatter produced by the root filling or other high-density intraradicular material may incorrectly suggest the presence of a fracture [8].Nair, Mandlik et al published clinical case reports in which the elusive VRF was diagnosed using CBCT [8].

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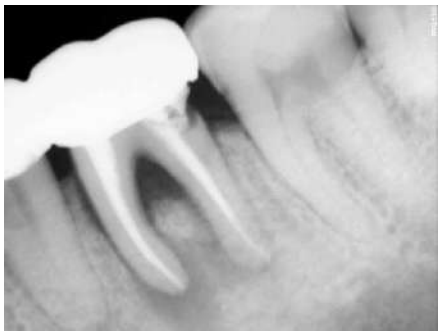

Fig: 3.1. Diagnostic radiograph Fig 3.2 CBCT image showing axial and sagital sections and fracture on mesial root *Courtesy Dr. Nair*

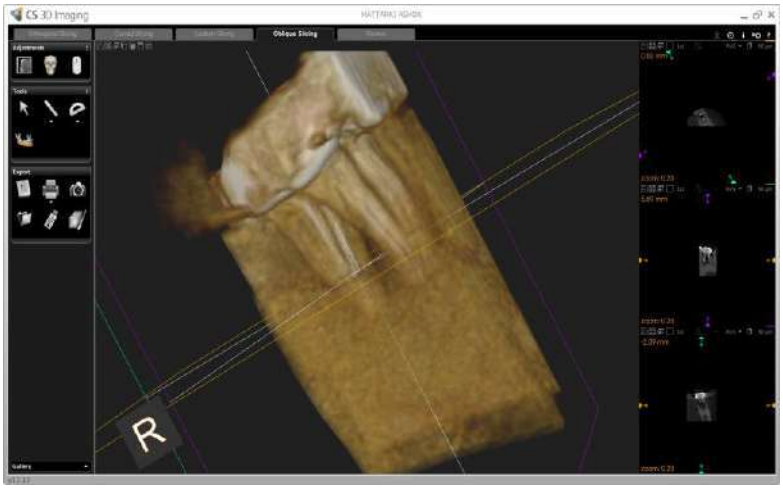

Fig: 3.3 CBCT image showing oblique section and fracture on mesial root *Courtesy Dr. Nair*

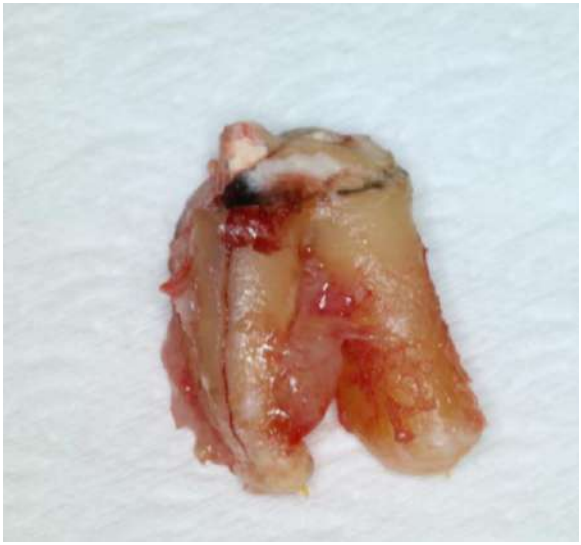

Fig: 3.4. Fracture evident after extraction *Courtesy Dr. Nair*

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Root Resorption

Root resorption is defined as the loss of dental hard tissuesas a result of osteoclastic activities [51]. Resorptive defects may spread within the root in all directions, and their sizes and the positions of radiolucency may not be detected on the radiograph (Fig. 3A and B) [52]. Although intraoral radiography is reasonably accurate in diagnosing internal and external cervical root resorption, CBCT scans enhance the diagnosis of the presence and type of root resorption [53].

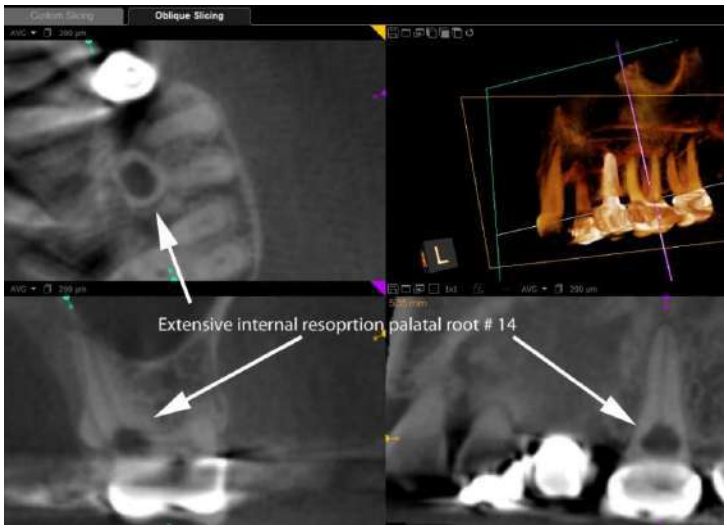

Fig: 4. CBCT showing Internal root resorption

CONCLUSIONS

In conclusion, CBCT technology aids in the diagnosis of endodontic pathosis and canal morphology, assessing root and alveolar fractures, analysis of resorptive lesions, identification of pathosis of non-endodontic origin, and presurgical assessment before root-end surgery. When compared with medical CT, CBCT has increased accuracy,higher resolution, reduced scan time, a reduction in radiation dose, and reduced cost for the patient [6]. As compared with conventional periapical radiography, CBCT eliminates superimposition of surrounding structures, providing additional clinically relevant information. Drawbacks of CBCT include limited availability, and significant capital investment. As CBCT technology evolves, clinicians will be able to adopt 3-D imaging into their diagnostic repertoire. Because accurate diagnostic information leads to better clinical outcomes, CBCT might prove to be an invaluable tool in the modern endodontic practice.However, endodontic cases should be judged individually,and CBCT imaging should be considered for situations in which informationfrom conventional imaging systems may not yield adequateamounts of information to allow for the appropriate management ofendodontic problems.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

FINANCIAL DISCLOSURE

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CASE REPORT OPEN ACCESS

FOUR ROOTED PERMANENT MAXILLARY SECOND MOLAR WITH TWO PALATAL ROOTS: A CASE REPORT

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Department of Conservative Dentistry and Endodontics Jaipur Dental college, Rajasthan, INDIA

ABSTRACT

Knowledge of the morphology and an awareness of unusual anatomy are essential for the successful endodontic treatment. Undetected anatomical variations of roots or root canals which remain untreated are the main reasons for endodontic failure. Clinical and radiographic evaluations should be done thoroughly before initiating endodontic treatment so that the clinician can modify the access cavity for stress free entry to the complex root canal anatomy. The present article describes a case in which anatomical variation existed in the form of additional palatal root in maxillary second molar. The literature review, clinical implication, need to recognize and methods to identify these variations are described in this article.

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KEY WORDS

Maxillary molars, palatal roots, morphological variations, endodontic treatment.

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INTRODUCTION

Sound knowledge of the root canal system and its frequently encountered variations help clinicians in achieving successful endodontics [1] .Anticipating the presence of variations should be a routine practice while performing endodontic therapy. Maxillary first molars have the most complicated root canal morphology amongst the permanent dentition; therefore, their anatomy has been evaluated extensively in various studies.

Maxillary first molars usually present with three roots and three canals, with a second mesiobuccal canal (MB2) canal seen in 18- 96.1% of the cases [2].Nevertheless, it can present with more or less number of roots or root canals than the accepted norm. Other variations for maxillary first molars include one, [2] four, [3] and five [4] roots, which may include unusual morphologies of root canal system within individual roots. Cases with five, [5] six, [6] seven, [7] eight root canals [8] or a C-shaped canal configuration [9] have also been reported earlier. Two-rooted maxillary first molar with two canals have rarely been reported. Such anatomic variations have been reported in limited number of studies for maxillary second molar.

This case series presents maxillary first molars with unusual morphologies of patients who reported to the Department of Conservative Dentistry and Endodontics: 1. two roots and two root canals, 2. three roots with five canals (2 Distobuccal canals), 3.three roots with five canals (2 palatal canals) and 4. three roots with 6 canals (3 mesiobuccal and 2 distobuccal canals). The use of adjuncts such as the operating microscope and Cone Beam Computed Tomography (CBCT) are imperative in the identification and location of additional canals and therefore must be used during treatment.

CASE DESCRIPTION

A 40 year old female patient reported to the clinic with the chief complaint of severe pain in the right upper back tooth region for the past two days. Patient gave a history of intermittent mild pain since one month. On clinical examination, tooth 27 had deep caries. The tooth was tender on percussion. Pre-treatment radiograph showed widening of the periodontal ligament space **[Figure-1]**. The crown was unusually large in appearance. A diagnosis of symptomatic pulpitis with symptomatic apical periodontitis was made and endodontic treatment was planned for 27. The tooth was anaesthetized and then isolated under rubber dam. The coronal access cavity was

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prepared using an endo-access bur. One mesiobuccal, one distobuccal and two palatal canal orifices (mesiopalatal and distopalatal) were located on the floor of the pulp chamber using an endodontic explorer (DG 16 endodontic explorer, Ash Instruments, Dentsply Gloucester, United Kingdom). The dentinal map at the floor of the pulp chamber gave the appearance of letter 'X **[Figure-2]**. The canal lengths were determined using radiograph and an apex locator (Root ZX;Morita, Tokyo, Japan). Cleaning and shaping was performed using ProTaper rotary instrument (Dentsply, maillefer, Switzerland) up to size F2 in crown down technique. Irrigation between each instrument was done using 3.5% Sodium hypochlorite and 17% EDTA. After the master cone selection **[Figure-3]** canals were obturated with the corresponding gutta percha and AH plus sealer (Dentsply, Mallifer, DeTrey Grabh Germany) **[Figure-4]**. The entire procedure was completed under operating microscope. Post endodontic restoration was completed and the patient was recalled for full coverage crown.

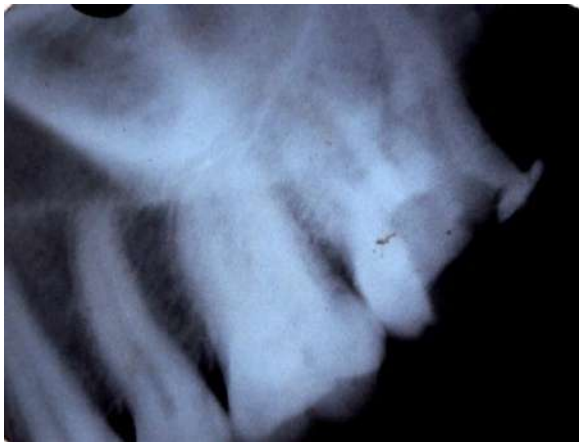

Fig: 1. Pre-operative radiograph

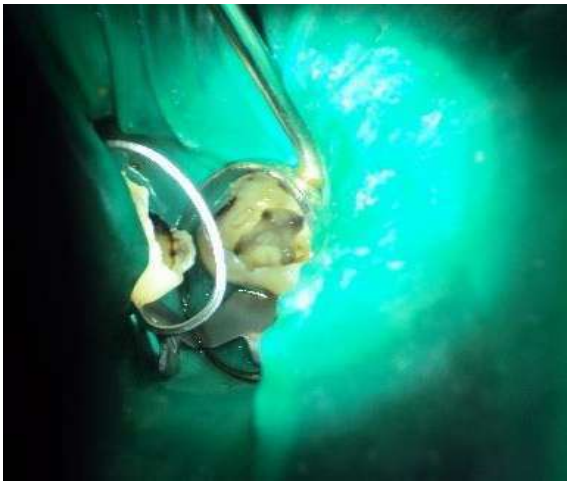

Fig: 2. Access cavity preparation (canal orifices and the dentinal map as seen under operating microscope)

DISCUSSION

The root canal morphology of teeth is often extremely complex and highly variable. However it is more likely to occur in the second or third maxillary molar [5,6,7]. There is a higher tendency towards fusion of two or three roots. Whenever two palatal roots exists in maxillary molars, one of them is the normal palatal root, the other is supernumerary structure which can be located either mesiolingually (radix mesiolingualis) or distolingually (radix distolingualis) [8]. Christie et al proposed a classification system of four-rooted maxillary molars, based on root separation level and root divergence, describing three types (type I- III). Type I molars have two widely divergent

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palatal roots that are often long and tortuous. The buccal roots are often cow-horn shaped and less divergent. Type II molars have four separate roots that are shorter, run parallel, have buccal and lingual root morphology, and have blunt root apices. Type III molars are constricted in root morphology with the mesiobuccal, mesiopalatal and distopalatal canals engaged in a web of dentin. The distobuccal root seems to stand alone and may even diverge to the distobuccal [3].

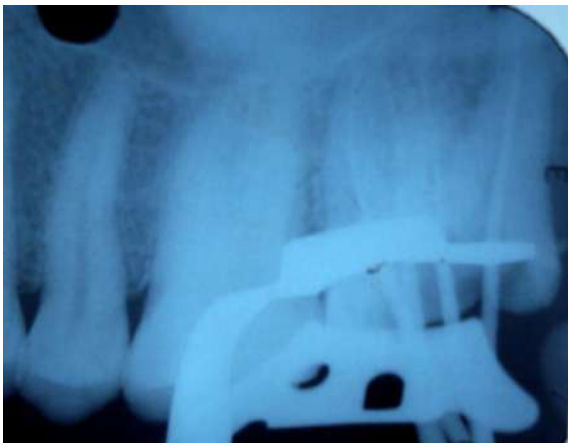

Fig: 3. Master cone selection radiograph

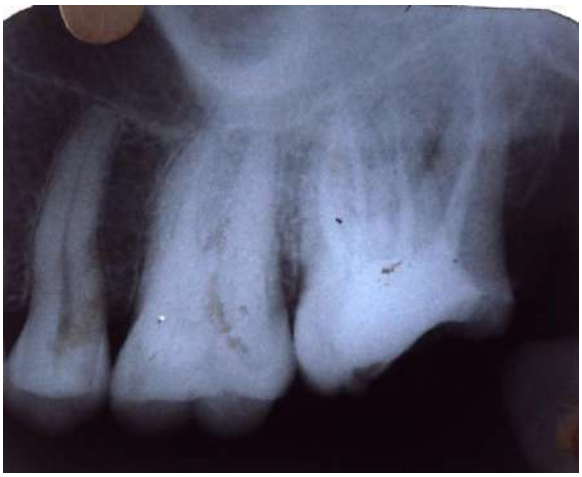

Fig: 4. Immediate post obturation radiograph

Stone and Stoner reported multiple root canal systems in maxillary molars such as a single palatal root containing two separate orifices, canals and foramina[9]. Maxillary second molar variants have already been reported in many clinical cases and in vitro studies and four roots with two separate palatal roots is found to be the least common among all the above[4]. In addition to Peikoff's results, a few less frequently occurred maxillary second molar variants have also been reported. Libfield & Rotstein's(1989) review and radiographic survey of 1200 teeth, reported (0.4%) incidence of maxillary second molars with four roots (two buccal and two palatal); rarer still in maxillary first molars[5]. Braulio et al reported a case of maxillary second molar with six canals[10]. Maxillary second molar with three buccal roots have been reported [11]. The prevalence of taurodontism is reported to range from 2.5% to 11.3% of the human population [12]. Kottoor et al. reported a case of maxillary second molar with five roots and five canals. Based on the anatomical relation of roots and their root canals a naming system was formulated by Kottoor et al and Albuquerque et al. This system is simple, yet extensive and appropriately names the internal and external morphology of maxillary and mandibular molars [13, 14]. Alavi et al.failed to find any four rooted maxillary molars among 268 maxillary molars in a Thai population.[15]. Al Shalabi et al. also did not find any teeth with extra palatal root in a sample of 83 teeth[16]. Even though the supernumerary root described here doesn't occur very often, an awareness of their presence is relevant in endodontics and surgical contexts. During clinical examination, if there is an extra cusp, cervical prominence or a deep groove, presence of an

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additional root should be suspected. Careful observation of pre-operative radiographs is a must in diagnosing variations in the morphology. Superimposition of anatomic structures on these roots of maxillary molars may result in failure to diagnose [6]. A properly designed and prepared access cavity is helpful for diagnosis and negotiation of the root canal morphology [17]. However, some of the common iatrogenic access opening errors are caused during the search for extra or missing canals. These errors include perforations and excessive tooth removal. If the clinician carefully examines the pulp chamber floor and wall anatomy with the help of loupes or an endodontic microscope, such iatrogenic errors can be minimized.

The present case report describes a new variant with four separate canals, mesiobuccal root, two individual palatal mesiopalatal and distopalatal with its own separate canal, and distobuccal root with a single canal. The access cavity was modified to a trapezoidal form to accommodate the orifice of the additional palatal canal and to achieve straight line access to all the canals. Up to now, the variant with four separate roots and four separate canals including two palatal was the least frequent abnormality, with its incidence ranging from 1.47.to 2.1%. Many diagnostic tools are also suggested to confirm these variations with each having their own limitations. These include: a)dye penetration contrast radiography b) CT scans with CBCT technique c)xeroradiography etc.[18,19]. Here the confirmatory diagnosis of the variation is so obvious by only visual examination, that the need for all other specialized techniques other than routine IOPA radiograph was not felt. The importance of unique coronal structure with regard to its large size and shape is stressed.

CONCLUSION

Anatomic variations can occur in any teeth in any form. Although the prevalence of accessory root in maxillary molar is low, it is challenging for diagnosis and successful endodontic therapy. As non treatment of these additional roots or root canals can lead to endodontic failure, every effort should be made to identify it . Thus thorough knowledge of morphology, an awareness of anatomic variations of the teeth and careful observation of radiographs are essential for the location and identification of additional roots and root canals which can be facilitated by using operating microscope, CBCT , xeroradiography, SCT and above all clinical skill for successful treatment..

FINANCIAL DISCLOSURE

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CONFLICT OF INTERESTS

The author declares no conflict of interest.

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CASE REPORT OPEN ACCESS

MANAGEMENT OF SURGICAL COMPLICATION DURING CYSTIC ENUCLEATION IN MAXILLARY CENTRAL INCISOR- A CASE REPORT

Pratik Kotadia, Srinidhi Surya Raghavendra, Sania Singh

Department of Conservative and Endodontics, Sinhgad Dental College and Hospital, Pune Maharashtra, INDIA

ABSTRACT

Radicular cyst is the most common odontogenic cystic lesion of inflammatory origin. It is also known as periapical cyst, apical periodontal cyst, root end cyst, or dental cyst. It arises from epithelial residues in the periodontal ligament as a result of inflammation. The inflammation usually follows the death of dental pulp. In the management of these lesions the endodontic treatment alone is not sufficient and it should be associated with enucleation. Trauma to the adjacent anatomical structure is a common complication in the enucleation procedure. This case report describes the management of exfoliated tooth during enucleation with bone graft and splinting as an adjunct.

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KEY WORDS

Apicoectomy; Enucleation; Radicular Cyst

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INTRODUCTION

Periapical lesions are the sequelae of bacterial infection of dental pulp [1]. Many times they are asymptomatic and are diagnosed during routine dental radiographic examination; however they can have clinical presentation in the form of acute pain in a tooth [2]. These periapical lesions can be granulomas, cysts or abscesses [3, 4]. The incidence of cysts among periapical lesions varies between 6 and 55 % [5]. A radicular cyst comprises 52-68% of all cystic lesions affecting jaws [6]. Most common location is apices of involved non-vital tooth, however other times it may be present on the lateral aspect of the root. These cysts can be seen at any age and can occur in periapical area of any teeth [7]. Shear [8] reported that they have particularly high incidence in the maxillary anterior region with male predilection. Non-surgical endodontic therapy is the first line treatment for the management of these lesions [9]. Oztan and Kalaskar et al. [10, 11] have confirmed that large periapical lesions including cysts can respond favorably to nonsurgical treatment using calcium hydroxide paste. But when root canal treatment is either not possible or fails, periapical surgery can be considered as a predictable option[12]. Hyun-Kyung et al.[13] in their retrospective observational study found that the most frequent management method for the radicular cyst was enucleation with apicectomy.The present clinical case describes the surgical management of infected radicular cyst in a 64 year old male patient using bone graft as an adjunct.

CASE DESCRIPTION

A 64 year old male reported to the Department of Conservative Dentistry and Endodontics with a chief complaint of pus discharge in the upper anterior region. On oral examination severe generalized attrition and abrasion along with sinus tract in was seen between 21 (Upper Left Maxillary Central Incisor) and 22 (Upper Left Maxillary Lateral Incisor **[Figure-1a]**. Sensibility testing (Electric Pulp testing) revealed loss of vitality with 21 and 22. Radiographic examination revealed large periapical lesion with 21 measuring 7×7mm in dimension **[Figure- 1b]**.

After obtaining informed consent from the patient non-surgical endodontic treatment was initiated with placement of calcium hydroxide (Prime Dental Products, Mumbai). Medicament was placed thrice with an interval of 7 days between each, however due to inability to obtain a dry canal and also due to persistent of sinus tract, decision to undertake a surgical intervention was made. The yellowish brown aspirate was sent for cytological examination and a histological diagnosis of a radicular cyst was established.

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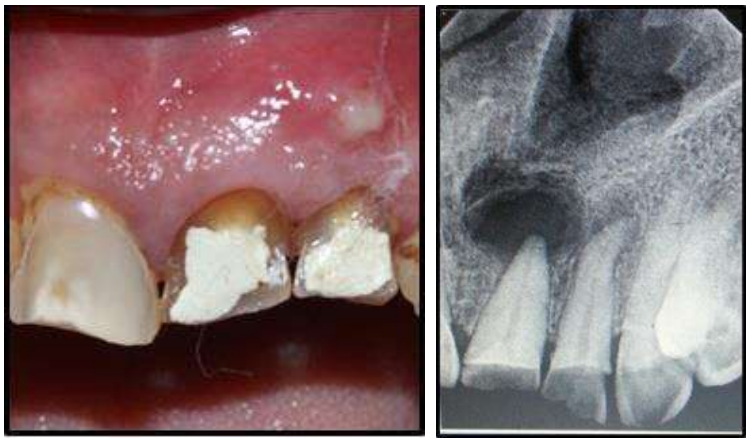

Fig: 1(a). Presence of Sinus. (b) Radiograph Showing Large Periapical Lesion

The surgical procedure was explained to the patient and the informed consent was taken. Bilateral infraorbital and nasopalatine nerve bock was given with lignocaine with 1:80,000 adrenaline (BiochemPharma, Mumbai) and a full thickness mucoperiosteal flap was raised from 11 (Upper Maxillary Right Central Incisor) to 23 (Upper Maxillary Left Canine). On flap reflection the cystic lesion was evident at the apical end of 21 along with dehiscence along the length of the root **[Figure- 2]**. Cystic enucleation was done in toto by packing wet gauze to separate the cyst from the bone **[Figure-3]**. However due to absence of buccal bone support and due to tight adherence of cystic lining on the lingual aspect, enucleation led to exfoliation of 21 in the process **[Figure- 4]**. The exfoliated tooth was held in gauze soaked in normal saline. The apicoectomy was done with ultrasonic tips (Vista Dental Products, USA) and the cavity filled with MTA (MTA Angelus, Brasil). This was followed by its replacement in the socket. The cystic cavity was filled with Bio-Oss bone graft material (Geistlich Bio-Oss, North America **[Figure- 5]** and flap was closed with interrupted sutures. In order to ensure stability of 21 during healing and bone formation, splinting was done using fibre splint (Ribbond THM, Ribbond Inc., Seattle, WA, USA) which was placed on the buccal aspect from 11 to 23 for 2 weeks **[Figure- 6]**.

