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Management of Maxillofacial Trauma due to Road Traffic Accident: A Case Study

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Abstract

Background: Being the most exposed part of the body, the face is particularly prone to trauma. Trauma to the facial region causes injuries to skeleton components, dentitions as well as soft tissues of the face. The incidence and pattern of maxillofacial fractures vary from country to country depending upon prevailing geographical, social, cultural and environmental factors

Case report: A 19-year-old male was brought to the Frances Newton Mission Hospital, Ferozpur with severe facial injury following a road traffic accident. Patient was conscious, oriented, GCS E4 V5 M6. Patient's relatives provided the history of road traffic accident injury on his face and Rt. hand and leg region. The patient was riding a bike when the incident occurred, was not under the influence of alcohol.

Results: Excellent results were obtained with primary suturing, as these types of injuries are prone to infection secondarily.

Conclusion: Maxillo-facial injuries even though causes severe damage, due to abundant blood supply of the facial region; healing is usually satisfactory and does not necessitate the need for removal of any soft/hard tissue unless it is non-vital.

Keywords: Maxillofacial injury, primary suturing, road traffic accident, trauma

INTRODUCTION

Being the most exposed part of the body, the face is particularly prone to trauma.¹ Trauma to the facial region causes injuries to skeleton components, dentitions as well as soft tissues of the face. The incidence and pattern of maxillofacial fractures vary from country to country depending upon prevailing geographical, social, cultural and environmental factors.² In developed countries increased incidence of trauma were reported due to road traffic accidents, however in developing countries and western world inter personal violence is the most causative factor.² The contributory factors in road traffic accidents include excessive speeding, use of alcohol, drugs, road conditions, poor light.

Road traffic accident (RTA) is a leading cause of morbidity and mortality in adults below the age of 50 years and the greatest numbers of cases are males in the 21–30 year age group.^{3–5} The treatment of trauma is more costly than of any other major disease.⁵ Annually, more than 1 million deaths are recorded worldwide annually, while non-fatal road traffic accidents are a major problem causing hospitalization and permanent disability to thousands of person each year.⁶

The causes of maxillofacial trauma vary and include road traffic accidents (RTAs), interpersonal violence, falls, sports and missile injuries.^{3,7,8} The relative contribution of each cause depends on such factors as geographical location, socio-economic factors and the seasons of the year.^{9,10} The contributory factors in road traffic accidents include reckless driving, excessive speeding, use of alcohol and other drugs, natural disease as well as road conditions.^{7,11,12}

Maxillofacial Injuries are associated with other injuries like brain, airway and cervical spine. Severity of injury, emergency and definitive treatment decides the outcome. Definitive treatment varies from simple suturing of wound to fracture reduction like for nasal bone fracture close reduction, for mandible fracture by inter-maxillary fixation by arch-bar or wiring with or without plating, for zygoma and maxilla fracture includes conservative and plating by ORIF.¹³ The goal of treatment in facial fractures is to achieve

anatomic reduction and restore function while increasing patient comfort and making postoperative care easier.¹⁴

The anatomical location of the maxillofacial bones poses a serious clinical problem once fractured. Thus, the knowledge of the distribution and treatment of maxillofacial fractures can be supportive of its adequate prevention.¹⁵

In the article, we presented a case report of management of maxillofacial trauma due to road traffic accident in the Frances Newton Hospital, Ferozepur, Punjab (India).

CASE REPORT

A 19-year-old male was brought to the Frances Newton Mission Hospital, Ferozepur with severe facial injury following a road traffic accident. Patient was conscious, oriented, GCS E4 V5 M6. Patient's relatives provided the history of road traffic accident injury on his face and Rt. hand and leg region. The patient was riding a bike when the incident occurred, was not under the influence of alcohol. His Vitals recorded were stable. Patient had an injury to both sides of the face causing a lacerated wound approx. 5 x 3 cm extending from lateral half of the upper and lower lip to the cheek region. Patient did not have any fracture of dento-alveolar segment involving the maxillary anterior and posterior region also. Vision in both eyes was normal. NCCT head and face showed normal study.

Prophylactic injectable antibiotics (cefoperazone + sulbactam & metronidazole) were started and primary closure was planned under local anesthesia. Patient was shifted to Operation Theater immediately; wound was irrigated thoroughly with normal saline and betadine solution with debridement of the exposed tissue. The surgery was done under the upper (Infraorbital and posterior superior nerve block and lower bilateral (inferior alveolar nerve block and buccal nerve block) local anesthesia block. A local anesthetic solution of 30 ml of 2% lignocaine with 0.1 ml of sodium bicarbonate was made. After aseptic precautions, with the patient sitting, the condylar notch was identified. The needle was inserted into the space below the midpoint of the zygomatic process till the needle hits the lateral pterygoid plate. After that, it was

inserted posterior and superior to get paresthesia of the mandibular region, and 3 ml was deposited. The needle then was withdrawn till subcutaneous plane to go back and hit the pterygoid plate. Then for accessing maxillary nerve, the needle was inserted anterior and lower to deposit around 3-4 ml. The infraorbital foramen, a thumb (or middle finger) was placed in the notch formed by the nasal bone and premaxilla, and the middle finger (or thumb) is placed on the rostral aspect of the facial crest. The foramen was located with the index finger halfway between and 1 to 3 cm caudal to an imaginary line connecting the thumb and middle finger. The ridge of the foramen is palpated beneath the ventral margin of the levator labii superioris muscle. The point of the needle is advanced along the surface of the maxilla and inserted about 1 inch (2.5 cm) into the canal. 4 ml of local anesthetic solution is deposited within the canal. Anesthesia was complete of full mouth on both sides in around 10 min.

The injured site contained multiple pieces of

foreign body such as wood and sand. After thorough debridement and removal of the foreign bodies, haemostasis was achieved over the lacerated wound margins.