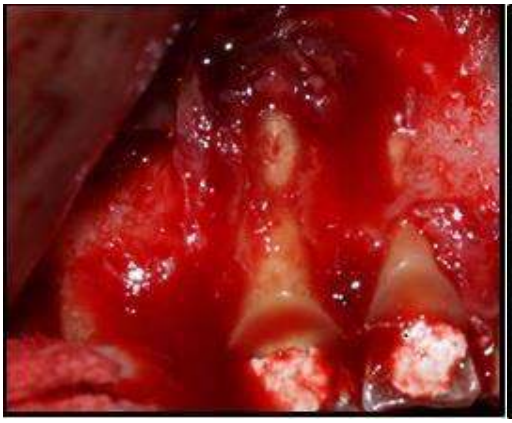

Fig: 2. Presence of dehiscence

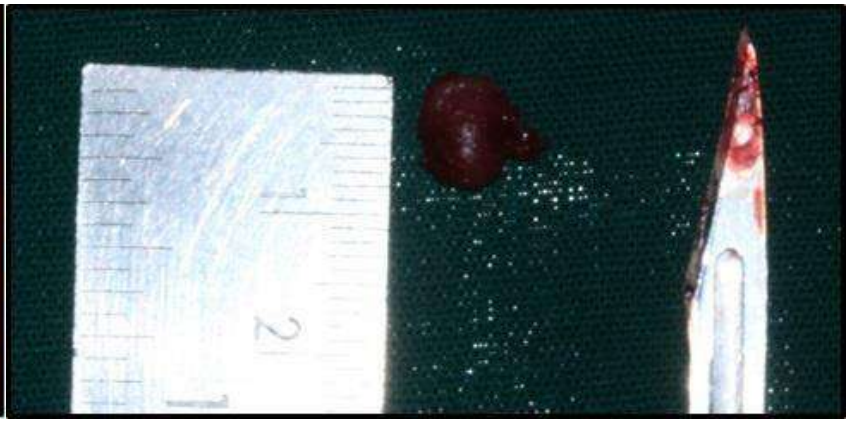

Fig: 3. In toto Cystic Enucleation with 21

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Fig: 4. Exfoliation of 21

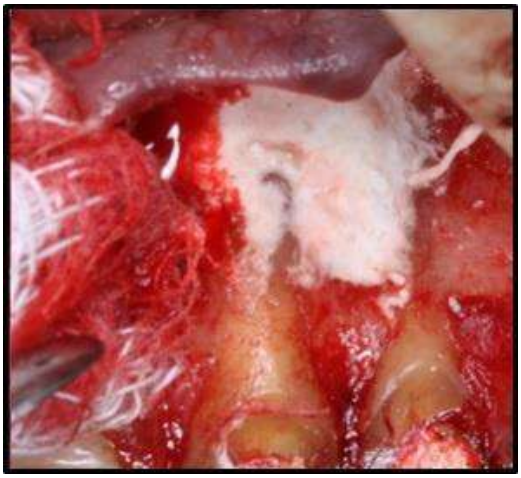

Fig: 5. Placement of Bone Graft

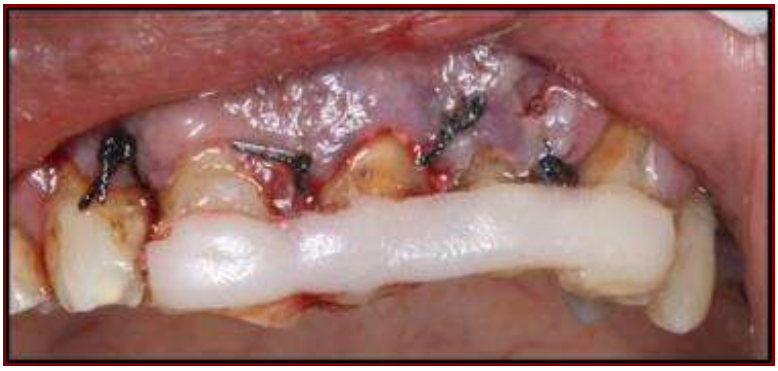

Fig: 6. Placement of Sutures and Splint

Sutures were removed after one week. Examination of 21 after removal of splint post 2 weeks revealed no presence of mobility. Follow up of the case after 6 months showed satisfactory healing of the lesion with no signs of mobility with 21**[Figure- 7]**.

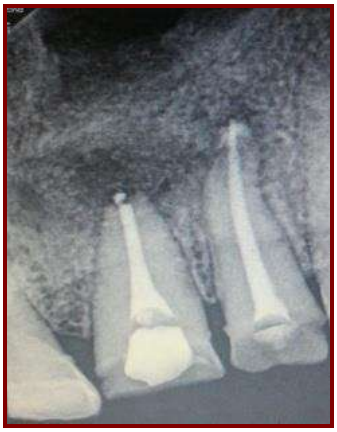

Fig: 7. Follow-Up after 6 Months

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DISCUSSION

A radicular cyst is an odontogenic cyst of inflammatory origin. They are believed to be formed from epithelial cell rests of Malassez (ERM), which are remnants of Hertwig's epithelial root sheath, present within the periodontal ligament. Although the source of the epithelium is usually an ERM, other sources, such as cervical epithelium, sinus lining, or epithelium lining of fistulous tracts, have been suggested [14]. Radicular cysts are inflammatory lesions leading to bone resorption and can reach great dimensions and become symptomatic when infected or with great size due to nerve compression [15].The choice of treatment of these lesions are determined by factors such as extension of the lesion, relation with anatomic structures, evolution, origin, clinical characteristic of the lesion, systemic conditions and cooperation of the patient. The first choice of treatment of these cysts although is a conventional endodontic procedure, however, in large lesions the endodontic treatment alone is not sufficient and it should be associated with decompression or marsupialisation or enucleation [16].

In the present case, endodontic treatment was initiated with interim calcium hydroxide dressings. The use of calcium hydroxide between sessions helps in reducing levels of bacteria better than that obtained with mechanical preparation, particularly by penetration of areas that are unreachable by instruments or irrigating solutions, such as dentinal tubules and ramifications. Calcium hydroxide has also shown clinical efficiency in reducing exudate due to its hygroscopic properties. Studies have shown that calcium hydroxide shows bactericidal activity after 2 weeks [17]. The periapical lesion, however, did not respond to the intracanal medicament as shown by the persistent sinus and weeping canal. This was probably due to the causative factor being located beyond the root canal system, viz.,within the inflamed periapical tissue, thus necessitating surgical intervention.

Full mucoperiosteal flap was used as it gives maximum access and visibility of the root with reduced likelihood of healing with scar formation [18]. Often, enucleation procedures might injure adjacent anatomical structures including teeth [19]. In the present case tooth 21 exfoliated while enucleating the cyst, due to the absence of buccal bone and also due to the tight adherence of the cystic lining on the lingual aspect of the tooth. Exfoliated tooth was stored in the normal saline as it has physiological osmolality and pH [20].

Bio-Oss is a xenograft material derived from bovine bone. It undergoes a heat treatment and chemical extraction process by which the organic components are removed but maintains the natural architecture of cancellous bone. The osteoconductive property leads to effective and predictable bone regeneration. Particles of size 100×200×500Ao are incorporated over time within living bone which provides long-term volume preservation. The biofunctionality of Bio-Oss is characterized by its topographic structure, hydrophilic properties and the biologic interaction that supports reliable bone formation [21].

The advantages of fibre splint are it allows physiological movement of the traumatized tooth to promote healing of the periodontal fibres and ensuring less chances of root resorption [22]. Ribbond is an ultrahigh molecular weight polyethelenefibre splint material. Woven arrangements of fibres transfers stresses efficiently throughout the fibre network, thus preventing fracture. They are highly bondable, with excellent aesthetic and are biocompatible[23]. 6 month follow up of the area showed absence of mobility with radiographic evidence of bone healing.

CONCLUSION

In the management of infected radicular cysts not responding to conventional endodontic therapy, periapical surgery can be considered as a viable option. In the present case complications during surgical procedure were managed using splinting and grafting as an adjunct.

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NIL

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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CASE REPORT OPEN ACCESS

A PRACTICAL APPROACH IN CONSERVATIVE MANAGEMENT OF VERTICAL CORONAL FRACTURE IN MOLAR: A CASE REPORT

Jimish Rajiv Shah, Srinidhi Surya Raghavendra, Kinjal Mahesh Gathani, Swati Ishwar Wadekar

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ABSTRACT

Coronal vertical root fracture with posterior teeth due to exogenous acute trauma is uncommon compared to its occurrence in anterior teeth. Endodontic and restorative management of such fractures is a challenge for the clinician. Newer advancements in adhesive techniques can provide successful intracoronal splinting of such teeth to reinforce the remaining tooth structure. This paper describes the diagnosis and management of a case of complicated vertical coronal fracture in mandibular first molar using splinting followed by buildup with a new fibre reinforced composite core.

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KEY WORDS

Coronal root fracture; Cuspal fracture; Fibre reinforced composite; Vertical root fracture.

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INTRODUCTION

Most common causes of coronal fracture in posterior teeth are chronic masticatory trauma due to presence of large restorations or un-restored endodontically treated teeth [1,2] or an acute trauma. When a visible separation is present at the interface of segments along the line of fracture, it is termed as "complete fracture" [3]. The fracture is seen in the crown and generally terminates near the cementoenamel junction (CEJ) or may extend apically into the root. When the coronal fracture extends mesiodistally there may be an involvement of a marginal ridge. A definite treatment plan for management of such teeth should involve proper history, diagnosis of signs and symptoms, examination of the fractured segment and the provision of necessary treatment with a suitable restoration that protect the remaining tooth structure [4].This case report shows a practical approach for the management of fractured fragments and preservation of remaining tooth structure in a case of posterior vertical coronal tooth fracture.

CASE REPORT

An 18-year-old female patient reported to the department of Conservative Dentistry & Endodontics, Sinhgad Dental College & Hospital, Pune with the complaint of pain in the lower right posterior tooth region since three days. She gave a history of trauma after she met with a traffic accident. On examination a vertical fracture in crown of right mandibular first molar with pulpal exposure was seen. The fracture line extended mesiodistally towards the mesiolingual aspect. The fragments were intact and in position. Wedging with a probe showed movement of the lingual segment indicating a complete fracture. After adequate isolation, remaining tooth surfaces were carefully examined for presence of other cracks or craze lines. Soft tissues around the tooth were examined for any defects such as swelling, dehiscence or fenestration to rule out any root fracture. Radiographs taken at different horizontal angulations suggested fractured fragments separated by a narrow radiolucent line extending to the CEJ **[Figure –1]**.

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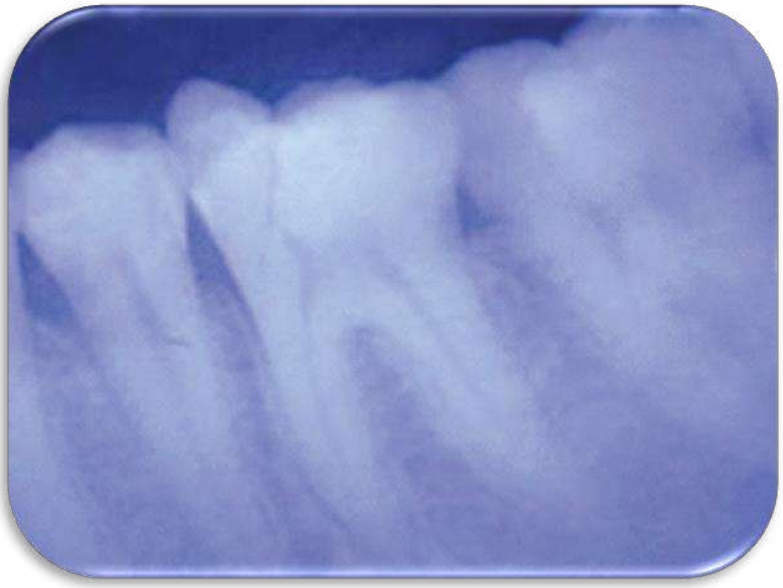

Fig: 1. Pre – operative radiograph showing fractured fragments separated by a narrow radiolucent line extending to the CEJ

The case was diagnosed as a vertical coronal fracture in the mesiodistal direction running upto the dentin and involving pulp but confined to the crown of the tooth. Treatment plan proposed was placement of molar band for stabilization around the crown followed by root canal treatment with restoration using a fibre reinforced composite as core material and covered by an onlay of nanofilled composite. This treatment plan was informed to the patient and consent obtained.

The tooth was adjusted out of occlusion in the same appointment. A preformed orthodontic stainless steel molar band (Ortho organizers, CA, USA) was cemented around the tooth (Cem – Zinc, D – tech Dental Technologies, Wagholi, Pune) to hold the fragments in position **[Figure– 2].**

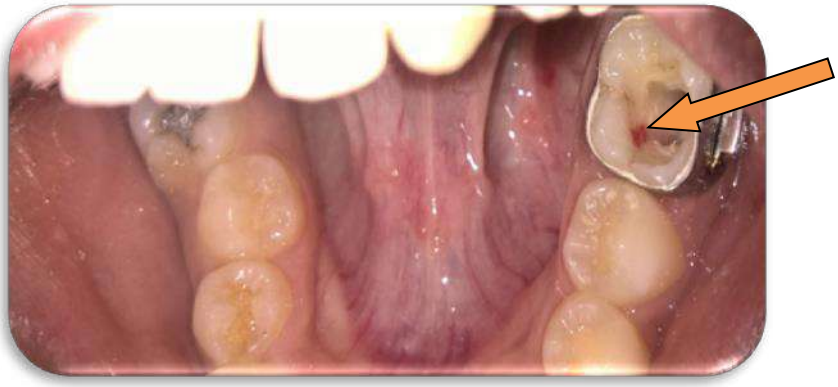

Fig: 2. Splinting of fractured cuspal fragment using orthodontic band followed by access opening

This stabilization also helped in isolation during the root canal treatment. Access opening was done, pulp was extirpated. Canals were prepared using hand protaper (Dentsply Mallifer, USA) and was temporized using temporary filling (Prime Dental Products, Mumbai, India). The patient was recalled after a week after which the canals were obturated using lateral condensation technique and RC fill, a ZOE based sealer (Prime Dental Products, Mumbai, India) **[Figure– 3]**.

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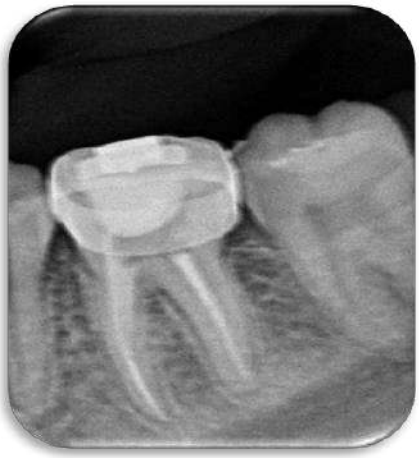

Fig: 3. Immediate post obturation radiograph

After performing the standard adhesive technique [acid etching carried out using 37% phosphoric acid (Etching gel, Prime Dental Products, Mumbai, India) followed by bonding using 5th gen single bottle adhesive system Tetric Bond (Ivoclar Vivadent Schaan, Principality of Liechtenstein)], the cavity surfaces were coated with a layer of low-viscosity resin composite (Protect Liner F, Kuraray, Japan). Curing was done using a combination of pulse and progressive curing technique. Restoration was done incrementally with fibre reinforced composite (everX Posterior GC, India) composite core and an onlay of nanofilled composite resin (Solare X, GC, India) **[Figure–4].** Orthodontic band was removed using band removing plier (Eltee, Libral traders, New Delhi). A full coverage crown was given after the post endodontic restoration **[Figure– 4].**

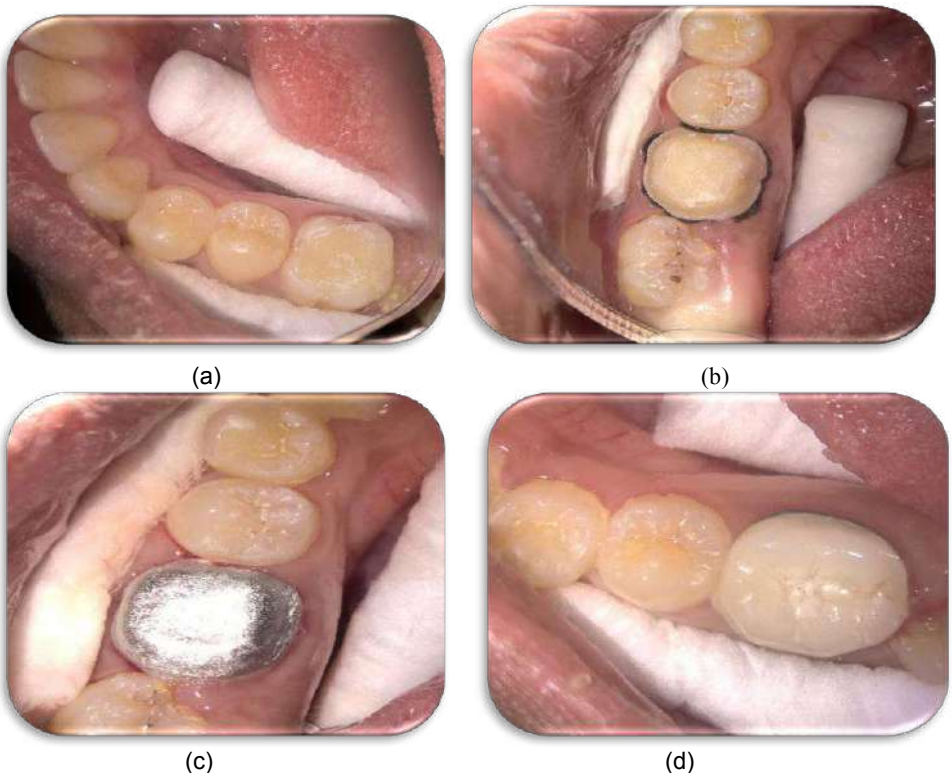

Fig: 4(a). Post endodntic restoration using everX Posterior & onlay of Solare X. (b) Crown preparation. (c) Metal Try-in. (d) Full coverage crown.

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A follow-up done at the end of 10 months showed satisfactory healing, suggesting the successful outcome of the treatment in saving the fractured tooth segment **[figure– 5].**

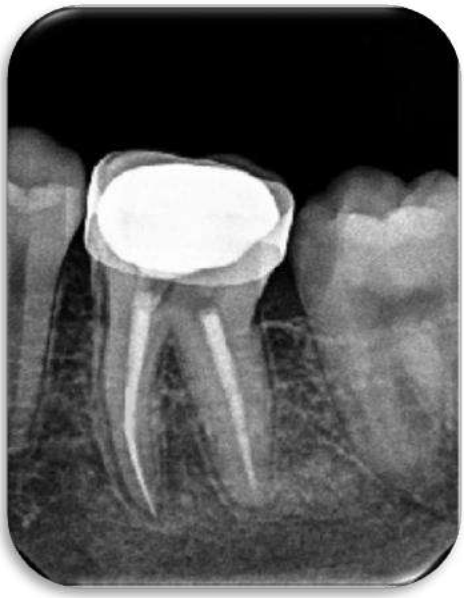

Fig: 5. 10 month follow up Radiograph

DISCUSSION

One of the most common causes of a posterior crown fracture is acute trauma[5]. Fractures involving both marginal ridges usually involve pulp and may extend apically into the root. Saving such fractured teeth remains a major concern for the clinician. Under occlusal load, coronal fracture increases the cuspal flexure. Resultant flexure not only weakens the tooth but also decreases the stiffness of the tooth. Hence, in such situations a temporary restoration should protect a tooth from further deterioration during endodontic treatment. Although traditional treatment approaches like the cast restoration or cuspal coverage has been suggested as the final restoration, studies have shown the importance of intracoronal strengthening of teeth to protect them against fracture[6]. Pane et al.[7] proved that stainless steel bands reduce cuspal flexure by one-half compared to teeth without bands and also doubled the fracture strength. The stainless steel band provides a good immediate treatment option to protect fractured teeth during root canal therapy. This paper discusses the use of stainless steel bands in restoring molar with cuspal flexure rather than extracting the fractured cusp followed by cuspal build up. The main objective of this case report is to stabilize fractured teeth from further weakening during access cavity preparation[8]. Post endodontic restoration remains an integral step in determining the final success of the endodontic therapy in the presence of fracture lines or cracks which are a cause of failure[9]. To increase the longevity of the treatment, the remaining tooth structure needs to be preserved and reinforced. Recent advances in bonding materials like fiber-reinforced composites (FRC) have been successfully used to prevent further fracturing of the traumatized tooth/cusps when incorporated in restorative adhesive resins. Miller TE in 1993 investigated the embedding of these fiber-reinforcement materials into dental resins and found that they provided for an increase in certain physical properties and for more durable tooth stabilization [10-11]. The fibrous assemblies can increase the effective fracture strength of the teeth. The fibers act as stiff bands when stretched over prefractured surfaces. This arrangement resists crack opening and creates a strong bridge between the fractured fragments [12].

Kangasniemi et al in 2003 stated that out of several different types of fiber reinforcement materials like kevlar, carbon, glass, ultra-high-molecular- weight polyethylene (UHMWPE) have also been used to provide fiber reinforcement[13].UHMWPE present in the form of a leno weave provides an increase in fracture strength. This is explained based on the combined effect of the fiber modulus and the interwoven structure (which has fibers oriented in multiple directions), allowing for the forces to be distributed over a wider area, thereby decreasing stress levels. The fibers provide multiple stress paths for redistribution of imposed stresses to intact portions of the

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teeth, and away from the bonded surfaces [9]. According to Karbhari VM et al in 2007, the UHMWPE braid and lenoweave- reinforced specimens did not fail through rupture but showed a deflection and bending of the beam [14]. Samadzadeh A et al in 1997 stated that the lock-stitch weave of the UHMWPE is the tight weave that allows the ribbon to maintain a structural integrity by minimizing weave and fabric shifting within the composite [15].

A pulse-curing technique can reduce stress development at the cavosurface margins, which avoids the formation of microcracks[16] and results in an improved marginal adaptation and improved physical properties of composite resin[17]. A combination of incremental placement of composite resin and UHMWPE fiber reinforcement system reduces polymerization shrinkage, reinforce the remaining tooth structure, and reduce the total composite volume [18]. everX Posterior gives maximum strength, with the optimum size and combination of glass fibers and barium fillers within a tough polymer matrix. The short fibers used in everX Posterior provide fracture toughness greater than collagen-reinforced dentine and almost double that of conventional composite. When everX Posterior was used as a substructure followed by nanofilled composite (Solare X) as overlay, the combination required greater load to fracture as compared to Solare X alone.
The possible reasons could be –

1. The fibres in everX Posterior increased the adhesion to overlying composite by providing added mechanical retention.
2. Fibres orientate into a horizontal plane within the cavity. Due to the strong adhesion between resin and silanated fibres in everX Posterior, the direction of the fibres minimizes shrinkage in the horizontal plane after placement[19].
3. Short fibres prevent fracture propagation in fillings and tooth structure
4. Reliable bond to any overlaying composite as well as to the tooth substance.

CONCLUSION

This paper presents a practical clinical technique for vertical coronal fracture management using stainless steel molar band and a fibre reinforced composite.

CONFLICT OF INTEREST

None

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FINANCIAL DISCLOSURE

None

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ARTICLE OPEN ACCESS

EFFECT OF ALCOHOLIC AND NON-ALCOHOLIC BEVERAGES ON COLOR STABILITY, SURFACE ROUGHNESS AND FRACTURE TOUGHNESS OF RESIN COMPOSITES: AN IN VITRO STUDY

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ABSTRACT

***Background**: Consumption of certain beverages may affect the esthetic and physical properties of the resin composite, thereby undermining the quality of restorations. Aim: To analyze the effect of four beverages (Sparkling Wine, Energy Drink, Jamun Juice and Cola drink) on color stability, surface roughness and fracture toughness of two different types of resin composites at various time intervals in vitro .**Materials and Methods**: A UDMA based composite and a Bis-GMA based composite were used. Each material was randomly divided into four subgroups of 10 samples each according to the beverages used (Sparkling Wine, Energy Drink, Jamun juice, Cola drink). The samples were immersed in each beverage for 10 minutes each day for 28 days. Color change and surface roughness measurements were noted at the baseline, 7th, 14th and 28th days. On 28th day, the samples were tested for fracture toughness using universal testing machine with a cross head speed of 0.1mm/min. The maximum load at specimen failure is recorded. **Results**: The maximum discolouration took place in UDMA based composite when immersed in energy drink ($p<0.05$) and the maximum change took place in the Bis-GMA based composite and results were statistically significant ($p<0.05$) in Cola drink for surface roughness and fracture toughness. **Conclusion:** UDMA based composites had highest discolouration in energy drink while Bis GMA based composites had more alterations in surface roughness and fracture toughness when immersed in Cola drink.*

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KEY WORDS

Beverages; color change; resin composite; surface roughness; fracture toughness; time

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INTRODUCTION

Esthetic failure is one of the most common reasons for the replacement of tooth colored restorations. Color changes in resin composites occur from intrinsic and extrinsic factors. Intrinsic factors involve chemical changes in the material whereas extrinsic factors like adsorption or absorption of stains pose a major problem for esthetic restorations. Surface roughness is one of the reasons for exterior discoloration [1]. Consumption of certain beverages may affect the esthetic and physical properties of the resin composite, thereby, undermining the quality of restorations [2]. The chemicals in them can lead to wear and surface degradation of composite restorations, resulting in external pigmentations. Due to its low pH, ethanol can produce erosion and also act as a plasticizer of the polymer matrix [3-4]. The effect of alcohol on the wear resistance of resin composites has not been studied extensively. Fracture toughness is an intrinsic characteristic of a material describing its resistance to crack propagation. The lower the fracture toughness, higher is the clinical probability of restoration failure under load. This is because fracture toughness defines the critical intensity level at which catastrophic failure occurs due to a micro-defect. Water sorption by a resin composite is dependent on the matrix resin, the filler and the properties of the interface between the matrix and filler [5]. The greater the resin content, the more the water that is absorbed [6]. It has been shown [7] that water sorption weakens the matrix and leaches the fillers into the aqueous media which can result in filler/matrix cracking and hydrolytic degradation of the filler surface [3]. It causes softening of the composite by penetration into the matrix followed by leaching out of unreacted monomer, degradation and leaching of filler components [8]. A number of different mechanical properties of resin composites have been described in various studies using different tests. However, limited information is available on the effect of different beverages on the fracture toughness of resin based composites. The aim of the study is to analyze the effect of four alcoholic and non-alcoholic beverages (Sparkling Wine, Jamun juice, Energy drink, and Cola drink) on the color stability, surface

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roughness and fracture toughness of two different types of resin composites (Bis-GMA and UDMA)at different time intervals in vitro.

MATERIALS AND METHODS

Forty six disk shaped samples (10 mm×2 mm) were prepared for each material using a Teflon mould [9]. The samples were cured as per the manufacturer's instructions. Forty samples were randomly divided into two groups;

- Group 1: Bis-GMA based composite {Tetric N-Ceram (Ivoclar Vivadent, Schaan; Liechtenstein)} A1 shade
- Group 2: UDMA based composite (G – aenial, GC, Tokyo, Japan), A2 shade. Six samples were kept as controls.

Each disk was polished using the Super-Snap polishing system (Shofu Inc, Kyoto, Japan). All the samples were stored at 37°C in distilled water for 24 hours for rehydration and completion of polymerization [10]. After 24 hours of storage, each material was randomly divided into four subgroups of 10 samples each, according to the beverages used.

- Group 1A and 1B: Sparkling wine (RiO fizzy wine)
- Group 2A and 2B: Jamun juice (Paper Boat Jamun Kala khatta, Hector Beverages Pvt Ltd)
- Group 3A and 3B: Energy drink (Red Bull GmbH)
- Group 4A and 4B: Cola drink (Thums Up, The Coca-Cola Company)

Fig: 1. Samples immersed in four different beverages

The samples were blotted dry using tissue paper and the baseline readings were obtained for Surface Roughness (Ra) and Color Change (ΔE) for each group. Surface roughness was measured using a Profilometer, Hommel Tester T500 (Hommelwerke GmbH) The diamond stylus tip of 4 µm radius was placed at the extremity of the disk shaped sample and it traversed the surface of the disk to trace a 10mm course, providing the first measurement of Ra in micrometers. Two additional measurements were taken by rotating the disk to 90° and the mean Ra was obtained from the three values [11]. Color change was measured using a Spectrophotometer, Spectrolino (Gretag Macbeth AG, Germany). The color was assessed using the CIEL*a*b* measuring system. The color measurements were performed at the center of the resin composite disks and repeated thrice. The ΔE values were obtained for each sample and the mean of the values was calculated [12].

After baseline readings, the samples were immersed in the respective beverages **[Figure–1]**. The immersion regimen followed was as follows: The samples in each group were immersed in the respective beverage for 10 minutes every day. For the remaining part of the day, the samples were kept immersed in distilled water. This regimen was followed for 28 days. Surface roughness and color measurements were checked on the seventh, fourteenth and twenty eighth days. The control samples were placed in distilled water only. Fracture toughness was measured after twenty eight days. Pre crack was made on the samples using diamond disk under water

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coolant till 5mm i.e, half of the disk **[Figure –2a]**. Two holes were made at equal distance with No. 2 round carbide bur as shown in figure. The specimens were secured in universal testing machine using guide pins placed through specimens holes **[Figure– 2b]**. Tensile loading was applied at a crosshead speed of 0.1 mm/min; the maximum load at specimen failure was recorded in MPa.

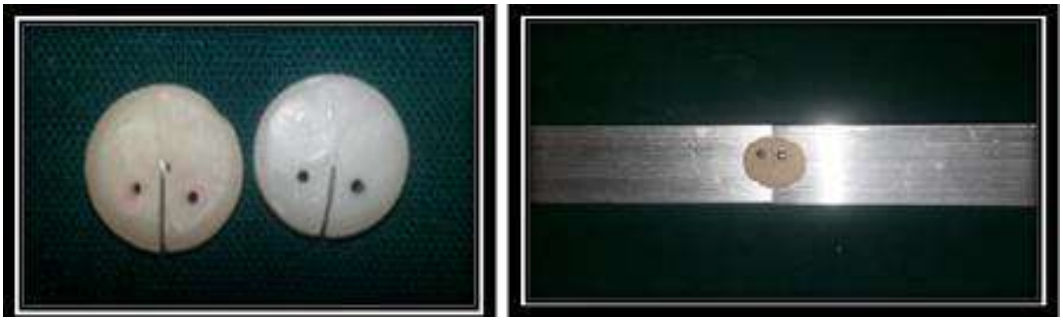

Fig: 2 (a). Pre crack was made on the samples using diamond disk; (b) Sample attached to jig

For statistical analysis, unpaired t test was done to compare the color difference, surface roughness and fracture toughness scores of two different composite materials at different time intervals in different media. Post Hoc Tukey's test was done to compare the effect individually. P value was set at 0.05.

RESULTS

When color changes between two resin composites was considered, the maximum discolouration took place in UDMA based composite as compared to Bis-GMA and results were statistically significant($p<0.05$) on 28th day of immersion in energy drink. However, with other drinks, results were not statistically significant. When discoloration in different beverages was considered, maximum discoloration took place in Energy drink > Cola drink > Jamun Juice > Sparkling Wine **[Figure-3].**

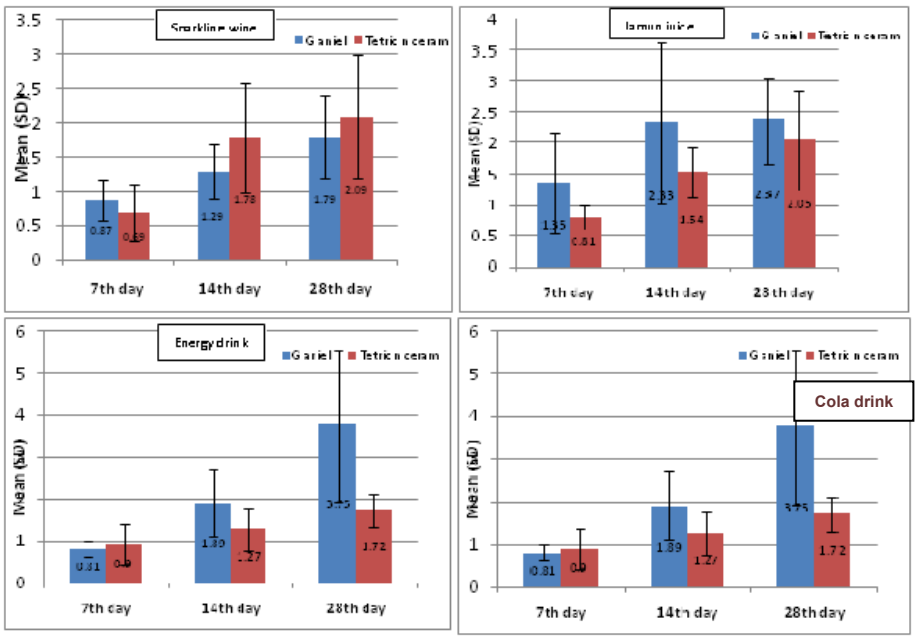

Fig:3. Comparison of Delta E in resin composite in different beverages over time

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When surface roughness between two resin composites was considered at the 14th day, the maximum change took place in the Bis-GMA based composite and results were statistically significant ($p<0.05$) in Cola drink. When surface roughness in different beverages was considered, maximum roughness took place in Cola drink > Jamun Juice > Energy drink > Sparkling Wine **[Figure-4].**

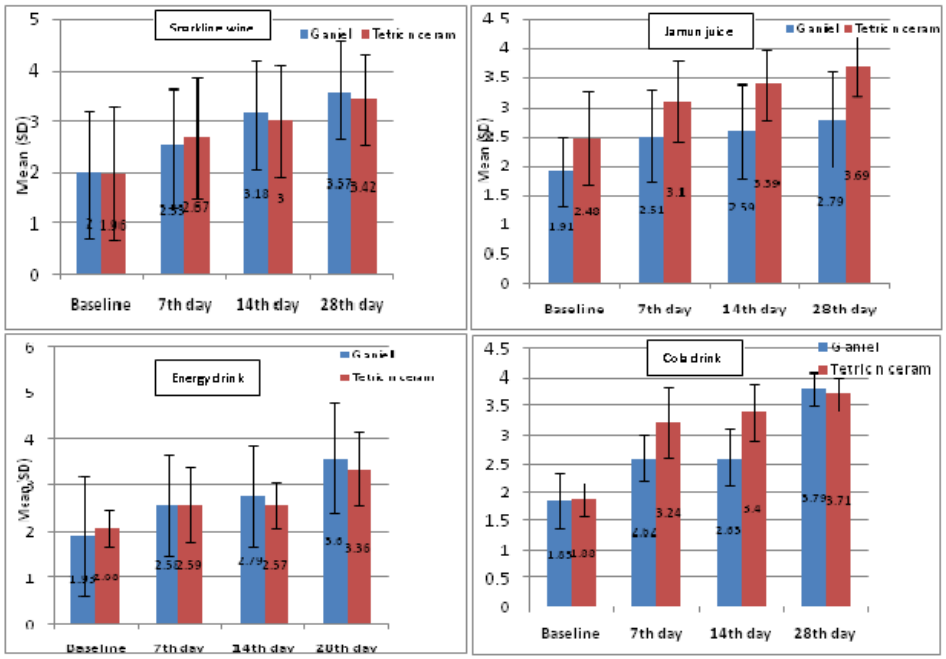

Fig: 4. Comparison of Ra in resin composite in different beverages over time

When facture toughness between two resin composites was considered on 28th day, the minimum fracture toughness was seen in the Bis-GMA based composite and results were highly statistically significant ($p<0.001$) in Cola drink. In other drinks, Bis GMA based composites had higher fracture toughness values as compared with UDMA based composites, but the difference was not statistically significant ($p<0.05$). Fracture toughness of different beverages was considered, Cola drink < Jamun Juice < Energy drink <Sparkling Wine **[Figure-5].**

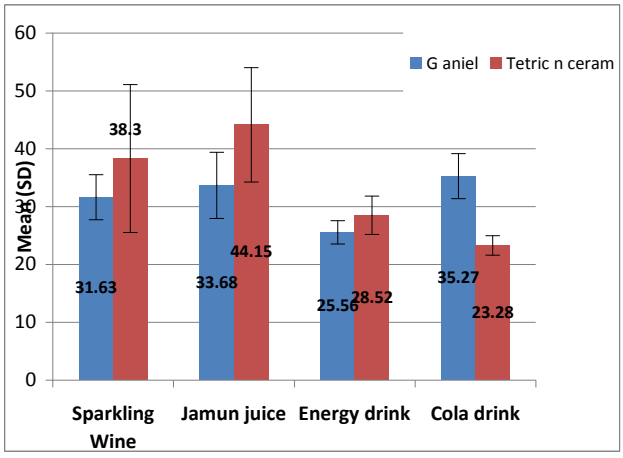

Fig: 5. Comparison of fracture toughness in resin composite in different beverages over time

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DISCUSSION

The present study was conducted on two resin composites; one, a Bis GMA based and the other a UDMA based composite. G – aenial, a new UDMA based resin composite, has not been researched on its degradation under acidic conditions, fracture toughness and color stability. In this study, surface roughness assessment was chosen because surface micromorphology would affect the staining susceptibility. The CIEL*a*b* system for measuring the chromaticity was chosen to record color differences, because it is well suited for the determination of small color differences [13]. CIE (Commission Internationale de l'Eclairage) Lab* system was established in 1976 and as International standard for color measurement in 1978. It consists of a three-dimensional color coordinate system by which every color may be identified. Of the three variables, L* is defined as the attribute by which a perceived color is judged to be equivalent to one of a series of grays ranging from black (0) to white (100). a* is defined as the difference in "a" between a specimen and a standard reference color.; if "a" is positive, there is more redness than greenness, if "a" is negative, there is more greenness than redness. b* is defined as the difference in "b" between a specimen and a standard reference color; if "b" is positive, there is more yellowness than blueness; if "b" is negative, there is more blueness than yellowness.

Commonly consumed alcoholic (Sparkling wine) and non-alcoholic beverages (Energy drink, Jamun juice and Cola drink) were used as the test media in this study, to evaluate the discoloration of resin composites in an in vitro setting. Among the alcoholic beverages, previous studies have evaluated the effect of red wine and cola drinks on the discoloration of a resin composite [13-15]. During consumption, food or drink comes in brief contact with the tooth surfaces before it is washed away by saliva. In previous studies, substrates usually contacted acidic foodstuff for a prolonged period of time. Here, the immersion regimen selected was to immerse each sample in the respective beverage for ten minutes each day. For the remaining part of the day, the samples were kept in distilled water to mimic the neutralizing effect of saliva. The measurement of color change and surface roughness was made at different time intervals (baseline, 7th, 14th, 28th days) to see the relationship of time on surface degradation.

According to the results of this study, both materials became stained and rougher after they were subjected to the immersion regimen. This can be ascribed to the capability of acid media to soften resin based restorative materials. In this study, UDMA based resin composite showed significantly more discoloration than Bis GMA based resin composite. Staining of resins by fluid pigments and beverages is caused by adsorption or absorption (the uptake of substances into or through tissues) of colorants by resins. According to Sham et al, [16] chemical discoloration is caused due to the oxidation of the polymer matrix or oxidation of unreacted double bonds in the residual monomers and the subsequent formation of degradation products from water diffusion. Filler loading plays important role in composite discoloration as well as with the material's strength [17-18]. The filler loading by weight of spectrum G – aenial is 73% where as that of Tetric N-Ceram is 76%. This might be the reason that in this study the spectrum of G – aenial had ΔE greater than Tetric N-Ceram. Some studies [19-20] have shown that heavier filler loading has a significant impact on the mechanical properties with the highly filled composites being the strongest while others [21] concluded that filler content has no role in fracture behavior. Time was found to be a critical factor for the color stability of tooth colored restorative materials. In this study, results showed that as the immersion time increased, the color changes became more intense. This result was similar to that reported by Abu-Bakr et al [22].

Values of ΔE* greater than or equal to 3.3 are visually perceptible and clinically unacceptable to 50% of the trained observers [23]. In this study, ΔE* of UDMA based composites in the Energy drink group crossed the 3.3 level at 28th day, whereas other groups were below 2 (28th day).

When different beverages were compared, Cola drink had more surface degradation. All the beverages used in the study were acidic with Cola being the most acidic (pH=1.57)>Jamun juice (pH=2.82)>Energy drink (pH=3.37)> Sparkling wine (pH=3.5). Lower pH was seen to increase the erosion in polymers and negatively affect the wear resistance of composite materials [24-25].Thus, the higher degradation that took place in Cola drink could be attributed to its lower pH. More surface roughness change in Cola drink is supported by an earlier study, in which cola caused a significant increase in surface roughness than sugar cane spirit (alcoholic graduation 39.00% v/v) [13].

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CONCLUSION

Both type of resin composites exhibited increased staining and surface roughness change, over time, on selective exposure to alcoholic and non-alcoholic beverages. Cola drink, among all three beverages, had more surface roughness and the most change in fracture toughness. Energy drink, among all three beverages, had more discolourations.

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None

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CONFLICT OF INTERESTS

None

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REVIEW ARTICLE OPEN ACCESS

IMAGING THE C- SHAPED CANALS

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ABSTRACT

Knowledge of the C-shaped canal configuration is essential to achieve success in endodontic therapy. Radiographic and clinical diagnoses can aid in identification and negotiation of the fan-shaped areas and intricacies of the C-shaped anatomy. The definition of the C-shaped root canal system is that the morphology of its horizontal cross- section is in the form of a C, with canals which may or may not be separate. C-shaped canal configuration is a variation that has a racial predilection and is commonly seen in mandibular second molars. The aim of this article is to review and discuss the etiology, incidence, anatomic features, classification, diagnosis and management of the C-shaped canal configuration.

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KEY WORDS

C-Shaped canal; Hertwig's epithelial root sheath; cemento-enamel junction; CT; Working length radiograph

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INTRODUCTION

One of the most important anatomic variations is the "C" configuration of the canal system. The C-shaped canal, which was first documented in endodontic literature by Cooke and Cox in 1979, is so named for the cross-sectional morphology of the root and root canal[1]. The definition of the C-shaped root canal system is that the morphology of its horizontal cross section is in the form of a C, with canals which may or may not be separate [2]. It occurs mainly in mandibular second molars, but has also been reported in maxillary first molars, first and third mandibular molars, and in mandibular lower pre-molars [3-4]. Molars with C-shaped root canal systems display fusion of the roots from the buccal or lingual aspect, with a radicular ridge opposite to a convex surface, and a root morphology which may be conical or square or in C-shaped [5-6].

ETIOLOGY

The shape and the number of roots are determined by Hertwig's epithelial sheath, which bends in a horizontal plane below the cemento-enamel junction and fuses in the center leaving openings for roots. Failure of the Hertwig's epithelial root sheath to fuse on the lingual or buccal root surface is the main cause of C-shaped roots, which always contain a Cshaped canal [7-9].

CLASSIFICATION

Melton's Classification: Melton et al in 1991 proposed the following classification based on the different configurations of the orifices in C-shaped canal systems [10]

Category I: Continuous C-shaped canal running from the pulp chamber to the apex defines a C-shaped outline without any separation
Category II: The semicolon-shaped orifice in which dentine separates a main C shaped canal from one mesial distinct canal.
Category III: Refers to those with two or more discrete and separate canals:

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• Subdivision I: C-shaped orifice in the coronal third that divides into two or more discrete and separate canals that join apically.
• Subdivision II: C-shaped orifice in the coronal third that divides into two or more discrete and separate canals in the midroot to the apex.
• Subdivision III: C-shaped orifice that divides into two or more discrete and separate canals in the coronal third to the apex.