Primary suturing of the wound was done using Polyglactin (Vicryl) & Nylon. Injury to the parotid duct was assessed and was found to be atraumatic. The laceration did not involve the parotid gland and hence facial nerve involvement was also ruled out. An initial approximation suture was placed at the upper lip junction for proper orientation of the soft tissue injury. Suturing was done in layers starting from the mucosa intraorally to the extra oral skin layer.

An antibiotic ointment was applied over the sutured wound margin and a pressure dressing was applied. The surgery took around 2 h to complete and postoperative radiographs demonstrated satisfactory approximation. Regular dressing was done over the sutured wounds after cleaning with povidone iodine solution. Patient was advised follow-up for daily dressing.



Figure 1 and Figure 2: Pre-operative photograph demonstrating the extensive soft tissue laceration



Figure 2: Post-operative photograph showing approximation of the soft tissue



Figure 3: Post-operative photograph after two months

DISCUSSION

Maxillofacial fractures occur in routine following RTAs. The etiology varies from country to country, even within the same country, and depends on the environmental, socioeconomic, and cultural factors. Even with the improvement of safety devices, maxillofacial fractures occur quite often with RTAs. The case report we presented, did not involve any facial bone fracture, but involved only soft tissue injuries.

RTAs are a major cause of maxillofacial trauma, Ferguson showed that 19.25 % RTA victims were 20 years and fewer than 8.75 % were 60 years and above 72 % were 20–59 years.¹⁶ Beyaztas and Alagozlu observed that the most common age group was 1–25 years (44.5 %).¹⁷ Kahoro observed peak incidence in the 21–30 year age group.¹⁸

The more frequent involvement of 21–50 year age group may be due to their involvement increased in travelling to work place and outdoor activities. The other causes of increased incidence of accidents in this age group may be their risk taking behavior along with lack of knowledge or in most of the cases, violation of traffic rules.

Trivedi and Seth found that 78.3 % of the fatal accidents involved males.¹⁹ Souzer et al. found that males constitute 71 % and females 29 % of the total RTA victims.²⁰ According to Tavis et al. overall male to female ratio is 4:3.²¹ The male to female ratio varied with the type of crash and differed by passenger and drivers. But in Indian society mostly males bear the burden of earning and hence are more prone to accidents due to

increased outdoor activity. Virtually all motorcyclists do not wear helmets and only a few drivers and passengers use seat belts.

The maxillofacial region is the most exposed part of the body and is more vulnerable to trauma. Facial fractures occur most commonly in males in the third decade of life.²² Reports reveal that 20 to 60% of all road traffic injuries involve some form of maxillofacial injury, and 62% involve motorcycles.²³

In the management of maxillo-facial injuries, irrigation is an essential component; to prevent infection since it aids in removal of debris and microorganisms. Thus, visible dirt and foreign bodies have to be removed using saline followed by rigorously washing the wound with high pressure saline irrigation. Facial wound debridement must be kept to a minimum as the blood supply to face is excellent providing an optimal healing environment. High-pressure saline irrigation can change the contaminated (or even dirty) wound into a clean- contaminated environment, making it suitable for subsequent primary closure. Saline usage is emphasized for the mechanical effect rather than any antibacterial activity.²⁴

Hydrogen peroxide can also be used for its effervescent and presumed antimicrobial effects. The effervescence is the result of oxygen bubbles created by the breakdown of hydrogen peroxide to water and oxygen by tissue catalase. This “bubbling” action enhances mechanical cleansing of necrotic debris from wounds.

Winter’s landmark article established that the formation of a dry scab on the superficial surface

of a wound impairs epithelization and he determined that a moist environment without scab formation enhances wound healing.²⁵ The patient was under the hospital environment for a period of over 5 days even after being operated for maxilla-facial injuries.

CONCLUSION

Maxillo-facial injuries even though causes severe damage, due to abundant blood supply of the facial region; healing is usually satisfactory and does not necessitate the need for removal of any soft/hard tissue unless it is non-vital. The youth of our country should be thoroughly and properly

exposed to the traffic rules, they should be made aware regarding safety measures to be followed while driving. Awareness programs should be arranged for the general population by the local governing bodies in order to make them aware regarding the first aid management of trauma victims. The public should be aware of utility of helmet while driving.

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Comparative Evaluation of Bite Force Recovery in Patients with Mandibular Fractures - An Observational Study

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Abstract

The mandible is one of the most involved bones of the craniofacial system to get affected by traumatic events or craniomaxillofacial deformities.

Aim: The research was undertaken to relate the recovery of maxillofacial masticatory apparatus post-fracture of the mandible.

Material and method: 37 patients who had undergone mandibular fractures are segregated into 6 groups based on the type of fracture the patient had attained. The fractures are reduced using closed reduction or ORIF. The patient groups were then evaluated for 12 weeks. Bite force was then evaluated at the incisor, left, and the right molar region at a head upright position. **Statistical analysis** Paired student t-test was performed on the given values.

Results: At the incisor region, maximum bite forces were recorded at 12 weeks, in patients that have undergone angle fracture (114.85N +/-35.90N). At the left & right molar region, the utmost bite force was recorded in patients with para symphysis fracture (239.9N +/-105.23N) and (234N +/-103.62N) respectively.

Conclusion: functional recovery of the masticatory system was best shown by patients with mandibular para symphysis fracture at 12 weeks postoperatively.

Keywords: mandibular fracture, bite force, trauma, craniomaxillofacial, mastication

INTRODUCTION

The lower jaw is one of the commonly affected long bones of the facial skeletal system to undergo trauma. The 2nd most common facial injury treated is mandibular fractures. According to several studies, they make up 36% to 70% of all fractures of the facial skeleton^{[1],[2],[3],[4]}. In addition to altering the skeletal architecture, mandibular fractures can also result in neurovascular injuries and modifications to the other parts of the masticatory apparatus, such as a tear or injury to the masticatory muscle^[5]. The main Aetiological factors for fracture of the mandible include road crash accidents, which account for a substantial number of cases in the Indian subcontinent, and intersubjective attacks and falls account for all remaining cases that are regularly seen in our clinic setups. Current literature includes multiple studies that analyse bite forces in individuals that have attained fracture of the mandible, but, detailed literature on the muscular and functional efficiency attained by the faciomasticatory system in each type of fracture at different locations has no existence.