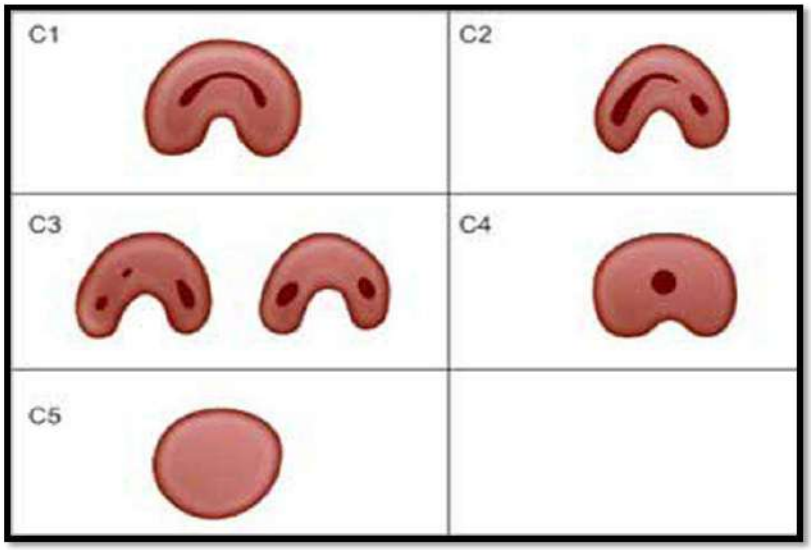

Fig: 1.Classification of C-shaped root canal configuration according to Melton

Fan et al in 2004 modified Melton's method of classification into the following categories-

- Category I (C1): The shape was an interrupted 'C' with no separation or division.
- Category II (C2): The canal shape resembled a semicolon resulting from a discontinuation of the 'C' outline, but either angle or should be no less than 60°.
- Category III (C3): Two or three separate canals and both angles, and were less than 60°.
- Category IV (C4): Only one round or oval canal in that crosssection.
- Category V (C5): No canal lumen could be observed (which is usually seen near the apex only).

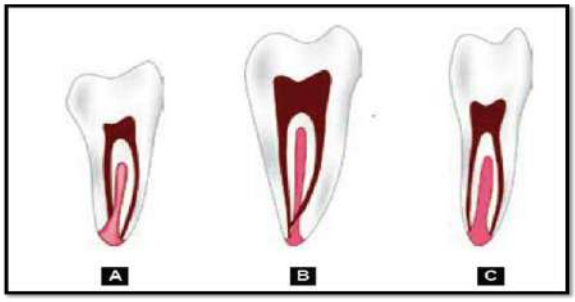

Fig 2: A to C: Fan's radiographic classification

Fan's Classification (Radiographic Classification) [11]
Fan et al classified C-shaped roots according to their radiographic appearance into three types.

1. Type I: Conical or square root with a vague, radiolucent longitudinal line separating the root into distal and mesial parts. There was a mesial and a distal canal that merged into one before exiting at the apical foramen (foramina).

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2. Type II: Conical or square root with a vague, radiolucent longitudinal line separating the root into distal and mesial parts. There was a mesial and a distal canal, and the two canals appeared to continue on their own pathway to the apex.

3. Type III: Conical or square root with a vague, radiolucent longitudinal line separating the root into distal and mesial parts. There was a mesial and a distal canal, one canal curved to and superimposed on this radiolucent.

RADIOGRAPHIC DIAGNOSIS

Good radiographic technique should alert the practitioner to unusual anatomy, such as C shaped canals. The C-shaped canal may have the appearance of a fused root with very fine canals. A pulp chamber that looks, unusual the dentine areas on the pulp floor map should give some idea of the location of root canals, and of the relationship of the floor to surrounding tooth structure. The preoperative awareness of a C-shaped canal configuration before treatment can facilitate effective management. Radiographic interpretation is overall more effective when based on film combinations ('preoperative and working length radiographs' or 'preoperative and final radiographs' or'all three radiographs') than on single radiographs. Among the latter, working length radiographs are more helpful **[Figure- 4]** than the preoperative **[Figure- 3]** and final ones, whereas preoperative radiographs are the least effective in diagnosing the C-shaped cases.

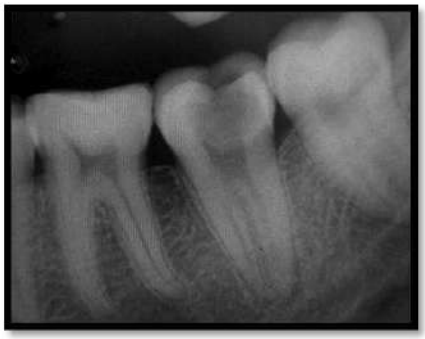

Fig. 3

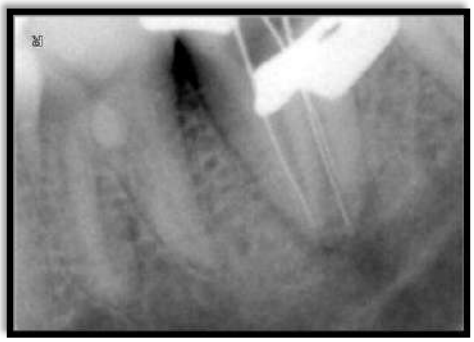

Fig. 4

Fig: 3and 4. The working length intraoral periapical radio wing the C-shaped canal as, Melton's category III

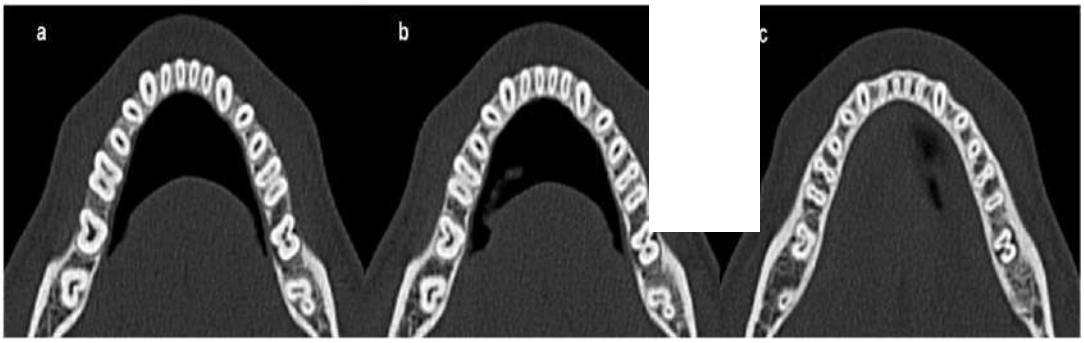

Fig: 5. CT image of C-shaped canal at various levels: (a) Coronal 3rd, (b) Middle 3rd, (c) Apical 3rd

Recent research used a spiral computed tomography **[Figure-6]** scan to diagnose the canal anatomy, but the dissolution of the image is not yet high enough to show irregular or fine canal structures.

DISCUSSION

A preoperative radiograph usually provides various clues in the identification of any variation in root canal morphology. However, there are differences in opinions on the value of a preoperative radiograph in diagnosing a C-shaped case. Cooke & Cox were of the opinion that it is not possible to diagnose C-shaped canals on

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preoperative radiographs [12]. Conversely, some investigators described four radiographic characteristics that can allow prediction of the existence of this anatomical condition: radicular fusion, radicular proximity, a large distal canal or a blurred image of a third canal in between. Hence, a C-shaped root in a mandibular second molar may present radiographically as a single-fused root or as two distinct roots with a communication [13-14]. When the communication or fin connecting the two roots is very thin, it is not visible on the radiograph and may thus give the appearance of two distinct roots. The radiograph may also reveal a large and deep pulp chamber, usually found in C-shaped molars.

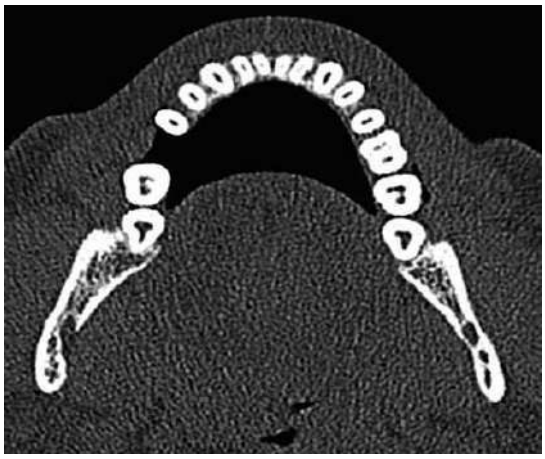

Fig: 6. CBCT image of C-shaped canal system in both mandibular left and right molar teeth

Fan et al. divided the radiographic appearance that the C-shaped teeth are present with three types. In type I, the C-shaped canal system actually appears as two distinct canals, because the isthmus that links the mesial and distal "main" canals is very thin and hence is not detected radiographically. In the radiographic type II, the mesial and distal canals assume their own individual course to the apex. Thus, there are apparently two distinct canals on the radiograph. In type III, one canal continues its course to the apex giving the image of a distinct canal whereas the other(s) proceeds very close to or within the fused area, that is, the "web" between the two main roots in the apical third. Hence, the canal may seem to exit into the groove radiographically. Wang et al. reported a higher incidence in the recognition of C-shaped canals using a combination of radiography and clinical examination under the microscope (41.27%) than using the radiography (34.64%) or clinical examination (39.18%) alone [15-16]. Working length radiographs are more helpful than preoperative and final radiographs in diagnosing C-shaped canals. In a true C-shaped canal, (single canal running from the orifice to the apex) it is possible to pass an instrument from the mesial to the distal aspect without obstruction [17-18]. In the semicolon type, (one distinct canal and a buccal or lingual C-shaped canal) whenever an instrument was inserted into any side of the C-shaped canal, it always ends in the distal foramen of the tooth and a file introduced in this canal could probe the whole extension of the C. When negotiating the C-shaped canal, instruments may be clinically centered. Radiographically, the instruments may either converge at the apex or may appear to be exiting the furcation, thus adding to the confusion and troublesome task of determining whether a perforation has occurred [20-21].

CONCLUSION

The C-shaped root canal configuration has an ethnic predilection and a high prevalence rate in mandibular second molars. Radiographic examination is effective for rule out the C shape canal morphology. Understanding the anatomical presentations of this variation will enable the clinician to manage these cases effectively.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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None.

FINANCIAL DISCLOSURE

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REVIEW ARTICLE OPEN ACCESS

MICROBIAL IDENTIFICATION IN ENDODONTIC INFECTIONS WITH AN EMPHASIS ON MOLECULAR DIAGNOSTIC METHODS: A REVIEW

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ABSTRACT

Accurate and definitive microorganism identification, including bacterial identification and pathogen detection, is essential for correct disease diagnosis, treatment of infection and trace-back of disease outbreaks associated with microbial infections. Bacterial identification is used in a wide variety of applications including microbial forensics, criminal investigations, bio-terrorism threats and environmental studies. Overwhelming evidence indicates that periradicular diseases are infectious disorders. The question now is no longer whether microorganisms are involved in the pathogenesis of such diseases, but which specific microbial species are. The list of microorganisms involved in periradicular diseases keeps expanding and has the potential to become increasingly more accurate during the next few years. Molecular methods have contributed significantly to the knowledge about the microbial species involved. Undoubtedly, a great deal of additional research is needed to define the specific role played by suspected endodontic pathogens in the etiology of each form of periradicular disease and to determine the best therapeutic measures for the pathogen's eradication. In addition, there is an emergent need to define markers that permit the clinician to know when he or she should conclude the treatment and to predict the outcome of the treatment. This paper will discuss briefly the methods of microbial identification with special reference to the molecular diagnostic technologies and their potential to explore the diverse microbiota associated with endodontic infections.

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KEY WORDS

Endodontic infections; Cultivation; Molecular diagnostic methods; PCR; FISH; Metagenomics

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INTRODUCTION

There is no greater association between a basic science and the practice of endodontics, than that of microbiology. Microorganisms cause virtually all pathoses of the pulp and the peri-radicular tissues. To effectively treat endodontic infections, clinicians must recognize the cause and effect of microbial invasion of the dental pulp space and the surrounding peri-radicular tissues. Knowledge of the microorganisms associated with endodontic disease is necessary to develop a basic understanding of the disease process and a sound rationale for effective management of patients with endodontic infections. Accurate and definitive microorganism identification, including bacterial identification and pathogen detection, is essential for correct disease diagnosis, treatment of infection and trace-back of disease outbreaks associated with microbial infections. Bacterial identification is used in a wide variety of applications including microbial forensics, criminal investigations, bio-terrorism threats and environmental studies. Epidemiological studies using sophisticated culture and molecular biology techniques have collectively shown that approximately 300 different microbial species can be found in infected root canals usually in combinations of 10-30 species. [1] Theoretically any one of these species would have the potential to be an endodontic pathogen. The question now is no longer whether microorganisms are involved with causation of endodontic infection, but which species are. [2] This article describes the molecular biological techniques with the potential to be applied decipher the diversity of microbiota associated with endodontic infections.

CHALLENGES IN BACTERIAL IDENTIFICATION

Traditional methods of bacterial identification rely on phenotypic identification of the causative organism using gram staining, culture and biochemical methods. However, these methods of bacterial identification suffer from two major drawbacks. First, they can be used only for organisms that can be cultivated in vitro. Second, some strains exhibit unique biochemical characteristics that do not fit into patterns that have been used as a characteristic

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of any known genus and species.

TRADITIONAL IDENTIFICATION METHODS

Culture

For more than a century, cultivation using artificial growth media has been the standard diagnostic test in infectious diseases. The success in cultivation of important pathogenic bacteria probably led microbiologists to feel satisfied with and optimistic about their results and to recognize that there is no dearth of known pathogens.[3] Making micro-organisms grow under laboratory conditions presupposes some knowledge of their growth requirements. Nevertheless, very little is known about the specific growth factors that are utilized by innumerous micro-organisms to survive in virtually all habitats, including within the human body. [4] A huge proportion of the microbial species in nature are difficult to be tamed in the laboratory. Certain bacteria are fastidious or even impossible to cultivate. [5] Updated analyses have indicated that presently 52 phyla can be discerned, of which 26 are candidate phyla, that is, they are uncultivable and known only by gene sequences.[6] Taking into consideration that known bacterial pathogens fall within 7 out of the 52 candidate bacterial divisions and that cultivation-independent approaches have shown that 40 to 75% of the human microbiota in different sites are composed of as-yet uncultivated bacteria,[7-9] it is fair to realize that there can be many pathogens which remain to be identified. Therefore, it is of concern that clinical microbiology continues to rely on cultivation-based identification procedures. [10]

Advantages and Limitations of culture

The main advantages of cultivation approaches are related to their broad-range nature, which makes it possible to identify a great variety of microbial species in a sample, including those that are not being sought-after. Still, cultivation makes it possible to determine antimicrobial susceptibilities of the isolates and to study their physiology and pathogenicity. However, cultivation-based identification approaches have several limitations: they are costly; they can take several days to weeks to identify some fastidious anaerobic bacteria (that can delay antimicrobial treatment); they have a very low sensitivity (particularly for fastidious anaerobic bacteria); their specificity may be also low and is dependent on the experience of the microbiologist; they have strict dependence on the mode of sample transport; they are time-consuming and laborious. Finally, the impossibility of cultivating a large number of bacterial species as well as the difficulties in identifying many cultivable species represent the major drawbacks of cultivation-based approaches.[3]

Difficulties in Cultivation

Lack of essential nutrients or growth factors in the artificial culture medium, toxicity of the culture medium itself, production of substances inhibitory to the target microorganism by other species present in a mixed consortium, metabolic dependence on other species for growth, disruption of bacterial intercommunication systems induced by separation of bacteria on solid culture media and bacterial dormancy, These are some possible reasons for bacterial unculturability:[5,11,12] Obviously, if micro-organisms cannot be cultivated, they cannot be identified by phenotype-based methods. Hence identification methods that are not based on bacterial culturability are required. This would avoid that many pathogens pass unnoticed when one is microbiologically surveying clinical samples. [3] There have been developments in approaches and culture media that allow cultivation of previously uncultivated bacteria. Strategies may rely on application of cultivation procedures that better mimic conditions existing in the natural habitat from which the samples were obtained. Recent efforts to accomplish this have met with some success by including the following: the use of agar media with little or no added nutrients; relatively lengthy periods of incubation (more than 30 days); and inclusion of substances that are typical of the natural environment in the artificial growth media. [13, 14]

Difficulties in Identification

Accurate identification of microbial isolates is paramount in clinical microbiology. For a given microbial species to be identified by means of their phenotypic features, this species has to be cultivated. However, one should be mindful that in some circumstances even the successful cultivation of a given microorganism does not necessarily mean that this micro-organism can be successfully identified. [3] For slow-growing and fastidious bacteria, traditional phenotypic identification is difficult and time-consuming. In addition, interpretation of phenotypic test results can involve a substantial amount of subjective judgment and personnel's expertise. Still, one major difficulty associated with microbial identification based on phenotypic features is that of divergence and convergence. Divergence occurs for strains of the same species, which are genetically similar, but have evolved to

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be different phenotypically. Convergence occurs for strains of different species, which are genetically different, but have evolved to have similarphenotypic behavior. In both situations, phenotypically based diagnostic tests would result in misidentification. [3]

Microscopy

Microscopy may suggest an etiologic agent, but it rarely provides definitive evidence of infection by a particular species. Microscopic findings regarding bacterial morphology may be misleading, because many species can be pleomorphic and conclusions can be influenced by subjective interpretations of the investigator. In addition, microscopy has limited sensitivity and specificity to detect microorganisms in clinical samples. [3] The knowledge of the endodontic microbiota is based mostly on culture studies. This is simply because of lack of real alternatives in the past. Microscopic studies have been used; however, they have severe limitations when it comes to deeper identification, to evaluate the composition, to characterize various micro-organisms, and to do further experimental studies on isolated species. [15] Microscopy of smears from the root canal is limited to main morphotypes. Microbial staining of histological sections has advantage of localizing the microbes in situ. Electron-microscopic pictures (transmission or scanning) have been valuable to distinguish main morphotypes in various locations. [3]

The scanning electron microscope (SEM) is an invaluable tool for describing biofilms because of its ability to provide an indiscriminate view on the surface topography at high-resolution and magnification. However, even high-resolution SEM examinations of biofilms are often compromised by the fact that matrix embedded bacteria cannot be easily visualized. Furthermore it is known that biofilm bacteria often lose their characteristic shape and size making them difficult, if not impossible, to identify. These limitations pose a problem when indisputable proof of the existence of bacterial biofilms growing in natural environments is required. In general, the presence of bacteria in a matrix is a sine qua non for the presence of a biofilm. If bacteria cannot be demonstrated to be present, the proposed existence of a bacterial biofilm remains questionable.

The use of confocal laser scanning microscopy on the other hand in combination with fluorescence in situ hybridization enables the visualization of matrix embedded bacteria in multi-layered biofilms. In a study by Schauddin, Carr et al., fluorescence in situ hybridization/confocal laser scanning microscopy and scanning electron microscopy were applied to visualize bacterial biofilm in endodontic root canals. The resulting fluorescence in situ hybridization/confocal laser scanning microscopy and scanning electron microscopy and pictures were subsequently combined into one single image to provide high-resolution information on the location of hidden bacteria. The researchers concluded that combined use of scanning electron microscopy and fluorescence in situ hybridization/confocal laser scanning microscopy as the potential to overcome the limits of each single technique. [16]

Immunological Methods

Immunological methods are based on the specificity of antigen-antibody reaction. It can detect micro-organisms directly or indirectly, the latter by detecting host immunoglobulins specific to the target micro-organism. The enzyme-linked immunosorbent assay (ELISA) and the direct or indirect immunofluorescence tests are the most commonly used immunological methods for microbial identification.[3] Advantages of immunological methods for identification of micro-organisms include:(a) they take no more than a few hours to identify a microbial species; (b) they can detect dead micro-organisms; (c) they can be easily standardized; and (d) they have low cost.[17] However, they have also important limitations as they can detect only target species, they have low sensitivity (about 104 cells), their specificity is variable and depends on types of antibodies used, and they can detect dead micro-organisms.[17, 18]

MOLECULAR GENETIC METHODS

Molecular biological methods have been recently used to decipher the diversity of the endodontic microbiota and many fastidious species and even uncultivated phylotypes have been disclosed. [19] During the last decade, numerous studies using various types of molecular biology techniques have been used to characterize more closely the microbial composition of the root canal microbiota. These methods have definitely showed that the root canal microbiota is much more complex than previously thought. This has made the clinical interpretations, diagnosis, and treatment strategies more difficult. Still, culture is a “gold standard” to identify specific targets for treatment

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and to evaluate treatment strategies due to its easier accessibility than the new techniques. Culture is also used in experimental models, which have disclosed the dynamics of the infections and the nature of micro-organisms. [15] Molecular diagnostic methods have several advantages over other methods with regard to microbial identification. [3]

- Detection of not only cultivable species but also of uncultivable microbial species or strains.
- Higher specificity and accurate identification of microbial strains with ambiguous phenotypic behavior, including divergent or convergent strains.
- Detection of microbial species directly in clinical samples, without the need for cultivation.
- Higher sensitivity.
- Faster and less time-consuming.
- They offer a rapid diagnosis, which is particularly advantageous in cases of life-threatening diseases or diseases caused by slow growing micro-organisms.
- They do not require carefully controlled anaerobic conditions during sampling and transportation, which is advantageous since fastidious anaerobic bacteria and other fragile micro-organisms can lose viability during transit.
- They can be used during antimicrobial treatment.
- When a large number of samples are to be surveyed in epidemiological studies, samples can be stored and analyzed all at once.