This study aimed to compare the operational recovery of the masticatory system after the attainment of mandibular fractures which are treated utilising either ORIF or the closed reduction technique with Erich's arch bar (open reduction and internal fixation). This research was done to evaluate the efficacy of the skeletal & masticatory system, operational recovery of muscles postoperatively and time required by a patient to attain a normal bite force adequate to carry out normal masticatory functions.

MATERIAL AND METHOD

Thirty-seven patients who visited the oral and maxillofacial surgery department between Oct 2021-22 were the subject of this investigation. Patients with unilateral isolated mandibular fractures or bilateral mandibular fractures of the same type between the ages of 18-50 were recruited for this study. All subjects were in generally good health. Both genders were taken into consideration

and all the patients had adequate dentition to undergo bite force evaluation. Patients with multiple fractures of the craniomaxillofacial region or different types of mandibular fractures were excluded from this study. Also, patients with inadequate dentition, myofascial pain dysfunction syndrome, dentofacial deformities, neurosensory deficits, and TMJ disorders were not chosen for this study.

This study was registered and ethical approval was obtained by the University Institutional Ethics committee {MVGU/ADM/2021/896(xv)}.

Consent was obtained from every patient selected to be a part of the study in the English language and the local language. Every patient underwent closed reduction using The Erich arch bar followed by open reduction & internal fixation with titanium load-sharing mini plates in accordance with Champy's rule of osteosynthesis.

Treated patients were then evaluated postoperatively using MonadTM strain gauge bite force measurement device at different intervals of time.

At one, two, four, eight, and twelve weeks, patients were evaluated postoperatively in a head upright position. The bite force was noted at the central incisor region, right & left molar region.

Mandibular angle fracture patients made up Group 1, mandibular body fracture patients made up Group 2, mandibular condylar fracture patients made up Group 3, mandibular para symphysis fracture patients made up Group 4, mandibular sub condylar fracture patients made up Group 5, and symphysis fracture patients made up Group 6.

STATISTICAL ANALYSIS

The data collected was entered in SPSS Software 18.0, (IBM, New York, United States of America). The significance level was settled at $p = 0.05$ or ≤ 0.05 were statistically insignificant. The significance of the research parameters on a continuous scale between the groups (intergroup analysis) on a metric parameter was calculated using the Student's *t*-test (paired, independent).

RESULTS

37 individuals that have undergone mandible fractures were involved in the research of which 10 patients were females and 27 were males.

At the left molar region, at 1 follow-up, the Greatest bite force was noted in patients with angle fracture (130.58N), and minimum bite force was documented in patients that have undergone condylar fracture (56.23N). At 2 weeks follow-up, maximum bite force was documented in a patient with para symphysis fracture (180.70 N), and minimum bite forces were documented in patients with sub condylar fracture (68.73N). At 4 weeks follow-up maximum bite force was noted in people

that have undergone para symphysis fracture (216.34N) and minimum bite force was noted in people that have undergone sub-condylar fracture (81.83N). At 8 weeks follow-up, maximum bite force was recorded in individuals with para symphysis fracture (235.15N), and minimum bite force was noted in people that have undergone sub-condylar fracture (90.10N). Finally, At 12 weeks of follow-up, maximum bite force was attained by individuals with para-symphysis fractures (239.9N) and minimum bite force was attained by patients with sub condylar fractures (90.60N). (Figure-1) (Table-1)

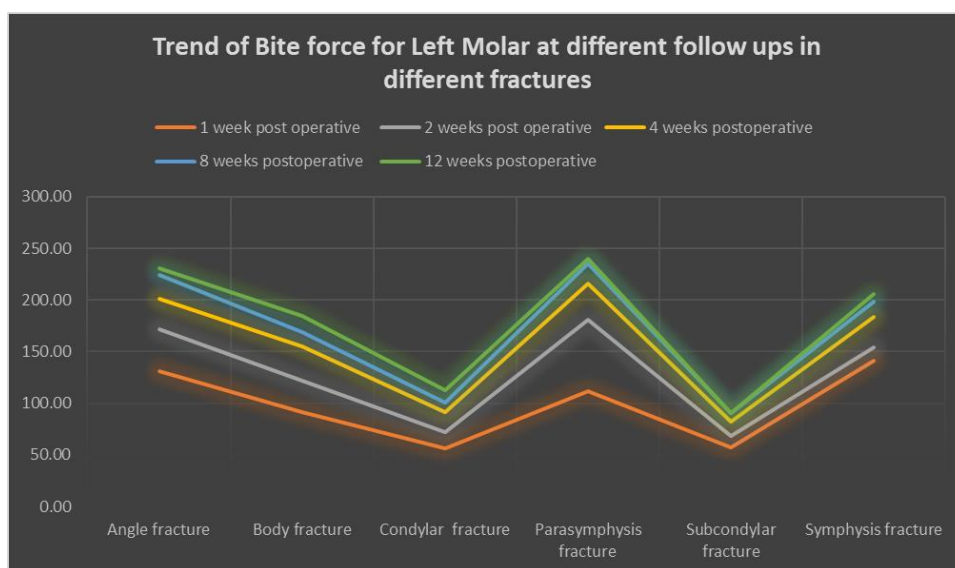


FIGURE-1

TREND OF BITE FORCE FOR LEFT MOLAR AT DIFFERENT FOLLOW UPS IN DIFFERENT FRACTURES						
	Angle fracture	Body fracture	Condylar fracture	Parasympysis fracture	Subcondylar fracture	Symphysis fracture
1 Week Post Operative	130.58	91.24	56.23	111.25	57.20	141.50
2 Weeks Post Operative	171.25	121.56	71.66	180.70	68.73	154.00
4 Weeks Postoperative	200.90	155.10	91.49	216.34	81.83	183.50
8 Weeks Postoperative	224.55	168.90	100.34	235.15	90.10	198.20
12 Weeks Postoperative	230.39	184.70	112.19	239.99	90.60	206.00

TABLE-1

The maximum bite force at the right molar area was obtained in patients with symphysis fractures at 1 week postoperatively (135.00N), whereas the

minimum bite force was recorded in patients with condylar fractures. At 2 weeks postoperatively, maximum bite force was recorded in patients with

symphysis fractures (176.10 N), and minimum bite force was recorded in patients with condylar fractures (67.15 N) at the right molar region. At 4 weeks postoperatively maximum bite force was recorded in patients with para symphysis fracture (207.73) and minimum bite force was recorded in patients with sub condylar fractures (81.23N). At 8 weeks postoperatively, maximum bite force was

recorded in patients with para symphysis fracture (223.51N), and minimum bite force was recorded in patients with sub condylar fracture (90.40N). And, At 12 weeks postoperatively, maximum bite force was attained by patients with para symphysis fracture (234.41N) and minimum bite force was attained by patients with sub condylar fracture (93.80N). (Figure-2) (Table-2)

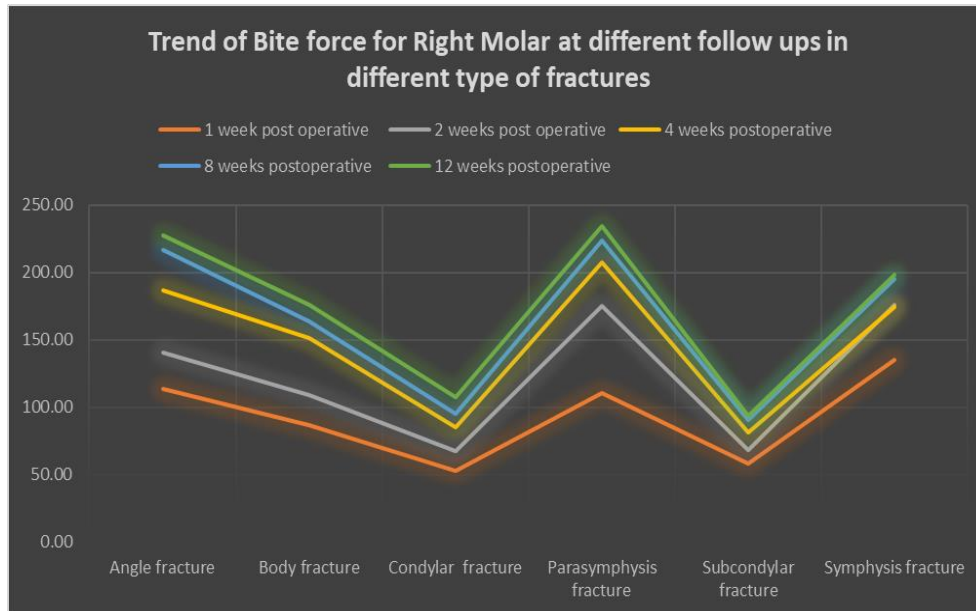


FIGURE-2

TREND OF BITE FORCE FOR RIGHT MOLAR AT DIFFERENT FOLLOW UPS IN DIFFERENT FRACTURES						
	Angle fracture	Body fracture	Condylar fracture	Parasymphysis fracture	Subcondylar fracture	Symphysis fracture
1 Week Post Operative	113.18	86.64	52.88	110.57	58.43	135.00
2 Weeks Post Operative	140.63	108.66	67.15	175.44	68.10	176.10
4 Weeks Postoperative	186.44	151.58	84.74	207.73	81.23	174.25
8 Weeks Postoperative	216.38	163.36	95.48	223.51	90.40	195.00
12 Weeks Postoperative	227.54	175.68	107.28	234.41	93.80	198.00

TABLE-2

At the incisor region, at 1 week postoperatively, Maximum bite force was recorded in patients with symphysis fracture (68.50 N), and the minimum bite force was recorded in patients with condylar fractures (27.80 N). At 2 weeks postoperatively, maximum bite force was recorded in patients with

symphysis fractures (87.65 N), and minimum bite force was recorded in patients with condylar fractures (39.78 N). At 4 weeks postoperatively maximum bite force was recorded in patients with symphysis fracture (92 N) and minimum bite force was recorded in patients with sub condylar fractures

(44.4N). At 8 weeks postoperatively, maximum bite force was recorded in patients with body fracture (98.20N), and minimum bite force was recorded in patients with sub condylar fracture (57.43N). And At 12 weeks postoperatively, maximum bite force was

attained by patients with angle fracture (114.85N) and minimum bite force was attained by patients with sub condylar fracture (68.40N). (Figure-3) (Table 3).