There are a plethora of molecular methods for the study of microorganisms and the choice of a particular approach depends on the questions being addressed. **[Figure- 1]**

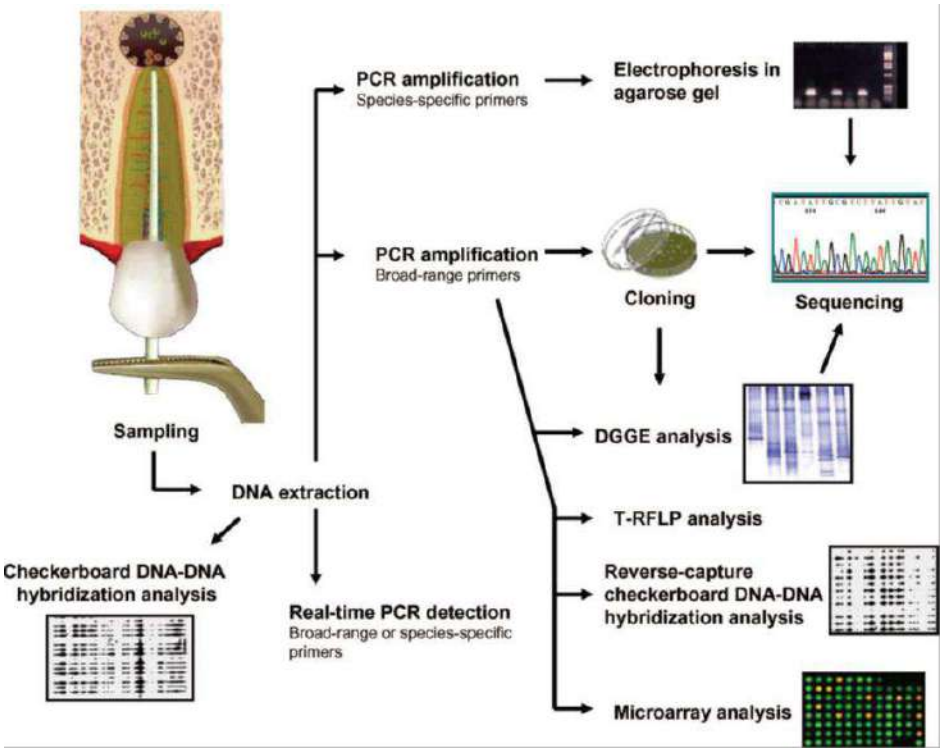

Fig: 1. Different molecular techniques that can be used to identify the diverse microbiota associated with endodontic infections

Broad-range polymerase chain reaction (PCR) followed by sequencing can be used to investigate the microbial diversity in a given environment. Microbial community structure can be analyzed via fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP). Fluorescence in situ hybridization (FISH) can measure abundance of particular species and provide

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information on their spatial distribution in tissues. Among other applications, DNA-DNA hybridization macro-arrays and microarrays, species specific PCR, nested PCR, multiplex PCR and real-time PCR can be used to survey a large number of clinical samples for the presence of target species. Variations in PCR technology can also be used to type microbial strains. [3]

Gene Targets for Microbial Identification

Molecular approaches for microbial identification rely on the fact that certain genes contain revealing information about the microbial identity. Ideally, a gene to be used as target for microbial identification should contain regions that are unique to each species. Following the pioneer studies by Woese [20], the genes encoding rRNA molecules, which are present in all cellular forms of life, namely, the domains Bacteria, Archaea, and Eucarya, have been widely used for comprehensive identification of virtually all living organisms and inference of their natural relationships. Ribosomes are intracellular particles composed of rRNA and proteins. The sizes of ribosomes are given in Svedburg (S) units, which represent a measure of how rapidly particles or molecules sediment in an ultracentrifuge. Bacterial and archaeal cells have 70S ribosomes composed of 30S and 50S subunits. The 30S subunit contains a 16S rRNA molecule, having approximately 1540 nucleotides. The 50S subunit contains a 23S rRNA molecule, having approximately 2900 nucleotides, and a small 5S rRNA molecule having only about 120 nucleotides. Fungi have 80S ribosomes composed of 40Sand 60S subunits. The 40S subunit contains 18S rRNA and the larger 60Ssubunit has 25S rRNA and 5.8S rRNA. [21] Large subunit genes (23S and 25S rDNA) and small subunit genes(16S and 18S rDNA) have been widely used for microbial identification, characterization and classification. The small subunit rDNA is among the most evolutionary conserved macromolecules in all living systems. The advantages of using small subunit rDNA is that it is found in all organisms, is long enough to be highly informative and short enough to be easily sequenced, particularly with the advent of automated DNA sequencers. [20] The small subunit rDNA contains some regions that are virtually identical in all representative of a given domain (conserved regions) and other regions that vary in sequence from one species to another (variable regions).[3] Variable regions contain the most information about the genus and species of the bacterium, with unique signatures that allow specific identification. The 16S rRNA of bacteria and archaea and the 18S rDNA of fungi and other eukaryotes have been extensively examined and sequenced and have been used to determine phylogenetic relationships among living organisms. In addition, data from rDNA sequences can also be used for accurate and rapid identification of known bacterial species, using techniques that do not require microbial cultivation. [3]

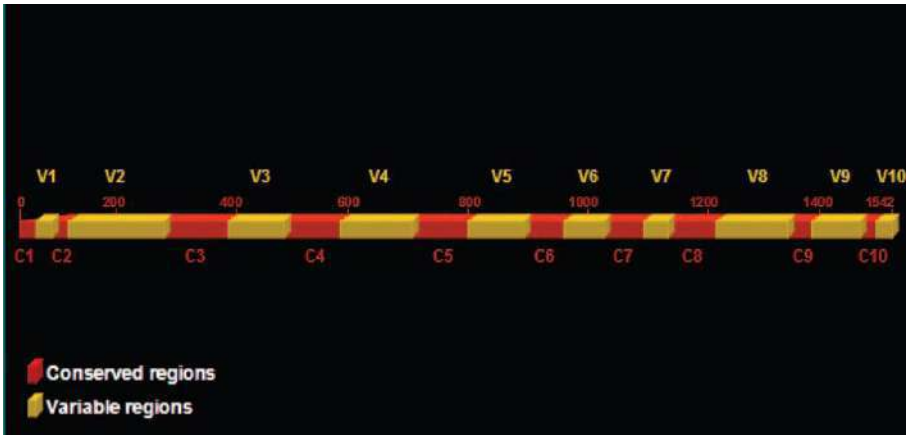

Fig: 2. Schematic drawing of the 16S rRNA gene (rDNA). Orange areas correspond to variable regions, which contain information about the genus and the species. Red areas correspond to conserved regions of the gene.

Polymerase Chain Reaction (PCR)

The PCR process was conceived by Kary Mullis in 1983 and ever since has revolutionized the field of molecular biology by being able to amplify as few as one copy of a gene into millions to billions of copies of that gene. The impact of PCR on biological and medical research has been remarkable, dramatically speeding the rate of progress of the study of genes and genomes. [22] Nowadays, it is possible to isolate essentially any gene from any organism using PCR, which made this technique a cornerstone of genome sequencing projects. The most

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widespread advance in clinical diagnostic technology has come from the application of PCR for detection of microbial pathogens.[23] The PCR method is based on the in vitro replication of DNA through repetitive cycles of denaturation, primer annealing and extension steps. The target DNA serving as template melts at temperatures high enough to break the hydrogen bonds holding the strands together, thus liberating single strands of DNA. Two short oligonucleotides (primers) are annealed to complementary sequences on opposite strands of the target DNA. Primers are selected to encompass the desired genetic bacterial, defining the two ends of the amplified stretch of DNA. In sequence, a complementary second strand of new DNA is synthesized through the extension of each annealed primer by a thermostable DNA polymerase in the presence of excess deoxyribonucleoside triphosphates. All previously synthesized products act as templates for new primer-extension reactions in each ensuing cycle. The result is the exponential amplification of new DNA products, which confers extraordinary sensitivity in detecting the target DNA. In fact, PCR has unrivaled sensitivity—it is at least 10 to 100 times more sensitive than the other more sensitive identification method. [18, 24] There are several methods to check if the intended PCR product was generated. The most commonly used method for detecting PCR products is electrophoresis in an agarose gel. Aliquots of the PCR reaction are loaded into the gel and an electrical gradient is applied through a buffer solution. The products migrate through the gel according to size, with larger products running a shorter distance in the gel because they experience more resistance in the gel matrix. The gel is usually visualized using ethidium bromidestaining and ultraviolet transillumination.[3] Numerous derivatives in PCR technology have been developed since its inception. For ex., nested PCR, Reverse Transcriptase PCR, Multiplex PCR, Broad-Range PCR and Real-Time PCR. These are discussed briefly in the following paragraphs.

Nested PCR

Nested PCR uses the product of a primary PCR amplification as template in a second PCR reaction and was devised mainly to have increased sensitivity. [25] The first PCR products are subjected to a second round of PCR amplification with a separate primer set, which anneals internally to the first products. This approach shows increased sensitivity allowing the detection of the target DNA several folds lower than conventional PCR. Increased sensitivity is because of the large total number of cycles. [3] In addition, target DNA is amplified in the first round of amplification, with subsequent dilution of other DNA and inhibitors present in the sample. The set of primers used in the second round of PCR results in additional specificity. The second reaction is performed with reduced background of eukaryotic DNA and other regions of the bacterial DNA. [24] Even if nonspecific DNA amplification occurs in the first round of amplification, the nonspecific PCR product does not serve as template in the second reaction, since it is highly unlikely to possess regions of DNA complementary to the second set of specific primers. [26] The major drawback of nested-PCR protocol is the high probability of contamination during transfer of the first-round amplification products to a second reaction tube and special precautions should be taken to avoid this.[3]

Reverse Transcriptase PCR (RT-PCR)

RT-PCR was developed to amplify RNA targets and exploits the use of the enzyme reverse transcriptase, which can synthesize a strand of complementary DNA (cDNA) from an RNA template. Most RT-PCR assays employ a two-step approach. In the first step, reverse transcriptase converts RNA into single-stranded cDNA. In the second step, PCR primers, DNA polymerase, and nucleotides are added to create the second strand of cDNA. Once the double-stranded DNA template is formed, it can be used as template for amplification as in conventional PCR. [27] The RT-PCR process may be modified into a one-step approach by using it directly with RNA as the template. In this approach, an enzyme with both reverse transcriptase and DNA polymerase activities is used, such as that from the bacteria Thermus thermophilus. [3]

Multiplex PCR

In multiplex PCR, two or more sets of primers specific for different targets are introduced in the same reaction tube. Since more than one unique target sequence in a clinical specimen can be amplified at the same time, multiplex PCR assays permit the simultaneous detection of different microbial species. Multiplex PCR assays have been used to minimize the time and expenditure needed for detection approaches. Primers used in multiplex assays must be designed carefully to have similar annealing temperatures and to lack complementarity.[3]

Real-Time PCR

Conventional PCR assays are qualitative or can be adjusted to be semi-quantitative. One exception is the real-time PCR, which is characterized by the continuous measurement of amplification products throughout the reaction. [28] There are several different real-time PCR approaches. The three general real-time PCR chemistries for

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amplifying and detecting DNA targets are SYBR-Green, TaqMan, and molecular beacon. [29,30] Real-time PCR assays allow the quantification of individual target species as well as total bacteria in clinical samples. The advantages of real-time PCR are the rapidity of the assay (30–40 min), the ability to quantify and identify PCR products directly without the use of agarose gels, and the fact that contamination of the nucleic acids can be limited because of avoidance of postamplificationmanipulation. [3]

Broad-Range PCR

PCR technology can also be used to investigate the whole microbial diversity in a given environment. In broad-range PCR, primers are designed that are complementary to conserved regions of a particular gene that are shared by a group of micro-organisms. For instance, primers that are complementary to conserved regions of the 16S rDNA have been used with the intention of exploiting the variable internal regions of the amplified sequence for sequencing and further identification. [31] The strength of broad-range PCR lies in the relative absence of selectivity, so that (in principle) any kind of bacteria present in a sample can be detected and identified. This aspect is in analogy to cultivation and in contrast to species-specific molecular approaches.48 Thus, broad-range PCR can detect the unexpected and in this regard it is far more effective and accurate than culture. Broad-range PCR has allowed the identification of several novel, fastidious or uncultivable bacterial pathogens directly from diverse human sites. [11, 32, 33] The analytical sensitivity of most broad-range PCR assays is in practice above 10, if not 100, gene copies per PCR, which is significantly lower when compared to most species-specific PCR assays. Because broad-range primers are used, there is a high risk for DNA from microbial contaminants to be amplified. A wide range of precautions is necessary to avoid contamination, including separate room for pre- and post-PCR work, UV decontamination of surface areas, uses of high-quality reagents and adequate sampling techniques and vials for clinical specimens. [3]

Denaturing Gradient Gel Electrophoresis

Techniques for genetic fingerprinting of microbial communities can be used to determine the diversity of different micro-organisms living in diverse ecosystems like infected root canals and to monitor microbial community behavior over time. A commonly used strategy for genetic fingerprinting of complex microbial communities encompasses the extraction of DNA,t he amplification of the 16S rDNA using broad-range primers, and then the analysis of PCR products by denaturing gradient gel electrophoresis(DGGE). In DGGE, DNA fragments of the same length but with different base-pair sequences can be separated. [34, 35] The DGGE technique is based on electrophoresis of PCR-amplified 16S rDNA (or other genes) fragments in polyacrylamide gels containing a linearly increasing gradient of DNA denaturants (a mixture of urea and formamide). [3] In DGGE, multiple samples can be analyzed concurrently, making it possible to compare the structure of the microbial community of different samples and to follow changes in microbial populations overtime, including after antimicrobial treatment. Specific bands can also be excised from the gels, re-amplified and sequenced to allow microbial identification. The DGGE method and its application in endodontic microbiology research have been recently reviewed by Siqueira JF Jr, Roĉas IN, Rosado AS. [36] Temperature gradient gel electrophoresis (TGGE) uses the same principle as DGGE, except for the fact that the gradient is temperature rather than chemical denaturants. [3]

Terminal-RFLP

T-RFLP is a recent molecular approach that can assess subtle genetic differences between microbial strains as well as provide insight into the structure and function of microbial communities. [37] T-RFLP analysis measures the size polymorphism of terminal restriction fragments from a PCR amplified marker. T-RFLP is a modification of the conventional RFLP approach. In T-RFLP, rDNA from different species in a community is PCR amplified using one of the PCR primers labeled with a fluorescent dye, such as 4,7,2',7'-tetrachloro-6-carboxyfluorescein (TET) or phosphoramidite fluorochrome 5-carboxyfluorescein (6-FAM). [38] PCR products are then digested with restriction enzymes, generating different fragment lengths. Digestion of PCR products with judiciously selected restriction endonucleases produces terminal fragments appropriate for sizing on high resolution sequencing gels. The latter step is performed on automated systems such as the ABI gel or capillary electrophoresis systems that provide digital output. [38] The use of a fluorescently labeled primer limits the analysis to only the terminal fragments of the enzymatic digestion. This simplifies the banding pattern, thus allowing the analysis of complex communities as well as providing information on diversity as each visible band represents single species. All terminal fragment sizes generated from digestion of a PCR product pool can be compared with the terminal fragments derived from sequence databases to derive phylogenetic inference.

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Through application of automated DNA sequencer technology, T-RFLP has considerably greater resolution than gel-based community profiling techniques, such as DGGE/TGGE. [3]

DNA-DNA Hybridization

DNA-DNA hybridization methodology employs DNA probes, which entail segments of single-stranded DNA, labeled with an enzyme, radioactive isotope or a chemiluminescence reporter that can locate and bind to their complementary nucleic acid sequences. DNA-DNA hybridization arrays on macroscopic matrices, such as nylon membranes, have been often referred to as "macroarrays." DNA probe may target whole genomic DNA or individual genes. Whole genomic probes are more likely to cross-react with non-target micro-organisms because of the presence of homologous sequences between different microbial species. Oligonucleotide probes based on signature sequences of specificgenes may display limited or no cross-reactivity with non-targetmicro-organisms when under optimized conditions. In addition, oligonucleotideprobes can differentiate between closely related species oreven subspecies and can be designed to detect uncultivable bacteria. [3] Socransky et al. [39] introduced a method for hybridizing large numbers of DNA samples against large numbers of digoxigenin-labeledwhole genomic DNA or 16S rDNA-based oligonucleotide probes on a single support membrane—the checkerboard DNA-DNA hybridization method. The checkerboard method permits the simultaneous determination of the presence of a multitude of bacterial species in single or multiple clinical samples. Thus, it is particularly applicable in large scale epidemiological research. In addition to the reported advantages of molecular methods, DNA-DNA hybridization technology has the additional feature that microbial contaminants are not cultivated, nor is their DNA amplified. [40] Because the numbers of contaminating micro-organisms are not increased, one may assume that, if present, they would be in numbers below detection limits of the checkerboard DNA-DNA hybridization method, which have been reported to be by the order of 103 to 104 cells. [39, 41]

DNA Microarrays

DNA microarray methods were first described in 1995 and essentially consist of many probes that are discretely located on a nonporous solid support, such as a glass slide.90, 92 Printed arrays and high-density oligonucleotide arrays are the most commonly used types of microarrays. DNA microarrays can be used to enhance PCR product detection and identification. When PCR is used to amplify microbial DNA from clinical specimens, microarrays can then be used to identify the PCR products by hybridization to an array that is composed of species specific probes. Using broad-range primers, such as those that amplifythe 16S rDNA, a single PCR can be used to detect hundreds of bacterial species simultaneously. When coupled to PCR, microarrays have detection sensitivity similar to conventional molecular methods with the added ability to discriminate several species at a time. [3]

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes has been developed for in situ identification of individual microbial cells. [42] This technique detects nucleic acid sequences by a fluorescently labeled probe that hybridizes specifically to its complementary target sequence within the intact cell. In addition to provide identification, FISH gives information about presence, morphology, number, organization and spatial distribution of micro-organisms. FISH not only allows the detection of cultivable microbial species, but also of as-yet uncultivable micro-organisms. [43] rRNAs are the main target molecules for FISH. This is because they can be found in all living organisms; they are relatively stable and occur in high copy numbers (usually several thousands per cell); and they include both variable and highly conserved sequence domains.[20] A typical FISH protocol includes four steps: the fixation and permeabilization of the sample; hybridization with the respective probes for detecting the respective target sequences; washing steps to remove unbound probe; and the detection of labeled cells by microscopy or flowcytometry. [44]

LIMITATIONS OF MOLECULAR METHODS

Molecular techniques have been used to overcome the limitations of cultivation procedures. Nonetheless, as with all methods, they are not without their own limitations.

The main limitations of PCR-derived technologies are: [3]

- Most PCR assays used for identification purposes qualitatively detect the target microorganism but not its levels in the sample. Quantitative results can however be obtained in real-time PCR assays.
- Most PCR assays only detect one species or a few different species (multiplex PCR) at a time. However, broad-range PCR analysis can provide information about the identity of virtually all species in a community.
- Like DNA-DNA hybridization, most PCR assays only detect target species and consequently fail to detect unexpected species. This can be overcome by broad-range PCR assays.

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- In addition to being laborious and costly, broad-range PCR analyses can be affected by some factors, such as biases in homogenization procedures, preferential DNA amplification and differential DNA extraction.
- Microorganisms with thick cell walls, such as fungi, may be difficult to break open and may require additional steps for lysis and consequent DNA release to occur.
- False positive results have the potential to occur because of PCR amplification of contaminant DNA. The most important means of contamination is through carryover of amplification product and special precautions should be taken to avoid this.
- False negatives may occur because of enzyme inhibitors or nucleases present in clinical samples, which may abort the amplification reaction and degrade the DNA template, respectively. Analysis of small sample volumes may also lead to false negative results, particularly if the target species is present in low numbers.

METAGENOMICS

The two fundamental questions in microbial ecology are who is there and what are they doing. Molecular biology methods have provided a great deal of information about the species composition in diverse environments. Now the important question to be answered refers to the role of different species in the consortium, what they are doing there. Data from 16S rRNA gene cloning libraries is astonishing as far as the identification of bacterial diversity is concerned. The challenge now is to develop methods to move beyond cataloging 16S rRNA gene sequences toward an understanding of the physiology and functional roles of bacteria in different environments. While the 16S rRNA gene often provides accurate identification, the other 99.95% of the genome provides the blueprint for the vast array of metabolic, structural, and virulence abilities of each bacterium. Because as-yet-uncultivated bacteria make up a large proportion of most environments, studies of the physiological and functional roles of the community members should also rely on culture independent approaches. Metagenomics is the culture-independent analysis of the collective microbial genomes (metagenome) in an environmental community, using an approach based either on expression or on sequencing. Metagenomics treats the genomes of all microorganisms present in a specific habitat as an entity. Theoretically, a metagenomic library will contain DNA sequences for all the genes in the microbial community. Metagenomic libraries permit analyses of species diversity based on a PCR-independent approach as well as a comprehensive description of functionalities of the whole ecosystem. Metagenomic analysis involves one of the three approaches; functional approach, sequence based approach and whole-genome shotgun sequencing. In the near future, metagenomic analysis of the oral microbiome will provide invaluable information about the physiological and functional roles of the oral microbiota, including bacteria that have not yet been cultivated. [45]

CONCLUSION

The oral cavity harbors one of the highest accumulations of micro-organisms in the body. Even though viruses, archaea, fungi and protozoa can be found as constituents of the oral microbiota, bacteria are by far the most dominant inhabitants of the oral cavity. There are an estimated 10 billion bacterial cells in the mouth. [46] A high diversity of bacterial species is evident from culture studies, but application of molecular biology methods to the analysis of the bacterial diversity has revealed a still broader and more diverse spectrum of extant oral bacteria. Taken as a whole, bacteria detected from the oral cavity fall into 12 separate phyla that comprised over 700different species or phylotypes. [47, 48, 49] Data based on culture-dependent and culture-independent approaches have revealed that there are presently 771 bacterial taxa in the oral cavity: 273 are named bacterial species, 412 phylotypes are known by 16S rRNA gene sequence only, and 86 are unnamed, partially characterized strains. Thus over 50% of the bacteria remain to be cultivated and fully characterized. This raises the interesting possibility that uncultivated and as-yet-uncharacterized species can play an important ecological role as well as participate in the etiology of oral diseases. [19] The introduction of molecular approaches in the oral microbiology research has brought about a significant body of new knowledge with regard to oral microbiota in health and disease. Despite of the great advances, endodontic microbiology is still undergoing a shift from a culture era to a molecular era. It obviously does not imply that culture has become obsolete. In fact efforts should be directed towards the cultivation of as-yet uncultivable species in an attempt to specify their role in the pathogenesis of periradicular diseases. Undoubtedly, the well-directed use of molecular methods will provide additional valuable information regarding the identification and understanding of the causative factors associated with endodontic diseases.PCR and other molecular biology techniques hold the hope of making the knowledge of endodontic infectious processes more accurate. Additionally, molecular methods have the potential to make diagnosis more rapid and directed evidence-based antimicrobial therapy a reality. The development of high density DNA

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microarrays, with hundreds of rDNA sequence probes specific to oral bacterial species and phenotypes will significantly enhance our ability to rapidly determine the composition of the endodontic infection and to establish association with particular signs and symptoms of the disease, thus enhancing the success rate of the endodontic therapy .[50]

CONFLICT OF INTERESTS

There is no conflict of interest amongst the authors.

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REVIEW ARTICLE OPEN ACCESS

CALCIFIC METAMORPHOSIS – A REVIEW

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ABSTRACT

Calcific metamorphosis (CM) is seen in dental pulp after traumatic tooth injuries. There is a deposition of hard tissues within root canal space and yellowish discoloration of clinical crown. The purpose of this article is to consider rationale for the management of pulpal tissues in teeth that exhibit CM and a short review of the same.

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INTRODUCTION

The management of trauma in the permanent dentition represents a significant challenge to the dental practitioner. Proper medical and dental histories, a thorough clinical examination, as well as a detailed history of dental trauma, will assist the dental provider in assessing orofacial injuries and are instrumental in formulating a proper diagnosis. Common squeal to dental trauma are pulp canal obliteration (PCO), also known as calcific metamorphosis (CM). It is recognized clinically as early as 3 months after injury, but in most instances it is not detected for about 12months [1-3]. Approximately 3.8% to 24% of traumatized teeth develop varying degrees of CM [4].