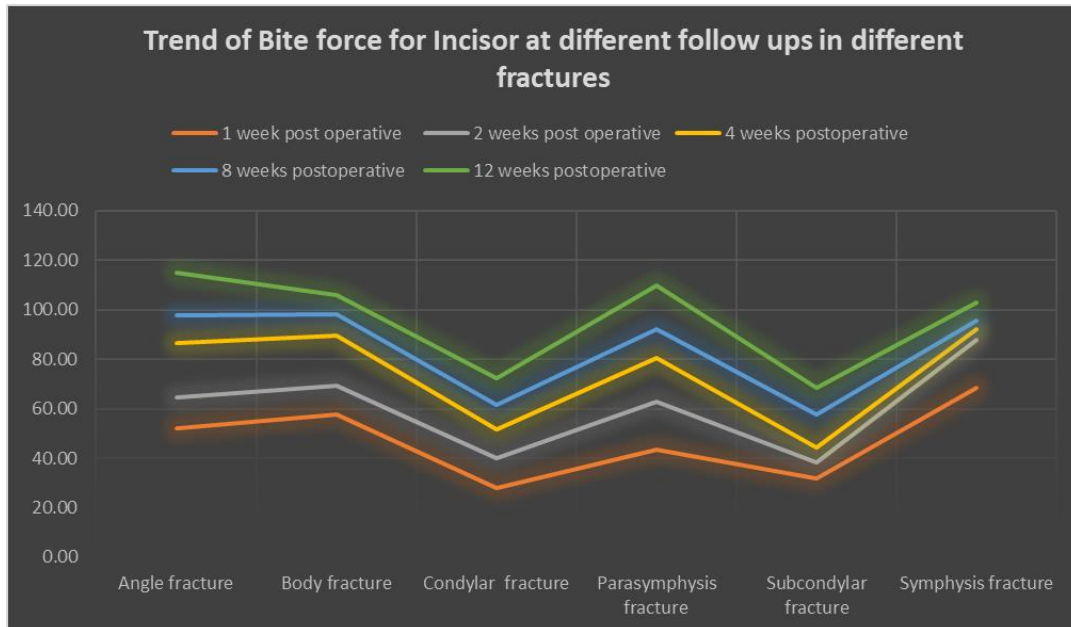


FIGURE-3

TREND OF BITE FORCE FOR INCISOR AT DIFFERENT FOLLOW UPS IN DIFFERENT FRACTURES						
	Angle fracture	Body fracture	Condylar fracture	Parasymphysis fracture	Subcondylar fracture	Symphysis fracture
1 Week Post Operative	51.96	57.50	27.80	43.24	31.73	68.50
2 Weeks Post Operative	64.45	69.44	39.78	62.90	38.40	87.65
4 Weeks Postoperative	86.61	89.70	51.74	80.68	44.40	92.00
8 Weeks Postoperative	97.70	98.20	61.44	91.99	57.43	95.65
12 Weeks Postoperative	114.85	105.74	72.45	109.80	68.40	102.80

TABLE-3

A significant difference was seen statistically in all the bite force values at 1, 2, 4, 8, and 12 weeks in groups 1, 2, 3, and 4 ($P < 0.05$) However, there were lesser statistically significant changes seen in groups 5 and 6.

DISCUSSION

Mandibular bone fractures are a significant patient source of worry, while not being a serious injury. This is because of the significant impact such fractures have on mastication, a function that can only be performed by the craniofacial muscular and skeletal systems. To determine the extent of harm to

the masticatory system induced by mandibular fractures and the impact of different mandibular fractures on the maximal bite forces, this study was done. Soft tissues injury, the dentoalveolar system, and significant skeletal elements of the face, such as the maxilla, mandible, naso-orbital-ethmoid complex, zygoma, or supraorbital structures, are

usually caused by trauma to the facial region. A significant fraction (>70 per cent) of all facial injuries are mandibular fractures alone [6]. The considerable reduction in bite forces that occurred after treatment for a fractured mandible may have been caused by surgical or trauma-related damage to the masticatory muscle or by the masticatory system's protective neuromuscular mechanisms.

In an analysis done by Yadav et al [7], it was found that 10% of women and 90% of men experience mandibular fractures, respectively. In the present study, there were three times as many males who experienced mandibular fractures as girls. The fact that women in our society do not engage in as many of the same physical activities as men do, once again, plays a role in the low frequency of females in our study.

In their study, Gandhi and Kattimani et al [8] found that RTA is a major contributing factor to the development of mandibular fractures. A similar study done by Friedrich et al [9] deduced the cause for mandibular fractures to be RTA as well. But a study done by Guimond et al [10] concluded that assault is the primary causative factor for the incidence of mandibular fracture. Road traffic accidents (RTA), which accounted for almost 80% of cases in the current study on the aetiology of mandibular fractures, were the other causes of mandibular fractures, followed by assault (15%) and falls (5 per cent). The fact that the study was carried out in industrialised nations may account for the variation in the etiologic factor when compared to previous studies.

In the current research, the maximum bite force at one week was attained by group 6 i.e., symphysis fracture. But, in the progressive follow-ups, the maximum bite force was attained by patients with para symphysis fracture, i.e., group 4.

A sudden spike was also observed during the study from the 4th week postoperatively to the 8th week postoperatively. This could be due to its correlation with the stage of bony callus formation that starts on the 11th day postoperatively till the 28th day and the bone remodelling phase which starts from the 18th day postoperatively and can last for several years. A similar observation was made in the present where, At the end of the 8th week, no significant changes were seen in the bite force values and similar values were also seen at 12 weeks postoperatively. The normal bone structure can eventually regenerate after a lengthy bone remodeling process that can extend for many months. [11],[12],[13].

It is essential to emphasize that the present study was a comparative evaluation of the bite force postoperatively for 12 weeks alone. Hence, Further studies are required to evaluate the bone formation post mandibular fractures as well as the functional recovery of the mandibular joint unit post a traumatic event for a longer duration of time.

CONCLUSION

The findings of this investigation were in line with the initial intent of the study. Patients with para-symphysis fractures regained maximum functional efficiency post a traumatic event. In patients with mandibular fractures, there is an initial decrease in the bite force but a sudden rise was observed at 4 weeks and eventually, the values were stable by the end of 12 weeks postoperatively.

Further studies need to be performed to deduce the maximum bite force that was obtained postoperatively.

Ethical Statement

University institutional ethics committee
MVGU/ADM/2021/896(xv)

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Comparative Evaluation of Dentinal Cracks Formation After Root Canal Preparation Using Three Different Rotary File Systems - A Stereomicroscopic *In Vitro* Study

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Abstract

Aim and Objective: The present in-vitro study was conducted with an aim to analyse and compare the incidence of dentinal cracks formation after root canal preparation with TruNatomy, One Curve and Hyflex EDM rotary files using a stereomicroscope.

Material and Methods: 60 human caries free single-rooted mandibular premolar with straight, fully formed apices verified radio graphically were selected for the study. Samples were grouped as file used for root canal preparation. Group 1: TruNatomy, Group 2: OneCurve, Group 3: Hyflex EDM, Group 4: Control -no preparation Done. The samples stained with methylene blue dye (0.5%). All roots horizontally sectioned at 3,6 and 9 mm from the apex. The sections were observed under stereomicroscope to determine the absence or presence of crack.

Result: The result of this study showed that all the groups except control group exhibited dentinal cracks least number of cracks was shown by group 1 (TruNatomy) followed by Group 3 (Hyflex EDM) and maximum was seen in Group 2 (OneCurve). The study also

showed that maximum dentinal cracks observed at 3 mm level from apex followed by at 6 mm and least at 9 mm in each of the group.

Conclusion: All the rotary files that were tested produced dentinal cracks. TruNatomy files group was associated with less number of cracks compared to OneCurve and Hyflex EDM files group. All the file showed more dentinal cracks at apical region compared to middle and coronal region of root.

Keyword: Dentinal cracks, Stereomicroscope, Rotary files

INTRODUCTION

The primary goal of endodontic therapy is to eliminate the infected dental tissue and disinfect the entire root canal using various instruments and materials. Root canal preparation with rotary endodontic files removes more root dentin and may result in weakening of dentin integrity, leading to a reduction in the fracture resistance of treated tooth. Root canal preparation procedures can damage the root canal dentin causing dentinal cracks.¹

NiTi rotary instruments have the advantages of increased flexibility and shortened working time, while instrument separation and dentinal crack formation are its major disadvantages.² Technological advancements in rotary nickel–titanium (NiTi) instruments has led to new design, concepts, and easier, faster, and better root canal shaping. Root canal shaping procedures and rotary instrumentation with NiTi instruments can induce crack formation.³

Crack is defined as a defect with complete crack lines extending from inner root canal space up to the outer surface of the root.^{4,5} Dentinal cracks or root fracture occur when the tensile stress in the root canal wall exceeds the tensile stress of dentin.¹⁴ Rotary NiTi files with large tapers can produce increased friction and stresses on the canal wall and cause dentinal cracks in root dentin.⁶

The newly developed TruNatomy instruments are manufactured from heat-treated NiTi alloy with parallelogram cross-section design.⁷ This file system uses a 0.8 mm NiTi wire instead of 1.2 mm NiTi wire, which offers maximum preservation of pericervical dentin and tooth integrity due to instrument geometry, regressive tapers, and the slim design.

One Curve is a heat-treated single-file system that was released in 2018. The heat-treated NiTi alloy is called C. Wire. C. Wire technology is reported to provide a controlled memory and a pre-bending

feature to the file for easier access to the root canal.⁸ The variable cross-sections with a triangular-shaped at the tip of the instrument and S-shaped near the shaft are claimed to allow effective cutting and centered trajectory.⁹

HyFlex EDM files are controlled memory instruments, manufactured using a unique process called electrical discharge machining (EDM).¹⁰ Electrical Discharge Machining uses spark erosion to harden the surface of NiTi files in the manufacturing process, which gives the files excellent flexibility and fracture resistance.¹¹ Spark initiated in this process is melting and vaporizing the material of the workpiece in its top layer.^{12,13} Like Hyflex CM files, HEDM files have controlled memory effect and regenerative properties.¹²

In this study we compare the incidence of dentinal cracks formation after root canal preparation with TruNatomy, One Curve and Hyflex EDM rotary files.

MATERIAL AND METHODS

The study protocol was approved by the Ethics committee, presenting with statement number RUHS-CDS/EC/2021/PG-The/013. Freshly extracted, single-rooted mandibular human premolar (n=60) with completely formed root and closed apices, with no cracks or structural anomalies were used for this study. The teeth were disinfected in a 0.1% thymol solution for 24 h. Throughout the experiment, the teeth will be stored in purified filtered water. Periapical radiographs of the teeth in the buccolingual and mesiodistal directions were obtained with the intention of visualising inflammatory resorptions and calcifications as well as the presence of a single root canal.

The coronal portions of the teeth were removed using a double-sided diamond disc with low rotation and under water refrigeration leaving roots of approximately 13 mm in length. The samples were inspected under a stereomicroscope with 10X

magnification to detect any pre-existing cracks or fracture lines. teeth with such findings were excluded from the study.

All the teeth were examined and compatible with a K-file#10 made from stainless steel (Dentsply Maillefer, Ballaigues, Switzerland). The length of the canal was determined by inserting the file until the tip became visible on the apical foramen. The canal length was defined as the distance between the tip of the file and the reference plane. The working length (WL) was calculated by subtracting 1 mm from the obtained length.

Roots were immersed into molten wax and then, all the samples were embedded in acrylic resin blocks. The wax on the root surface was cleaned with the help of a curette prior to the polymerization of the acrylic resin. A silicone impression material (Vinyl Polysiloxane impression material, 3M ESPE, Seefeld, Germany) covered the root surface. All the roots were then embedded into acrylic resin again. Initially, the root canals were irrigated with 2 mL of a 3% sodium hypochlorite solution (NaOCl). The glide path of all the samples was made with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland). The teeth were worked in a wet environment. One operator performed all root canal instrumentation. The specimens were randomly divided into four groups (n=15) based on instrumentation technique used.

GROUP 1 (n=15) : 15 premolars were instrumented with TruNatomy files system (Dentsply Sirona, Maillefer, Ballaigues, Switzerland) using crown-down technique. Canal orifice was shaped using TruNatomy Orifice Modifier and a glide path was achieved using #17 TruNatomy Glider till the working length. Subsequently, #20 TruNatomy small files and #26 TruNatomy Prime shaping files at speed of 500 rpm and a torque of 1.5Ncm were used to complete the preparation till the working length.