Etiology and incidence

CM is defined as a pulpal response to trauma that is characterized by rapid deposition of hard tissue within the root canal space[5].. CM occurs commonly in young adult because of trauma. It is usually in the anterior region of the mouth. It is more common in extruded, intruded and laterally luxated rather than subluxated and concussed teeth. Also C.M. depends upon the type of injury. Greater the injury, greater the intensity of either pulp canal obliteration or pulp necrosis. Moderate injury could result in partial PCO. Partial PCO usually diagnosed within first year after trauma while total PCO observed from 2-6 years after trauma.

Table: 1. The frequency of necrosis following C.M. in permanent teeth.

Study	Mean observation period	No. of Units started	No. of teeth with CM	No. of teeth with pulpal necrosis
Holcomb & Gregory (1967[6].	4	88 patients	41	6(7%)
Andreasen (1970) [7].	1-12 (3.4)	189 luxated teeth	42	3(7%)
Stalhane & Hedegard (1975) [8].	13-21	76 teeth with C.M.	76	12(16%)
Jacobsen & kerekes (1977) [9].	10-23(16)	122 traumatized teeth	122	16(13%)
Andreasen et al (1987) [10].	1-10 (3.6)	637teeth	96	1(1%)
Robertson et al (1996) [11].	7-22(16)	82 traumatized teeth	82	7(8.5%)

ve observations, studies indicate that 1% - 16% will develop pulpal necrosis following C.M.

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Clinical Features (figure-1)

Clinically a tooth with CM is darker in hue than adjacent teeth and exhibits dark yellow color because of decrease in translucency from a greater thickness of dentin. [12]. Tooth is asymptomatic unless and until there is periapical infection.

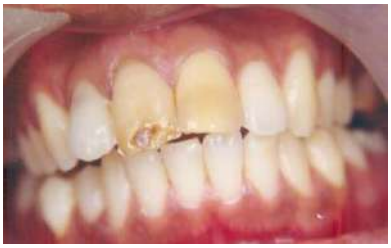
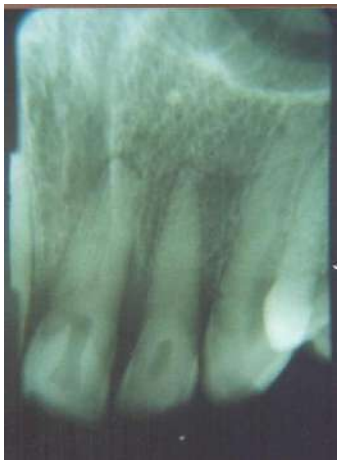


Fig: 1. Clinical features of CM

Radiographic Feature (figures- 2 & 3)

There will be either total or partial obliteration of pulp canal space with normal periodontal membrane space and intact lamina dura. Sometimes thickening of periodontal ligament space or periradicular radiolucency may be observed with or without subjective symptoms. Clinically, the apparent radiographic diameter of the canal does not always correspond to its true width. Kuyk and Walton[13]. measured the canal diameters of 36 teeth from radiographs and then compared them with the true widths of the canals as measured by histological sections. They found that all sections of the roots demonstrated a canal histologically, although some regions had no canal visible radiographically. Complete radiographic obliteration of the root canal space does not necessarily mean the absence of the pulp or canal space; in the majority of the cases, a pulp canal space with pulpal tissue is present[2,14].



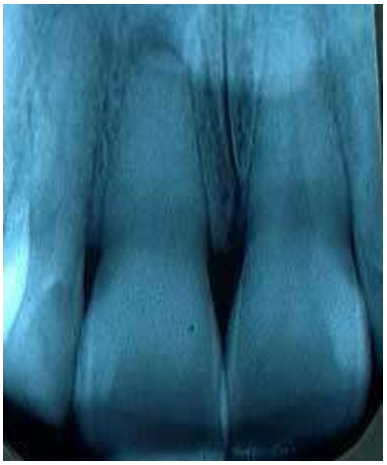


Fig: 2. and 3. Radiographic features of CM

Histopathology

Histopathologic studies designed to assess the pulpal status of teeth with CM have failed to show any inflammatory component indicative of pathologic process[2,12,15]. This may be a result of multiple causes, like poor tissue fixation, specimen sectioning, investigators interpretation etc. Histopathologically 3 types of calcific tissue occlude the pulp lumen. These include: dentin- like, bone- like, and fibrotic tissue. Blackwood's investigation has led to the conclusion that the hard tissue is primarily dentinal in character. Torneck (1990) [2] described calcific metamorphosis as a tertiary dentin response to trauma that is highly irregular in pattern and contains a maze of small irregular spaces and cul-de-sacs that extend from the pulp chamber to the apical foramen. Holan (1998) [17] described tube like structures that extended along the entire length of the pulp canal. These were separated from the root dentine by normal pulp tissue but connected to the dentin in some of the sites

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evaluated. The structures had a histologic appearance of osteodentin, with cellular inclusions in ring like formations.

Mechanism of hard tissue formation

The exact mechanism is not yet clear. Several hypotheses have been proposed to explain this phenomenon. Sundell et al (1985) produced (as per **Figure-4** takes into consideration of) several hypotheses that have been put forward.

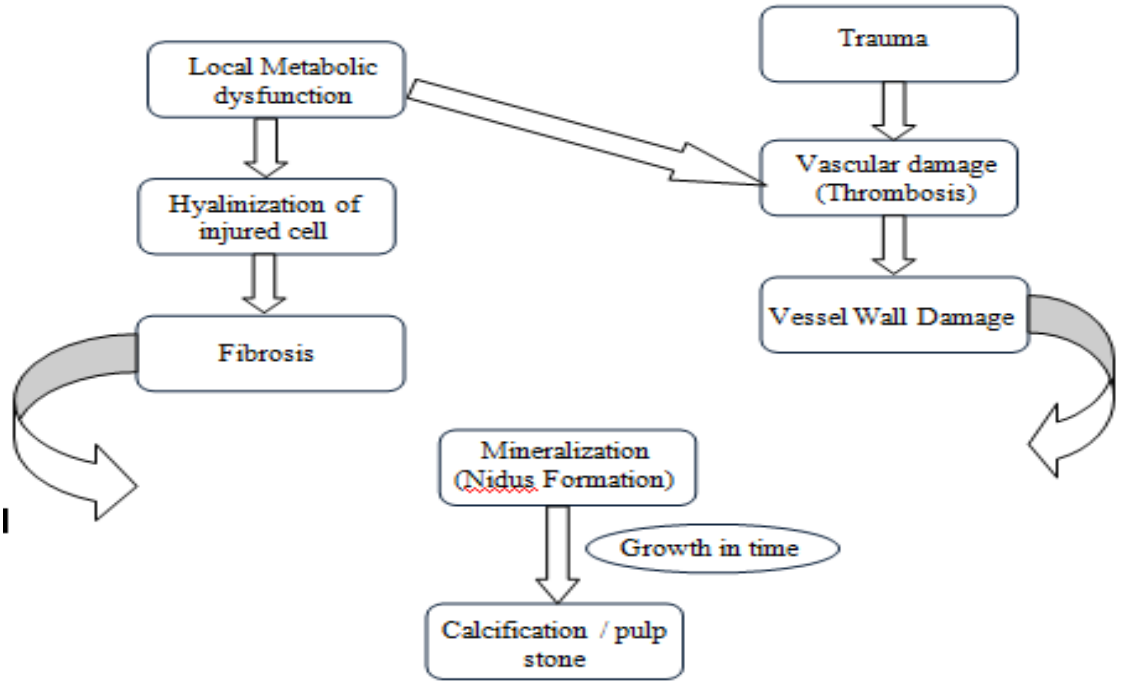

Fig. 4. Mechanism of hard tissue formation

After trauma there will be vascular damage. Sometimes local metabolic dysfunction will also lead to vascular damage, causing thrombus formation. This will act as a nidus and as the time progresses there will be calcification.

Ten Cate (1998) [18] also gave a mechanism for hard tissue deposition. He identified this process as a deposition of tertiary or reparative dentin in response to trauma. During development of tooth, the undifferentiated ectomesenchymal cells of dental papilla will differentiate into two daughter cells. The first daughter cell will differentiate into odontoblasts under the epithelial influence and lay down dentin while the 2nd daughter cell that is not exposed to epithelial influence persist as a subodontoblast cell which under certain influences differentiate into odontoblast like cells and deposit dentin like hard tissue.

Differential Diagnosis

1) Canal calcifications do not necessarily have pathologic origin; they can be result of normal aging of the pulp.
2) Diffuse calcification is seen in mild to severe periodontal diseases.
3) Also certain factors such as alkaline pH of Calcium hydroxide bases, unset composite monomers, hand or mechanical condensation pressure, thermal conductivity and microleakage may stimulate localized reparative dentin deposition leading to eventual obliteration of the pulp canals.
4) It is apparent in disease process like caries.
5) Some nutritional influences like excessive vitamin D cause calcification of pulp.

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6) In chronic pulpitis, the pulp tends to become obliterated by the elaboration of reparative dentin in root canal.

Diagnosis

For diagnosing calcific metamorphosis, thorough dental history and radiographic interpretation is must.Other test like sensibility to electrometric pulp testing shows no significant difference from contralateral tooth except lateral luxation cases which responds feebly to electric pulp tester.

Does the tooth need root canal therapy?

In 1965, Patterson and Mitchell [12] felt that a tooth that had signs of calcific metamorphosis due to trauma should be regarded as a potential focus for infection and that root canal therapy should be initiated. However, further research and clinical observation provided the foundation for current guidelines. The Naval Academy study [6] found that over a four year period only 3/41 (7.3%) of teeth with CM developed pulpal necrosis, and as a result the only definitive criterion for endodontic treatment was the appearance of a periapical radiolucency. Jacobsen and Kerekes [9] conducted a study of 122 traumatized teeth in which partial canal obliteration was identified in 36% of the cases and total canal obliteration in 64%. Only 13% eventually developed pulpal necrosis. Smith [19] performed a literature review and found that teeth with calcific metamorphosis have a low incidence of periapical pathosis development (0-16%) and recommended delaying treatment until symptoms or radiographic changes develop. The development of

CM following trauma does not justify prophylactic root canal therapy [6, 9,11]. According to Fischer (1974) [20] CM was a response to trauma with progressive hard tissue formation, with maintenance of vital tissue & pulp space observed up to the apical foramen. He argued, that such cases require endodontic treatment because of reduced cellular content leading to decreased ability for healing, therefore making the pulpal tissue more susceptible. Worman has described CM as either a reparative or retrogressive change. According to him, root canal treatment is not only futile but also contraindicated, for this obliteration in itself is a perfect root canal treatment. Lundberg & Cvek (1980) [15] evaluated 20 pulps from traumatized permanent incisors with reduced pulpal lumen under microscope. The tissue changes were characterized by a varied increase in collagen content and a marked decrease in number of cells. They concluded that tissue changes in the pulp of teeth with CM do not indicate the necessity for root canal therapy.

Management of canals with calcific metamorphosis

It is similar to management of pulpal spaces with any form of calcification. To locate the calcified orifice, first mentally visualizes and projects the normal spatial relationship of the pulp space onto a radiograph of calcified tooth. Then, the two dimensional radiographic image is correlated with the three dimensional morphology of the tooth [21]. There after the access preparation is initiated, with the rotary instrument directed toward the presumed location of the pulpal space. This approach requires knowledge of normal pulp chamber location, tooth canal anatomy and long axis of roots. Accurate preoperative radiographs are essential. Periodic assessment of bur penetration and orientation should be done radiographically.

Location and Penetration of root canal

DG-16 explorer (SybronEndo) is the most important instrument for orifice location. It will not penetrate and stick in solid denting, however, if orifice is present, firm pressure will force the instrument slightly into the orifice and it will resist dislodgement or catch.

Number 6 K- file is used to negotiate the canal but it is very fine and lacks stiffness. Alternate option is to use canal pathfinder such as canal Pathfinder (JS Dental) or instrument with greater shaft strength, such as pathfinder CS (Kerr). Use of magnification in the form of enhanced glasses or a microscope is also preferred by many practitioners. Examining the color changes in the floor with high magnification will aid in locating the canal orifice. Chelating agents such as REDTA (Roth drug), R C prep (Premier dental products), Glyde (Dentsply) are seldom of value in locating the orifice but can be useful during canal negotiation [22].

Tips in locating & negotiating calcified canals

- Copious irrigation with 2.5% - 5.25% sodium hypochlorite which enhances dissolution of organic debris, lubricates the
  Canal and keep dentin chips and pieces of calcified material in solution.
- Advance instrument slowly in calcified canals.

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- Clean the instrument on withdrawal and inspect it before reinserting it into the canal.
- Use chelating pastes or solutions to assist in canal penetration.
- Use ultrasonic instruments in the pulp chamber to loosen debris in the canal orifices.
- Flare the canal orifice in crown-down fashion.

Esthetic concerns

If the tooth with trauma becomes discolored and the patient has esthetic concerns, external bleaching should be considered first. However, since the decrease in translucency and acquisition of a yellowish color is due to irregular reparative dentin formation, external bleaching of the enamel may not achieve a clinically successful result. Intentional root canal treatment may be performed to facilitate internal bleaching. This may be carried out whether the pulp is vital or necrotic. Rotstein and Walton felt such teeth could be bleached with fair esthetic results A study by Friedman et al [23] found that after a recall period of 1-8 years, 79% of internally bleached teeth had clinically acceptable clinically acceptable or better esthetics.

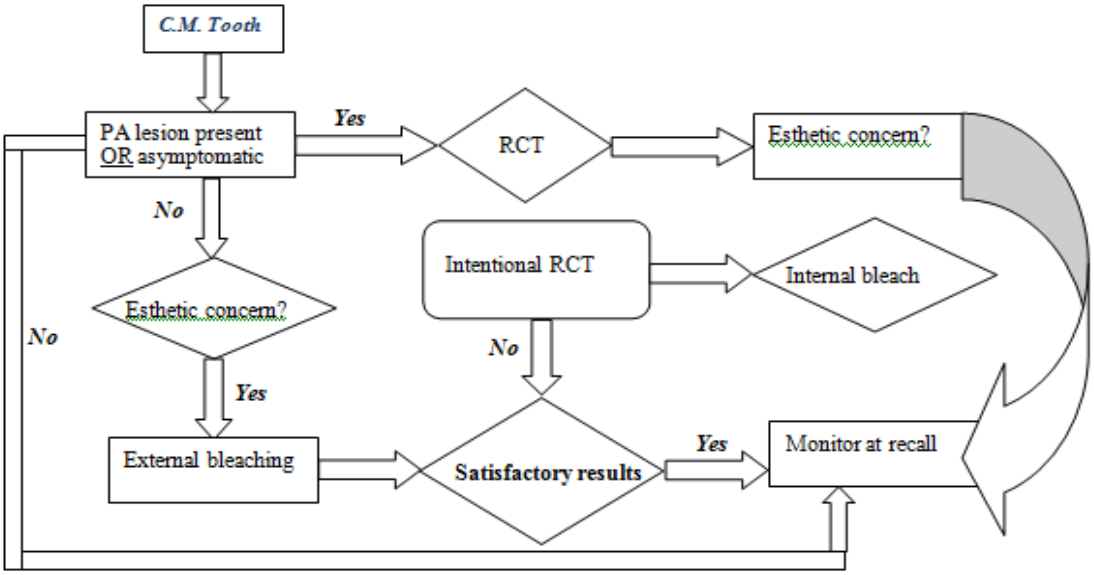

Fig: 5. CM clinical decision

SUMMARY

Although there are different opinions on the management of pulps exhibiting canal obliteration, studies indicate that the incidence of pulpal necrosis in these teeth is between 1% to 16% only. Pulpal necrosis, periradiular pathology or symptoms along with esthetic concerns are the definitive criteria for proceeding with endodontic treatment.

CONFLICT OF INTERESTS

There is no conflict of interest amongst the authors.

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REVIEW ARTICLE OPEN ACCESS

SILVER NANOPARTICLES: A NEW PERSPECTIVE IN ENDODNTIC THERAPY

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ABSTRACT

Silver has been in use in medicine since time immemorial because of its antimicrobial properties. But due to the emergence of antibiotics the use of silver has been declined. Several pathogenic bacteria have developed resistance against various antibiotics. This has led to reemergence of silver. Recently nano science and nanotechnology are gaining tremendous popularity. The small size of nanoparticles provides larger surface area and hence increases the effectiveness of nanoparticles. Silver nanoparticles are used in medical and dental applications ranging from silver based wound dressings, silver coated medicinal devices like catheters, endotrachial tubes, bone cements, in gels, lotions, cosmetics, in dental restorative materials, endodontic cements, dental implants caries inhibitory agents, and in prosthesis. The purpose of this article is to discuss briefly the potential role of silver nano particles in endodontic therapy.

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KEY WORDS

Silver nanoparticles; Root Canal disinfectant; root canal irrigation; Antimicrobial agent; Nano Sealer

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INTRODUCTION

For thousands of years, silver has been used in medicines as an antimicrobial agent. The use of silver as an antibacterial agent lessened with the discovery of antibiotics. The evolution of antibiotic-resistant pathogens has brought a revival in silver-based applications.[1] The use of silver nanoparticles as antimicrobial has become very popular. Silver has been widely used in medical and life-care polymers and exhibits antimicrobial action against gram positive, gram negative bacteria and fungi. This has stimulated incorporation of antimicrobials into dental materials such as silver, silver ions and silver nanoparticles (AgNPs). Some researchers used silver nanoparticles in dental restorative and endodontic materials to make them antimicrobial.

"Nano" is a Greek word synonymous to dwarf, meaning extremely small. Nanoparticles are clusters of atoms in the size range of 1-100nm. Nanotechnology modulates metals into their nanosize. This drastically changes the chemical, physical and optical properties of metals. Nanoparticles have been introduced as materials with good potential to be extensively used in biological and medical applications. Inorganic nanoparticles and their nano-composites are used as good antibacterial agents.[2] As per Humberto HH**,** particle size, size distribution, shape and surface chemistry of silver nanoparticles determine their performance and they determine in vivo distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. [3] The small size and large surface area of the nano-particles can lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. This aggregation may lead to the loss of properties associated with the nanoscale nature of the particles. Agglomeration and particle sizes of AgNPs are responsible for cytotoxicity. Smaller AgNPs (3 nm) are more cytotoxic than larger particles (25 nm) at a concentration of 10 µg/mL.[4] For better efficacy size, shape and morphology are important. Recent advances in Nanotechnology help in modulation of size and shape of nanoparticles.

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Antimicrobial actions of silver nanoparticles

The mechanism of the inhibitory action of AgNps on microbes is still not fully understood. Recently, it has been suggested that the antimicrobial mechanism of AgNps may also be related to membrane damage due to free radicals that are derived from the surface of the nanoparticles. This bactericidal activity also appears to be dependent on the size, shape and concentration of the AgNps. Silver nanoparticles bind to sulfur- containing proteins in biological molecules, resulting in pore formation in cell membrane or defect in cell membrane through the formation of reactive oxygen species (ROS) in the vicinity of bacterial cell membrane causing cell permeability and death.[5] As per other researchers it interacts with phosphorus- containing compounds such as DNA and various cellular enzymes such as cytochrome oxidase, NADH- succunate- dyhydroginase that affects cell division process and leading to cell death.[6] Both mechanisms depend on Ag release. Researchers have studied silver nanocomposites with antimicrobial, antifungal, antiviral applications in the medical field. But as compared to other fields application of silver nanoparticles in dentistry is less.

APPLICATIONS OF SILVER NANOPARTICLES IN DENTISTRY

Main intention of incorporation AgNPs into dental materials is to avoid or at least to decrease the biofilm formation and microbial colonization.[7] Silver nanoparticles are used in dental prostheses, implantology and restorative dentistry.[8,9,10,11,12,] Recently it is under research for its potential use in endodontic materials.

Silver nanoparticles in conservative dentistry

'Dental caries is one of the most common infectious diseases, in which demineralization of hard tooth tissues occurs by the acid produced as a result of fermentation of carbohydrates by bacteria. In today's scenario restorations with composite resins have predominantly replaced dental amalgam restorations. But composite resin restorations is polymerization shrinkage, which produces gap between the restoration and tooth structure, leading to recurrent caries and the final failure of restoration. Silver nanoparticles have been considered as antibacterial components in dental resin composites. Composite resins containing zinc oxide and silver nanoparticles can significantly inhibit the growth of two important oral cavity microorganisms: Streptococcus mutans and Lactobacillus. [13] Patrícia Bolzan Agnelli das Neves et al evaluated physical properties and antibacterial activity of a light-activated composite modified with silver nanoparticles. . Discs were produced with unmodified resin (control group - CG) and modified resin with silver nanoparticles. It was concluded that the discs with silver nanoparticals were less conducive to biofilm growth, without compromising the strength in compression and surface roughness.[14] Cheng et al.[15] studied the effect of AgNPs incorporation, at different concentrations, to a composite resin, in order to investigate its mechanical properties and biofilm formation. In this study, composites were synthesized with AgNPs at 0.028, 0.042, 0.088, and 0.175%. Mechanical properties of composites with AgNPs at 0.028% and 0.042% were similar to those with no AgNPs. Besides that, counts of colony forming units (CFU) for total streptococci and S. mutans, using AgNPs at 0.042%, were 75% smaller than the control group without AgNPs. These data suggest that AgNPs incorporation to composite resins enables good mechanical properties and notable antimicrobial potential, even at low concentration. [16] To evaluate the influence of AgNPs incorporation on bond strength to dental substrate, Melo et al. [17] added AgNPs, at 0.1% by mass, to an adhesive system. The results have shown that AgNPs did not compromise the bond strength, at the same time that it decreased metabolic activity on biofilm, compared to control group without AgNPs. In this study it was also observed reduction of CFU for total microorganisms**.** The longevity of tooth restorations may increase and it helps in reduction of bacterial biofilms on teeth and restorations. It has been showed by many researchers that addition of AgNPs in specific concentration not affects mechanical properties and cytotoxicity of composite resins and adhesive systems.**[16]** The biocompatibility of AgNPs-containing restorative materials was studied by Zhang et al. [18] have studied the effects of AgNPs incorporation on human gingival fibroblast viability., at 0. 05% by mass, to a primer and an adhesive. It has been shown that AgNPs addition did not affect the cytotoxicity of primer and adhesive tested, evidencing the clinical applicability of this antimicrobial**.** Thus many studies showed that the antibacterial effects of AgNPs-containing restorative materials might decrease the development of recurrent caries.

Silver nanoparticles and endodontic materials

Bacteria are the main etiologic agent of pulpal infection and periradicular lesion formation. [16, 19, 20, 21] The microbiota of infected root canals is polymicrobial and is dominated by Gram-negative anaerobes. [22, 23] The residual bacteria in root canal are connected with significantly higher rates of treatment failure. [23] Since elimination of bacteria in root canals is the key to treatment success [24], endodontic materials should ideally provide some antimicrobial activity [25, 26], in order to improve the prognosis of endodontically treated teeth

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.[27] Various materials have been used as root canal fillings, among which gutta-percha is one of the most used. [28] This material has been proved to present slight antibacterial property, provided by the zinc oxide in its components; however, this does not provide to gutta-percha an effective bactericidal potential. [29,16] Shantiaee et al. [27] tested the biocompatibility of this new material, by comparing the cytotoxicity of nanosilver-coated gutta-percha and normal gutta-percha on mouse fibroblasts. In this study, after 24 hours, nanosilver-coated gutta-percha presented cytotoxicity similar to normal gutta-percha and, after one week, it reached the lowest level of cytotoxicity among the tested materials. Dianat and Ataie have introduced nanosilver gutta-percha, the standard gutta-percha that is coated with nanosilver particles . The nanosilver gutta-percha demonstrates significant antibacterial effect against Enterococcus (E.) faecalis, Staphylococcus (S.) aurous, Candida (C.) albicans and Escherichia (E.) coli.[30]

The applications of nanotechnology are not limited to filling materials but have been extended to endodontic applications. A bioceramic based nanomaterials (EndoSequence BC sealer) composed of calcium silicate, calcium phosphate, calcium hydroxide, zirconia and a thickening agent, has been developed recently. Nanoparticles have improved the handling and physical properties. During the hydration reaction in the root canal, a nanocomposites structure of calcium silicate and hydroxyapatite is formed. The hydration reaction and setting time is affected by the availability of water and setting time may be prolonged in overly dried canals. Nano sized particles facilitate delivery of material from 0.012 capillary needle and adopt to irregular dentin surfaces. It sets hard in a matter of a few hours providing excellent seal and dimensional stability. Upon setting, it forms of hydroxyapatite; providing biocompatible and bioactivity. The highly alkaline pH (12.8) gives antimicrobial properties as well Another example is a silicon based sealer (Gutta-Flow Sealer) with an addition of gutta-percha powder and silver nanoparticles. This material is available in the form of uni-dose capsule that can be mixed and injected.This nano-sealer has good biocompatibility, dimensionally stable and sets within half an hour. This material has been reported to improve the sealing capability and better resistance to bacterial penetration. For infection point of view, the antibacterial activity of endodontic sealers can be very beneficial. Recently, antibacterial quaternary ammonium polyethyleneimine (QPEI) nanoparticles have been incorporated into existing sealers such as AH plus, Epiphany and Guttaflow. [31]

Other important step in the endodontic treatment is the chemomechanical debridement of pulpal tissue and pathogenic bacteria. In this stage, irrigant solutions should be used, for dissolving tissue and disinfecting the root canal system. [32] For this purpose, sodium hypochlorite (NaOCl) has been used for more than 70 years, and it remains as one of the most common solutions. [33] However, if NaOCl passes beyond the apex, it is extremely toxic to the periapical tissues. [34] Lotfi et al. [35] compared the antibacterial effect of NaOCl and AgNP solution against Enterococcus faecalis, which is a bacterium often isolated from failed endodontic treatment cases. They have observed that there were no significant differences among 5.25% NaOCl and 0.005% AgNPs, suggesting that this solution, in a remarkably lower concentration, possesses the same bactericidal effect as 5.25% NaOCl; hence, it could be used as a new intracanal irrigant..

Another important endodontic material is the mineral trioxide aggregate (MTA), used in many indications such as perforations sealing, external/internal root resorption repair, and apexification. In spite of being a material of wide application, the antimicrobial properties of MTA are controversial, and they seem to be limited. [36,37] Aiming to improve its antimicrobial potential, Samiei et al.[38] modified MTA by adding AgNPs, at 1% weight. Its effect against oral bacteria and fungi species was assessed. Results have showed that AgNPs-containing MTA possesses higher antimicrobial effect against Enterococcus faecalis, Candida albicans, and Pseudomonas aeruginosa, compared to unmodified MTA. To be used for antisepsis in root canal therapy longer than one session, silver nanoparticles must be able to penetrate dental tubules and the lateral channel systems and accessories. In this way, an adequate fluidity is necessary to facilitate penetration and draining of the radicular canal. [39]

CONCLUSION

The impact of nanotechnology on the field of dentistry is creating major changes with respect to improvement of health. Use of silver nanoparticles has had its greatest effect on restorative dentistry by contributing to the enhancement of antimicrobial properties of dental materials. Silver has been most extensively studied and used since ancient times. The uses of silver nanoparticles are varied and many. They are already entrenched for many commercial applications, certain medical applications and in dentistry. In dentistry it has been advocated as an antifungal agent used against all type of microbes. AgNPs possess great prospective due to their antibacterial, antifungal, antiviral, and anti-inflammatory properties. However, the pitfall of silver nanoparticles is that they can

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induce toxicity at various degrees. It is demonstrated that higher concentrations of silver nanoparticles are toxic and can cause various health problems. [40] AgNPs containing dental materials specially root canal irrigants and root canal filling materials present good antimicrobial properties. Many researchers have done in vitro studies. Since laboratory conditions do not entirely reproduce oral conditions in vivo studies are of great significance. Use of animal models and clinical studies to get a better understanding of the antimicrobial properties is necessary. [16] Studies should be carried out to determine the optimal concentration of this silver compound, in order to assure the antimicrobial effect without increasing its cytotoxicity and also to interrogate the Ag ion release and long-term properties of the AgNP-containing dental materials. Although AgNP is a promising antimicrobial, there are only a few studies employing it in endodontic materials. Considering that endodontic treatment success is highly connected to the elimination of bacteria, research involving AgNPs incorporation to root canal filling materials and intracanal irrigating solutions should be encouraged. Researchers must study the most suitable method of silver nano particles incorporation into endodontic materials.

CONFLICT OF INTEREST

There is no conflict of interest amongst the authors.

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CASE REPORT OPEN ACCESS

THINKING BEYOND EXTRACTION: HEMISECTION WITH PRF AS AN ALTERNATIVE TREATMENT OPTION

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ABSTRACT

Background: *The recent times have seen a changed perception in patients' outlook towards retaining their natural dentition. This has increased the number of root amputation procedure performed by dentists. Such procedures have led to a more conservative and definitive treatment option over radical treatments like extraction.* ***Case description:*** *The first case is of a 55year old female with pain and swelling in relation to 46 where root canal treatment was initiated somewhere else. The tooth was tender on percussion and Grade II mobile. IOPAR showed bone loss and furcation involvement pertaining to the mesial root only. The second case is of a 35 year old male who reported with pain in relation to 36 that had an 8-mm deep periodontal pocket. IOPAR revealed severe vertical bone loss and external root resorption of the distal root.* ***Discussion:*** *Hemisection may be considered a viable treatment in many cases. Platelet Rich Fibrin, which a second-generation platelet concentrate has excellent healing properties and acts as a scaffold material. It has been used as an adjunct to healing of extraction sockets but it's used in an extraction socket post hemisection has not been widely reported. This article discusses two such cases that have been performed successfully with hemisection and PRF scaffold.* ***Clinical significance:*** *Hemisection can be considered as a conservative treatment approach towards retaining the tooth and the alveolar ridge. PRF in such instances can be used as a valuable adjunct.*

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Hemisection; PRF; Root amputation

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INTRODUCTION

Advances in dental technology and imaging have led to a plethora of options to salvage a diseased tooth. Patients are now becoming more aware of the importance of preserving the natural dentition. However, retention of a severely mutilated tooth requires a thorough sequential treatment plan utilizing a multi disciplinary approach.

As mandibular first molars are the first permanent teeth to erupt in the oral cavity, they are prone to maximum abuse. This makes them more susceptible to dental caries, vertical fracture of the tooth, attachment loss, or furcation involvement [1]. Though problems like these significantly complicate the treatment procedure and the final prognosis, extraction is not necessarily an option. In such cases, resection type of surgical therapy may be considered as a viable and a definitive treatment modality as it provides better access to the remaining tooth structure, thereby enabling subsequent periodontal and prosthetic rehabilitation [2].

According to the American Academy of Periodontology, hemisection is defined as the surgical separation of a multirooted tooth in such a way that the root and the associated portion of the crown may be removed [3]. Endodontic treatment of the retained root is done prior to hemisection and the furcation area is made self-cleansable by removing any irregularities on the sound root surface [4]. Several adjuncts have been tried to accelerate the process of bone healing following tooth extraction. Platelet rich fibrin (PRF) is one such autologous material, which was first described by Choukroun in 2001[5,7]. PRF has been used as a scaffold to promote bone growth and maturation. It also has shown excellent wound healing and haemostatic properties. Since the mandibular permanent molar is subjected to high occlusal stresses, the hemisected tooth loses its ability to bear such high loads [1]. Hence, it is mandatory to adequately restore such teeth with an extracoronal restoration.

Hemisection is indicated [6]in cases with severe vertical bone loss involving only one root of a multi-rooted tooth, furcal destruction due to various causes, iatrogenic perforation through the floor of pulp chamber or vertical root fracture of one root to name a few.

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It is contraindicated [6] when there exists anatomical aberrations like fused roots where separation is difficult and in cases where the root to be retained cannot be endodontically manipulated. In this article, series two cases are discussed where hemisection was considered as a treatment option. PRF was used to promote and expedite the process of bone healing.

CASE REPORT

Case 1

A 55-year-old female patient reported to the Department of Conservative Dentistry and Endodontics with pain and swelling in the right lower back tooth region. She gave history of initiating endodontic treatment few months back, which was discontinued. On clinical examination the tooth in question #46 was tender on percussion with grade II mobility. On radiographic examination the tooth showed furcation involvement with bone loss in relation to the mesial root extending upto the furcation and periapical rarefaction **[Figure-1a]**. Since the patient was keen to preserve the tooth, a thorough treatment planning was done explaining the patient the prognosis and the need for non surgical root canal treatment and hemisection to conserve the tooth.

Following working length determination and cleaning and shaping, Calcium hydroxide (Dentocal) was placed. Patient was recalled after 3 weeks. She was completely asymptomatic with no signs of swelling. The distal canal was obturated with guttapercha and AH plus sealer **[Figure-1b]**. The mesial canals were left unobturated. Hemisection was carried out in the subsequent appointment by sectioning the tooth with a long shank tapered fissure diamond bur using a high-speed airotorhandpiece (NSK-JAPAN) under water cooling. A vertical cut was directed towards the furcation area and the tooth sectioned into two halves **[Figure-1c]**. Following this, the mesial roots were extracted. The distal root was smoothened. Granulation tissue present in the mesial root socket was curreted with Universal GraceyCurrete no.2 and irrigated with saline. The PRF was obtained by the protocol given by Choukron et al. 10 ml of patient's blood was collected into dried monovettes (Vacuette, Greiner Bio-One, Austria). The collected blood was immediately centrifuged for 10 min at 3000 rpm. The entire clot without depriving it of the red thrombus was employed in the socket using thin sterile forceps. Figure of 8 sutures were placed at the wound site **[Figure-2]**. The retained tooth was relieved occlusally to reduce the forces acting along the distal root. The patient was kept under follow up for 3-4 weeks.

During the follow up visit, radiograph was taken **[Figure-2 g]**. Wound healing was uneventful and the mobility of the retained tooth had reduced considerably. Tooth preparation for a metal ceramic fixed prosthesis involving 45, the remaining distal half of 46 **[Figure-3]** was done and the bridge was cemented using Type I GIC (GC Corp. Tokyo).

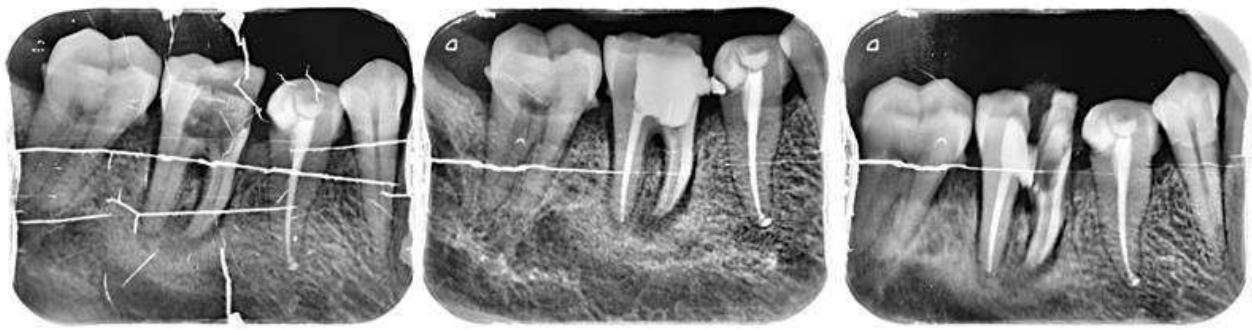

Fig: 1. a) Pre-op b) Obturation of the distal canal c) IOPA- vertical cut made

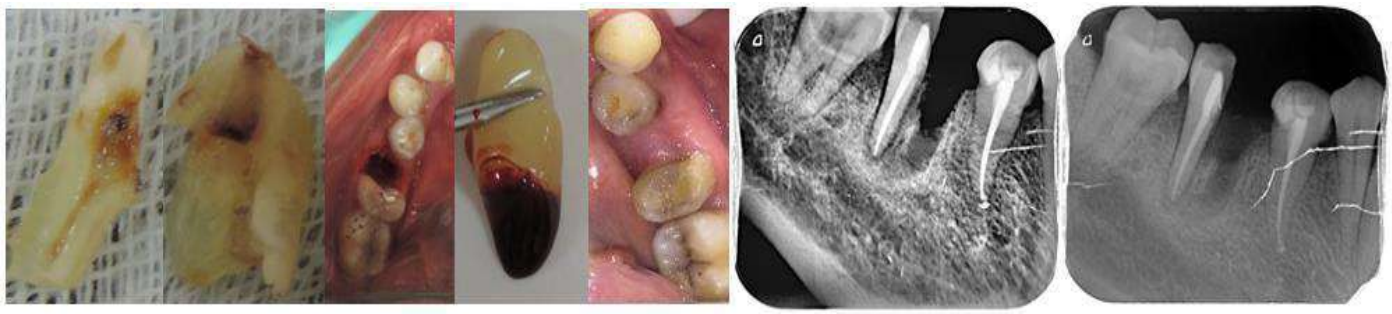

Fig: 2. a,b)Tooth fragments c) Hemisection d)PRF placement e)Post op photograph after 3 weeks f)Immediate Post op IOPAR, g) 3 weeks follow up IOPAR

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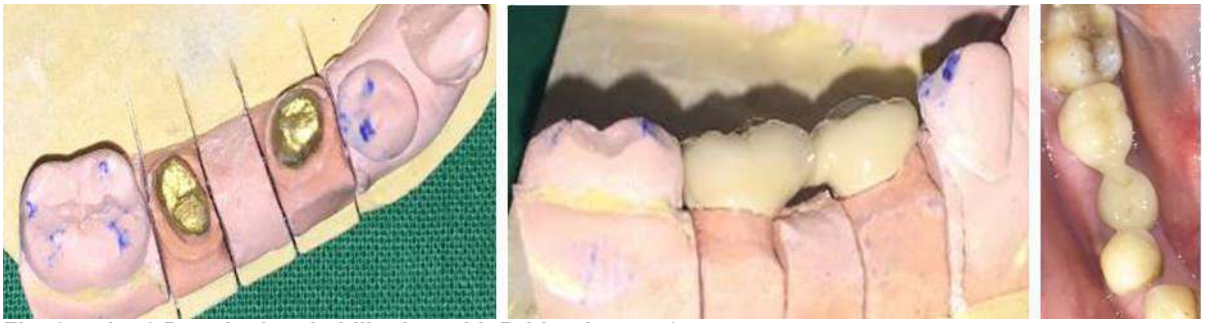

Fig: 3. a ,b, c) Prosthetic rehabilitation with Bridge *irt* 45, 46

Case 2

A 30-year-old male patient reported to the Department of Conservative Dentistry and Endodontics with pain in his left lower back tooth. On clinical examination, the tooth in question #36 looked intact, without any clinical signs of dental caries. An 8mm deep periodontal pocket was found on the distal side on probing. On carrying out vitality tests, there was a delayed response to both Electronic Pulp Vitality Tester (Digitest, Parkell Inc. New York) and cold tests using Endo-Frost (ColteneWhaledent).

Radiographic evaluation showed severe vertical bone loss in respect to the distal root with evidence of external root resorption. Since the patient was keen on preserving the tooth, a decision was made to perform root canal treatment and hemisection of the distal root, after the completion of endodontic therapy.

After administration of local anesthesia, disocclusion and isolation, an access was prepared. Following orifice location and determination of working length, glide path was established with #10 and #15 hand K-files (Mani). The mesial canals were prepared up to 25-6% while the distal canal was prepared up to 30-6% using Hyflex files, using a lubricant (Endoprep-RC, Tamil Nadu, India). Copious irrigation was done using 2.5 % sodium hypochlorite. 2 ml of saline was used as a final irrigant and the fit of the cones verified with a radiograph. Obturation was done in both the mesial canals using AH Plus sealer. The distal canal was not obturated **[Figure-4]**

In the next appointment, post space preparation was done in the mesiolingual canal and a light transmitting post if 1.1 mm diameter (Reforpost, Angelus) was luted using dual cure resin (ParaCore, ColteneWhaledent). Core build up was done using composite resin **[Figure-5]**..

Hemisection was carried out in the subsequent appointment by sectioning the tooth with a long shank tapered fissure diamond bur using an airotorhandpiece under water cooling. A vertical cut was directed towards the furcation area and the tooth sectioned into two halves. Following this, the distal root was extracted. The defects present on the sound mesial root were smoothened **[Figure-6]**.. The granulation tissue was curetted and socket irrigated with saline. PRF was obtained and placed in the extraction socket in a similar manner as described previously. Figure of 8 sutures were given to stabilize it **[Figure-7]**.

Patient was recalled for a definitive prosthetic rehabilitation. Tooth preparation was done with respect to the 36 and 37 for a Metal ceramic bridge. The three unit fixed partial prosthesis **[Figure-8]** was cemented using Type I GIC. (GC Corp., Tokyo.

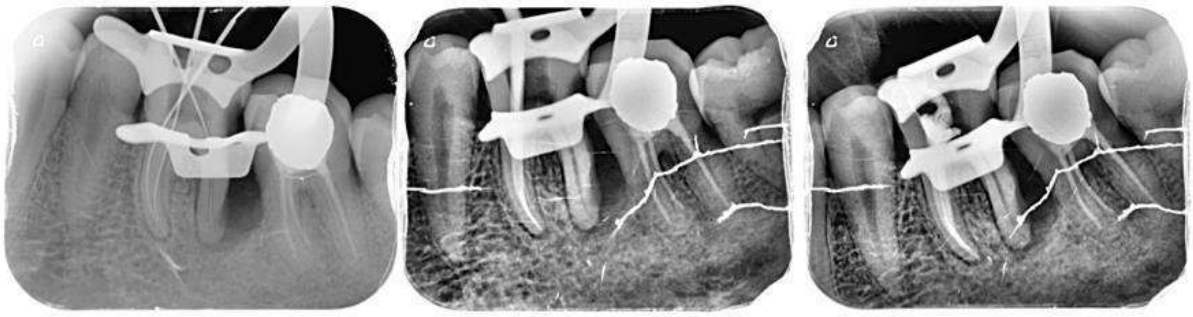

Fig: 4. a) Working length determination b) Master cone selection c) obturation of the mesial canals

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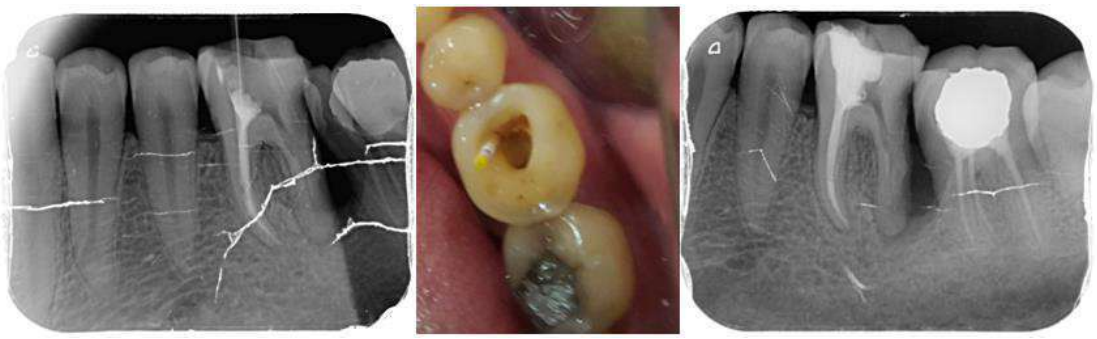

Fig: 5. a,b) Post selection c) post-and-core build up done

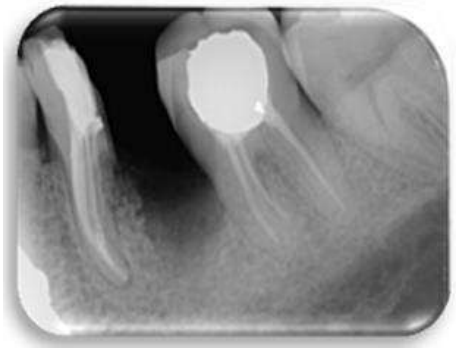

Fig: 6. Immediate post-op IOPAR

DISCUSSION

The success of a clinical procedure is based on thorough clinical knowledge, diagnosis and a multi disciplinary treatment plan. Various root amputation procedures have been carried out to retain a multirooted tooth with a questionable prognosis.While hemisection involves the removal or separation of a root with its accompanying crown portion in mandibular molars, radisection is the terminology given for removal of roots in maxillary molars. Another term that is frequently used is bicuspidization, which is the separation of mesial and distal roots of mandibular molars along with its crown portion, which are both retained individually[8]. However, this procedure was found to have a very poor long-term prognosis and is rarely recommended as a treatment option nowadays. Since, root amputation procedure on mandibular molars poses multiple difficulties to the operating clinician; hemisection is a more predictable treatment procedure. Another advantage of retaining one half of a mandibular molar is to provide occlusion and prevent the supra-eruption of the opposing maxillary tooth and maintain the proprioceptive ability of the tooth.