GROUP 2 (n=15): The root canals were prepared with the One Curve file in a continuous rotary motion at a speed of 300 rpm and a torque of 2.5 N/cm. with an estimated working length. Size. The OneFlare (25/.09) file used to enlarge the orifice and after that OneGlide (14/.03) file is inserted to the working length according to the manufacture

instructions. the OneCurve 25/.04 and 25/.06 files are used to shape the canal to full working length.

GROUP 3 (n=15): The following sequence of Hyflex EDM file used to prepare the canal with Xsmart endomotor. Hyflex EDM orifice opener is used to enlarge the orifice and after that Hyflex EDM glide path is inserted to the working length according to the manufacturer's instructions. Hyflex EDM 20/.05 and 25/.08 files are used to shape the canal to full working length. The Hyflex EDM files are used in a gentle in and out motion with a rotational speed of 500rpm and 2.5 Ncm torque.

GROUP 4 (n=15): left unprepared

A complete rotation with light pecking in and out motion will be done for instrumentation. With the use of each instrument, the canal was irrigated with 2 mL of 3% NaOCl and 5 ml of saline and 5 ml of 17% ethylene diamine tetra acetic acid between each instrument change, followed by final rinse with 2 ml of distilled water. The instruments were used only once according to the manufacturer instructions.

Sectioning and Microscopic Examination

The samples stained with methylene blue dye (0.5%) for 24 hours and washed in running water and after that washed with distilled water.

All roots were marked at 3,6 and 9 mm from the apex using a marker. All roots were horizontally sectioned at 3,6 and 9 mm from the apex with rotating diamond disc positioned perpendicularly to the root canal axis, under copious water cooling. Each slices are examined under 40x magnification using stereomicroscope to detect the presence or absence of dentin micro-cracks. Pictures were taken with camera attached to stereomicroscope and examined the sections for dentinal cracks. PowerPoint presentation for each root sections would be prepared with three images on each slide for blind study by examiner.

Defects that extending from the inner canal lumen was considered as dentinal crack having been produced by the instrument. The data collected will be subjected to statically analysis.

OBSERVATION AND RESULT

Normality of data was checked using Shapiro wilk test. Thus, inferential statistics were performed using parametric tests of significance. Pearson Chi-square test was used to determine the differences between groups. The results regarding the presence

of dentinal defects were expressed as the number and percentage of samples with microcracks in each group. Level of statistical significance was set at $p < 0.05$.

The results showed that at 3mm level, the number of teeth in which cracks were observed were 2 (13.3%) in group 1, 5 (33.3%) in group 2, 3 (20.0%) in group 3 and 0 (0.0%) in group 4.

At 6mm level, the number of teeth in which cracks were observed 1(6.7%) in group 1, 3 (20.0%) in group 2, 2 (13.3%) in group 3, and 0 (0.0%) in group 4.

At 9mm, the number of teeth in which cracks were observed 1(6.7%), 2(13.3%) in group 2, 2 (13.3%) in group 3, and 0 (0.0%) in group 4.

At all three horizontal sections, TruNatomy has shown (8.88%) less number of cracks followed by Hyflex EDM (15.15%) and maximum in OneCurve (22.2%) though $p > 0.05$ thus, statistically insignificant difference has been found.

In all file systems, more cracks has been found at 3mm (TruNatomy 13.3%, Hyflex EDM 20% and OneCurve33.3%) followed by 6mm (TruNatomy 6.7%, HyflexEDM 13.3% and OneCurve 20%) and least at 9mm (TruNatomy 6.7%, Hyflex EDM 13.3% and OneCurve 13.3%) though $p > 0.05$ thus, statistically insignificant difference has been found.