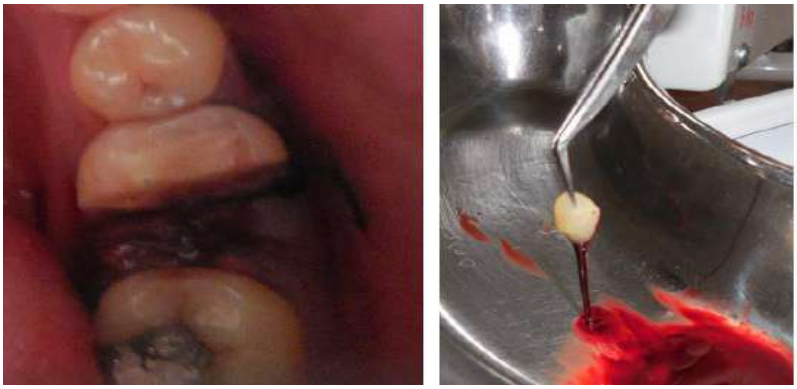

Fig: 7. a) Hemisection and suturing and b) PRF placement

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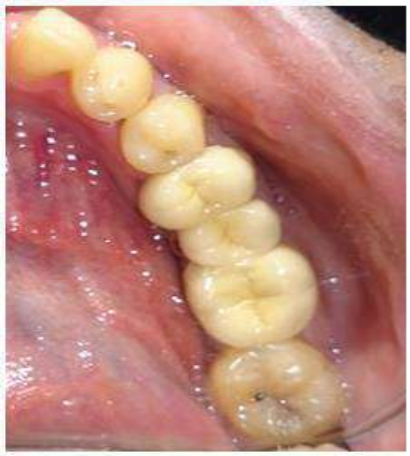

Fig: 8. Three unit fixed partial denture *irt* 36, 37.

The mesial root of a mandibular molar shows more predictable outcome as it has more surface area making it more stable periodontally. However, the distal surface of the root presents a concavity making it more difficult to restore and cleanse with a dental floss and a toothbrush. Retaining the meisal root requires the fabrication of a self-cleansable prosthesis like a hygienic/sanitary pontic. The distal root on the other hand, is generally more conical in shape and easier to maintain [9]. For a faster and an uneventful healing to occur, platelet concentrates like Platelet Rich Fibrin (PRF) can be used. It contains growth factors and cytokines that play a key role to combat inflammation and aid in bone healing. Furthermore, it acts as a scaffold or matrix for regeneration of bone cells [10]. A recent study evaluated the effects PRF on bone regeneration after extraction. The authors concluded that there was a definite improvement in bone regeneration pertaining to cases treated with PRF when compared to the control group, where no PRF was placed, at immediate post-op, 1, 3 and 6 months [11].

In both the cases discussed above, a three-unit bridge with hygienic/sanitary pontics was used to rehabilitate the missing halves and restore the function and esthetics. According to Stein [12] and Essman[13], sanitary pontic is the pontic of choice in hemisection cases because it is self cleansable. The occlusal table was reduced in size in both the cases to further minimize the forces acting on the retained abutments.

CONCLUSION

Hemisection can be considered as conservative approach towards retaining the tooth and the alveolar ridge. A thorough knowledge of the indications and contraindications albeit to proper diagnosis and case selection is essential. The use of PRF as an adjunct to the healing of extraction sockets can provide additional benefits of faster healing and bone regeneration.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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AN OVERVIEW OF REGENERATIVE MATERIALS IN THE TREATMENT OF ENDO-PERIO LESIONS: A REVIEW

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ABSTRACT

Endodontic-periodontal combined lesion is a true challenge. Its management requires thorough understanding of wound healing process involving both endodontic and periodontal complex. The treatment of endodontic-periodontal combined lesions requires both endodontic therapy and periodontal regenerative procedure. Traditional approaches to treat periodontal and endodontic defects include nonsurgical debridement of root surfaces or root canals, as well as surgical approaches that provide better access to clean the root surfaces and apical lesions and to reshape the surrounding bone/root apex. This article reviews the etiology of endo-perio lesions, biologic rationale behind current techniques used for tissue/bone regeneration, reviews the most common materials and techniques, and attempts to explain the factors that influence the outcomes of these therapies.

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KEY WORDS

Endodontic-periodontal lesions; GTR; Barrier Membranes; Bone Grafts; Bone regeneration

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INTRODUCTION

Endodontic-periodontal lesion usually develops due to the pathologic/inflammatory intercommunication between pulpal and periodontal tissues via open structures such as apical foramina, lateral, accessory canals, and dentinal tubules. Formulating a differential diagnosis among combined lesions has been challenging and the diagnostic steps should include thorough patient-reported dental history, visual inspection for presence of sinus tract and severe inflammation in association with large restoration and anatomic anomalies such as palatal grooves, radiographic confirmation with tracing the sinus track, results of clinical findings including percussion and palpation, routine periodontal assessment for presence of mobility or deep probing depth, testing for coronal cracks, and pulp vitality testing. The pulp sensitivity tests are accepted as being reliable in differentiating between pulpal and periodontal disease. However, it is also recognized that false responses might be elicited with available tests, particularly in cases of multi-rooted teeth that might have partial pulp necrosis. Therefore, completing the entire recommended diagnostic steps is critical in management of endodontic periodontal lesions. [1]

The treatment of endodontic-periodontal combined lesions requires both endodontic therapy and periodontal regenerative procedure. The success rate of the endodontic-periodontal combined lesion without a concomitant regenerative procedure has been reported to range from 27% 37%. This is significantly lower than the reported success rate of 95% with conventional nonsurgical root canal therapy. [1] Therefore, case selection and the appropriate treatment modalities are essential for the successful treatment outcome. Guided tissue regeneration (GTR) therapy introduced in 1980s has been widely used to regenerate lost periodontium from periodontal disease. GTR therapy has also been implemented in the endodontic surgeries as a concomitant treatment during the management of the endodontic-periodontal lesions. [1]

Regeneration is the most desirable outcome for any therapy. However, this is also the most difficult result to achieve. Consequently, a wide variety of treatment modalities have been developed, all with the goal of attaining tissue/bone regeneration. Regenerative procedures frequently include the use of barrier membranes and bone grafting materials to encourage the growth of surrounding lost tissues like periodontal ligament, bone cementum and connective tissue, while excluding unwanted cell types such as epithelial cells. [2] To help promote tissue/bone regeneration and healing, the local application of growth factors/cytokines and host modulating agents are being used to maximize the healing potential. Growth factors and hormones including platelet-rich plasma

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(PRP), bone morphogenic proteins (BMPs), platelet-derived growth factor (PDGF), parathyroid hormone (PTH), and enamel matrix proteins (EMD) have shown promise in enhancing regeneration, although their long-term predictability remains questionable, and their anticipated benefits are moderate. [2]

ETIOLOGICAL CLLASIFICATION AND DIAGNOSIS OF ENDO-PERIO LESIONS

Simon classified the Endo-Perio lesions according to the etiology whether pulp or periodontium is primarily the culprit of combined lesions, as: [3]

- Primary endodontic lesions
- Primary endodontic lesions with secondary periodontic involvement
- Primary periodontic lesions
- Primary periodontic lesions with secondary endodontic involvement
- True combined lesions

Depending on their pathology Simon et al listed factors that need to be considered to diagnose an endo-perio lesion : [3,4]

- Inadequate endodontic treatment
- Coronal leakage
- Trauma
- Resorptions (non-infective, transient, pressure induced, chemical induced, replacement, extracanal,infective)
- Perforations
- Developmental malformations

Von Arx & Cochran (2001) proposed a classification of bone defects associated with endodontic surgical cases. The same authors identified membrane application techniques based on typical periradicular lesions classified by their location, extension or pathway of infection. Another classification by Dietrich et al. (2002) proposed a subdivision on the basis of pathogenetic and morphologic criteria of perio-endo lesions.[5] **[Table-1]**.

Table: 1. Bone defects

Localisation and characteristic of the defect		*Classification by VON ARX (2001)*	*Classification by DIETRICH(2002)*
Bone defect confined to periapical region		Class I	
	Lingual/palatal cortex not eroded	Class I a	
	Lingual/palatal cortex eroded (through and through defect)	Class Ib	
Apico marginal lesion		Class II	Class I Class I/1: purely periodontal Class I/2: combined periodontal-endodontic Class I/3: purely endodontic Class II:periapical lesion of purely endodontic origin and characterised by pre-operative probing depths within the normal range . usually with a fistula close to the gingival margin. classIIA: presence of bony bridge classIIB: bony bridge above the defect after surgery. Class III: apical defect with bony dehiscence (etiology is not infectious)
	Periapical and concomitant marginal lesions without communication	classIIa	
	Periapical and concomitant marginal lesions with communication	classIIb	
Lateral juxtaradicular lesion		Class III	
	Without communication to marginal lesion	Class IIIa	
	With communication to marginal lesion	Class IIIb	

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The diagnosis of Endo-Perio lesions becomes difficult when a complete history is unavailable. Once the lesions progress to their final involvement they give a similar radiographic picture and the differential diagnosis becomes more challenging. Rud et al, Hirsch et al, Gutmann and Harrison have concluded, along with the prognostic factors is the extent and the size of the periapical lesion. [6,7,8] A delay in or alteration of healing might occur when the lesion size is greater than 5mm. [5,9,10] The radiographic image of bone resorption, including the apical and furcal or marginal regions, may confuse rather than aid in making the diagnosis. In general it is easier to determine the origin of the lesion if the pulp sensitivity test is done, because the test results usually will rule out an endodontic etiology. However pulp tests may not always be reliable. This is particularly true when the periodontal diseases are primarily responsible for challenging the pulpal status. It has been suggested that when doubt exists about pulp's status, a test cavity can be made. A non-vital or Endodontically treated tooth associated with a combined lesion presents a greater challenge in diagnosis. Location and extent of the pockets, probing depths and furcation invasions are essential for differential diagnosis. [11]

CLINICAL FEATURES

Acute manifestations of root canal infections can result in rapid and extensive destruction of periodontium. Dental abscesses can form and may drain anywhere from the neck of the tooth to the apex, sinus tract may be evident with seeping of purulent exudates. The periodontium can be extensively damaged at the sites of the periapical infections. Following proper endodontic therapy, such lesions frequently heal without a persistent periodontal defect. Endodontic and periodontal abscesses may resemble each other clinically, differing only in the point of origin and specific path of infection. In most instances, periapical abscesses occur singly, the involved tooth may be extruded and exhibits tenderness to percussion and mobility. This tooth usually gives no response to the pulp sensitivity test. The periodontal abscesses are manifested by increased probing depths, suppuration, increased tooth mobility and loss of fibrous attachment. The teeth may be vital to the sensitivity tests; however, a periodontal abscess may also occur in the absence of any previous periodontal disease, following perforation of the lateral wall of the root during endodontic therapy. [11]

RATIONALE OF TREATMENT

After conventional endodontic and periodontal therapy, wound healing takes place as repair or regeneration. The healing is greatly influenced by the cell type that repopulates the wound first. PDL cells, alveolar bone cells, and cementoblasts are all capable of periodontal regeneration whereas the epithelial cells produce repair /or long junctional epitheliam formation.Epithelial cells are capable of migrating 10 times faster than periodontal cells, that is the reason periodontal therapy results in formation of long Junctional epithelium and dominate the initial healing phase. The cells of cementum, PDL and alveolar bone can establish themselves with regenerative potential, only if epithelial downgrowth is restricted. This same principle applies to endodontic defects for ex. Root end surgery. An added advantage in endodontic defects is that the periodontium is healthy. The periodontal defect is mostly an open wound and endodontic lesion is primarily a closed wound. In endodontic situations the tissue is removed for surgical access whereas periodontal treatment is initiated in diseased tissues.

Gerald et al successfully treated a peri-radicular defect and a soft tissue fenestration using DFDBA and a non resorbable membrane.[12] Britain et al treated chronic lesions in fox wounds using open flap debridement(OFD) alone, OFD with bioabsorbable porcine derived collagen, OFD with membrane and bovine bone matrix and found that bioabsorbable collagen membrane with or without bone matrix resulted in increased amounts of bone, PDL and cementum compared to open flap debridement alone. [13]

BONE GRAFTS FOR REGENERATION

Bone replacement grafts are most commonly used for periodontal regeneration. Owing to their osteogenic properties, they can promote tissue/bone regeneration either through osteoinductive or osteoconductive mechanisms. The grafts can be categorized into autogenous, allografts, alloplast and xenograft sources.

Autografts

Autografts are considered as gold standard for bone replacement. They are obtained from the same host from

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other sites such as mandibular symphysis, maxillary tuberosity, extraction sockets, ramus, tori, iliac crest or tibial plateaue. Block grafts, particulate graft or bone chips can be harvested. They can be cortical, cancellous or cortico-cancellous. Cancellous grafts have a capability to revascularise sooner than cortical grafts due to spongy architecture. [14] Revascularisation of these grafts has high strength that decreases with time. After several weeks to 6 months post implantation. cortical autografts have shown to be 40% to 50% weaker than normal bone when strength is compared. This graft material possesses osteogenic, osteoinductive and osteoconductive properties. Autografts possess viable osteogenic cells and osteoconductive properties due to presence of bone morphogenic proteins (BMPs) and porous mineralised component of bone. This graft avoids immunological reactions. Disadvantages are infection, hypersensitivity, chronic post operative pain at donor site, harvesting tissue requires additional surgery at donor site. [15]

Allogenic bone grafts

A bone allograft refers to a graft between genetically dissimilar members of the same species. A non vital osseous tissue is procured from tissue banks that process the donor tissues from a genetically indifferent individual of the same species. Main benefit of this graft is avoidance of secondary donor site, reduced surgical time, reduced blood loss unlimited supply of graft maretial. Commonly used methods for sampling and processing are freeze dring and Tuloplast ® process that reduce the risk of transmission. [16, 17]
Three types of allogenic bone material are available: [18]

- Fresh frozen
- Freeze dried bone allograft (FDBA)
- Demineralised freeze dried bone allograft (DFDBA)

They provide collagen type I and are capable of carrying organic components of bone. Bone morphogenic proteins are found that have osteogenic potential (BMP-1 to BMP-13) in DFDBA. Ddemineralization uncovers the BMPs and collagen which encourage new bone formation. DFDBA is available as putty, sponge and gel forms. [19] Bioactivity of DFDBA depends on donor's age, grafts harvested from younger individuals and have higher osteogenic potential. Most appropriate particle size is 100-400 um smaller particle size has enhanced osteogenic potential. This appropriate particle size provides adequate surface area for vascularization and bone formation. Large particle size(10000-2000um) hinder the vascularization and very small particles get resorbed faster. Saad AY, Abdellatief EM. treated osseous defects of periapical leasions with failed endodontically treated teeth using FDBA showed no adverse response. Case reports using DFDBA have demonstrated formation of hemopoetic marrow and mature [20-26]

Xenografts

Xenografts are derived from the species other than human. It refers to the bone tissue harvested from one species and transplanted to other species. They have a potential of osteoconduction. Anorganic bone matrix (ABM) is de-organified bovine bone and is demineralised using low temperature chemical extraction process and other process useing high temperature which is >1500°c. It consists of a crystalline structure and calcium phosphate ratio which is identical to human bone. Disadvantage is that they are highly brittle and have very less favourable healing. [27-30] Calcium carbonate containing materials are obtained from natural coral species Porites. They are highly porus , provides surface area for resorption and replacement of bone. It provides as a source of carbonate and requires no processing. Additional feature is that it doesn't undergo fibrous encapsulation and results in higher osteoconductivity. Coralline calcium carbonate shows regeneration of periodontal ligament, clinical attachment and greater defect fill. Xenografts are capable of altering the mechanical and biological property of healing bone as they have a very low resorption rate.

Taschieri et al studied the efficacy of xenogenic bone grafting with guided tissue regeneration on the management of bone defects after surgical endodontics and observed 78% of defects healed successfully. [31] In another study conducted by the same researchers about guided tissue regeneration in management of through and through lesions following surgical endodontics, the results showed 88% success and only 57% success in control which received no periodontal regenerative therapy. This also proved that there is no requirement of sufficient host bone around the defect for regeneration. [32]

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Alloplasts

Alloplastic bone graft materials are synthetic materials developed to overcome the inherent problems associated with autograft use. These are synthetically manufactured graft materials made of polymers, ceramics composites and metal. Major advantages of alloplasts are that there is no risk of disease transmission, can be manufactured in various particle sizes, no rejection. Alloplasts can be manufactured in various forms and with varying physicochemical properties. They can be made available in both resorbable and nonresorbable forms and can be customized with varying levels of porosity and pore sizes. Alloplastic materials are mainly osteoconductive without intrinsic potential for osteogenesis or osteoinduction. Their rough surface and large particle size provides a scaffold for bone formation, repair or growth. Choice of alloplasts for endodontic applications are β TCP, bioactive glass, poly methacrylate (PMMA/HEMA) polymers, porous/non porous hydroxyapatite (HA). [33,34]

MEMBRANES

Occlusive barrier membranes are used to exclude the epithelium and connective tissue fibroblasts from a periodontal wound. This allows connective tissue and bone cells to repopulate first resulting in periodontal regeneration. Membranes also prevent contamination and collapse/ disruption of wound. Membranes are available as resorbable and non- resorbable. Ideal membrane blocks the epithelial ingrowth, tissue stability, space maintenance, clinical manageability.[35]

Nonresorbanle membranes

Non-resorbable membranes include polytetrafluoroethylene (PTFE) and titanium mesh. One drawback in the use of this type of membrane is the necessity for its removal with a second stage surgical procedure. However, this disadvantage may be overshadowed by the advantages offered. These membranes provide an effective barrier function in terms of biocompatibility, they can maintain the space beneath the membrane for a sufficient period, they are more predictable in their performance, they have a reduced risk of long-term complications, and they are simple to manage clinically. Nonresorbable membranes also offer a unique characteristic. Their structure can be varied with changes in porosity if a more adaptable and tissue-compatible alternative and multiple designs are commercially available and can be further developed on demand. We will discuss three predominant non-resorbable membranes: the expanded and dense forms of PTFE (e- and d-PTFE) and titanium mesh. [36]

Titanium mesh (Ti-mesh)

Titanium has been used extensively in numerous surgical applications because of its high strength and rigidity, its low density and corresponding low weight, its ability to withstand high temperatures and its resistance to corrosion. This metal is highly reactive, and can be readily passivated to form a protective oxide layer, which accounts for its high corrosion resistance. The low density of titanium provides both high-strength and lightweight dental materials. Titanium mesh (Ti-mesh) has excellent mechanical properties for the stabilization of bone grafts beneath the membrane. Its rigidity provides extensive space maintenance and prevents contour collapse; its elasticity prevents mucosal compression; its stability prevents graft displacement; and its plasticity permits bending, contouring, and adaptation to any unique bony defect. [36] Various studies have shown that Ti-mesh maintains space with a higher degree of predictably, even in cases with a large bony cavity. [37- 40] In addition, it is believed that the smooth surface of Ti-mesh makes it less susceptible to bacterial contamination than resorbable materials. The stiffness of Ti-mesh can maintain space better than other membrane, but may result in mucosal irritation that leads to exposure of the membrane. This space maintenance and resistance to collapse is influenced by the thickness of the Timesh, and as such, an appropriate thickness must be balanced with the likelihood of irritation when using Ti-mesh for GBR. [36] Another common feature of commercially available Timesh membranes is its macroporosity (in the millimeter range). This is thought to play a critical role in maintaining blood supply and is believed to enhance regeneration by improving wound stability through tissue integration and allowing diffusion of extracellular nutrients across the membrane [41- 43]

Poly tetrafluoroethylene (PTFE) membrane

According to its structure, PTFE can be divided into two types: expanded-PTFE (e-PTFE) and high density-PTFE (d- PTFE). Expanded PTFE(e-PTFE) if exposed, may harbor bacteria and needs to be removed immediately in

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case of inflammation. High density PTFE (d-PTFE) has highly dense pore structure and restricts any bacterial infilteration in the bone augmented site. If exposed this membrane doesn't get contaminated and primary closure is not mandatory. [44]

Bioresorbanle membranes

Resorbable materials that are used as membranes all belong to the groups of natural or synthetic polymers. Of these, collagen and aliphatic polyesters, such as polyglycolide or polylactide, are best known for their medical applicability. Collagen is derived from a number of sources and is treated in various ways for membrane fabrication. Polyglycolide or polylactide can be made in large quantities with different physical, chemical, and mechanical properties. As the name suggests, resorbable materials offer the advantage of being resorbed by the body, thus eliminating the need for second-stage removal surgery. [36] In principle, stiff resorbable membranes promote a similar degree of bone regeneration and bone formation as non-resorbable membranes. [45,46] The disadvantages of resorbable materials, however, are their unpredictable degree of resorption, which can significantly alter the amount of bone formation .[47] When the membranes are exposed and/or associated with inflammatory reactions in the adjacent tissue, the enzymatic activity of macrophages and neutrophils causes the membrane to rapidly degrade, thereby affecting the structural integrity of the membrane and causing decreased barrier function and less bone regeneration or bone fill; this is particularly problematic when grafting in conjunction with implant placement, as the implant becomes unstable [36]. When the bone defect is not supported by a physical barrier, bone regeneration fails. Even if the membranes are initially able to keep the space, they generally lose strength, collapse into the space and lead to a failed reconstruction; for example, when treating periodontal defects, resorbable membrane may have a tendency to collapse. [48]

GROWTH FACTORS

Growth factors are natural cell products that are released or activated when cell division is needed. This action typically occurs during such events as wound healing or tissue regeneration. Activated platelets at the wound margins release several growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-a, epidermal growth factor etc. Cells adjacent to the injured site also are induced to release growth factors such as insulin-like growth factor-I, PDGF, TGF-a and TGF-a within a few hours after injury. In periodontal regeneration, the coronal re-establishment of the periodontal ligament (PDL) is required together with corresponding cementum and supporting alveolar bone. Thus, agents which promote periodontal ligament fibroblast (PLF) proliferation and migration as well as collagen biosynthesis would appear to be mediators for enhancing new PDL formation. When combinations or cocktails of different factors are used, greater repair is achieved than when individual factors are applied. [49]

CONCLUSION

It should be emphasized that combined endodontic-periodontal lesions present a clinical dilemma to the clinician and are challenging as the endodontic and periodontal tissues share an embryologic, biologic and functional interrelation. Although traditional nonsurgical periodontal therapy and regular endodontic therapy can be predictably used to arrest mild to moderate defects, it might be inadequate for the treatment of disease characterized by deep pockets or wide circumferential apical defects caused by endodontic infection or surgery. Although traditional surgical procedures provide better access in these situations, there is still a disadvantage to both techniques in that tissue repair is the probable outcome. Many techniques and materials are available to promote regeneration, including bone replacement grafts, barrier membranes, and host modulating agents. Currently, regeneration attempts are widely variable in terms of their ability to predictably regenerate the lost tissue/bone in all types of defects or for all situations. Knowledge of the factors that can negatively affect regeneration outcomes and subsequent careful case selection can help to optimize successful regenerative attempts. Moreover, a critical need still exists for a therapy that can enhance the regeneration in a predictable fashion. This article reviewed the currently available techniques and materials for tissue/bone regeneration, as well as their advantages and disadvantages

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CONFLICT OF INTEREST

There is no conflict of interest amongst the authors.

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