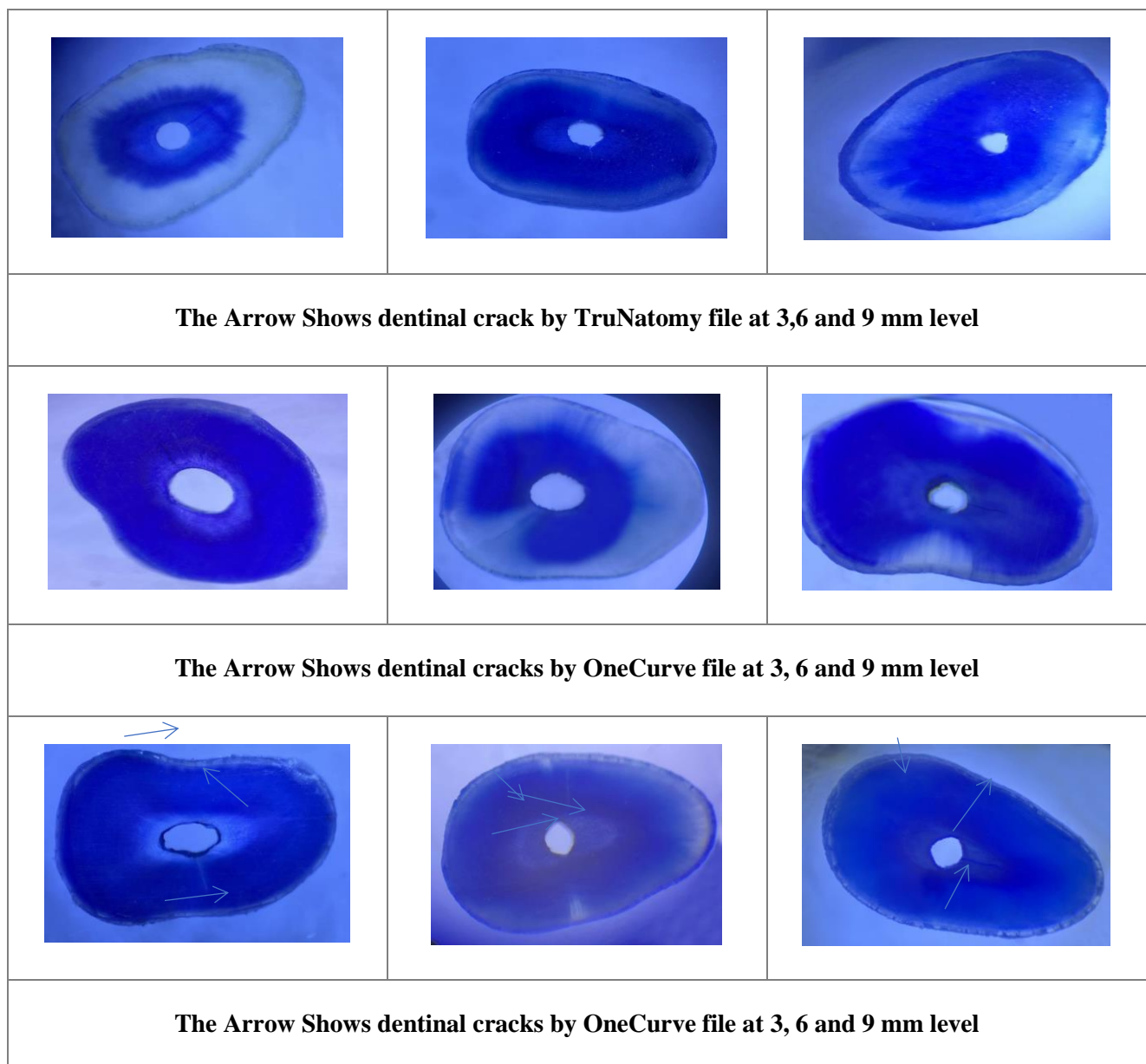


Table 1: Intergroup comparison of the number of teeth in which cracks were observed on the horizontal sections at 3mm

			Cracks at 3mm		Total
			Absent	Present	
Group	Group 1	N	13	2	15
		%	86.7%	13.3%	100.0%
	Group 2	N	10	5	15
		%	66.7%	33.3%	100.0%
	Group 3	N	12	3	15
		%	80.0%	20.0%	100.0%
	Group 4	N	15	0	15
		%	100.0%	0.0%	100.0%
Total		N	50	10	60
		%	83.3%	16.7%	100.0%
P value			0.100, ns		

^aChi square test, Level of significance at $p < 0.05^*$

Graph 1: Intergroup comparison of the number of teeth in which cracks were observed on the horizontal sections at 3 mm

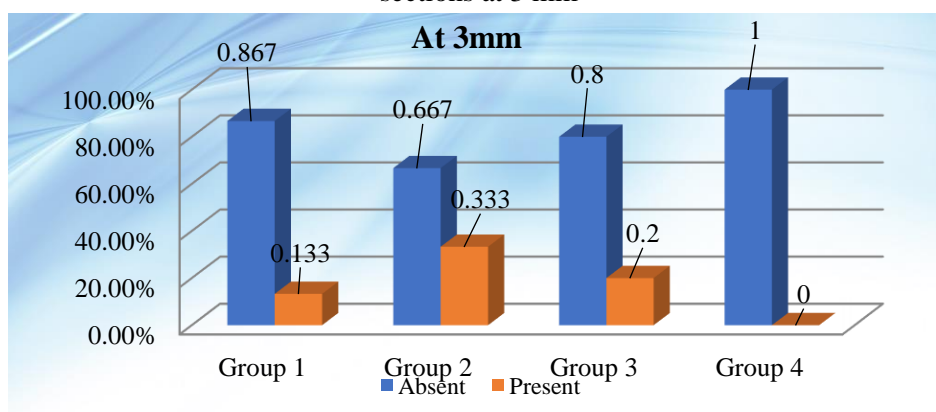


Table 2: Intergroup comparison of the number of teeth in which cracks were observed on the horizontal sections at 6 mm

			At 6 mm		Total
			Absent	Present	
Group	Group 1	N	14	1	15
		%	93.3%	6.7%	100.0%
	Group 2	N	12	3	15
		%	80.0%	20.0%	100.0%
	Group 3	N	13	2	15
		%	86.7%	13.3%	100.0%
	Group 4	N	15	0	15
		%	100.0%	0.0%	100.0%
Total		N	54	6	60
		%	90.0%	10.0%	100.0%
P value			0.295, ns		

^aChi square test, Level of significance at $p < 0.05^*$

Graph 2: Intergroup comparison of the number of teeth in which cracks were observed on the horizontal sections at 6 mm

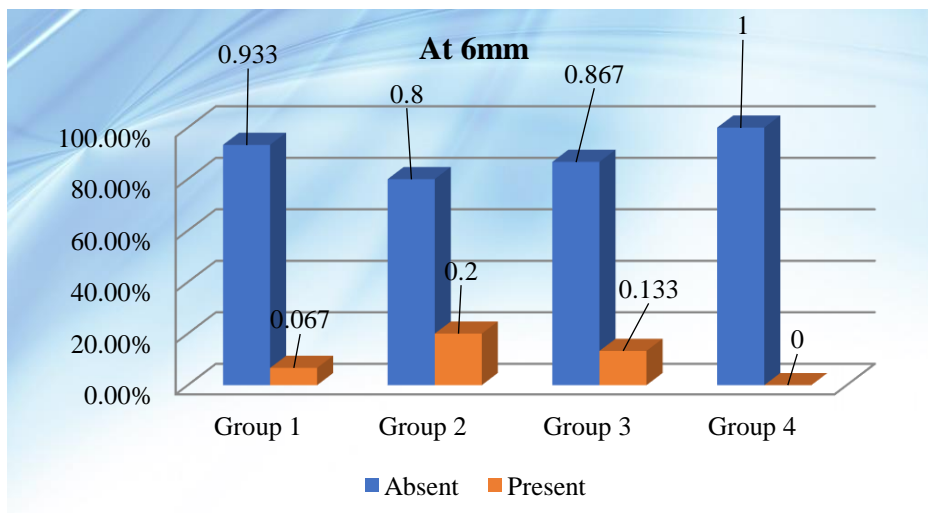
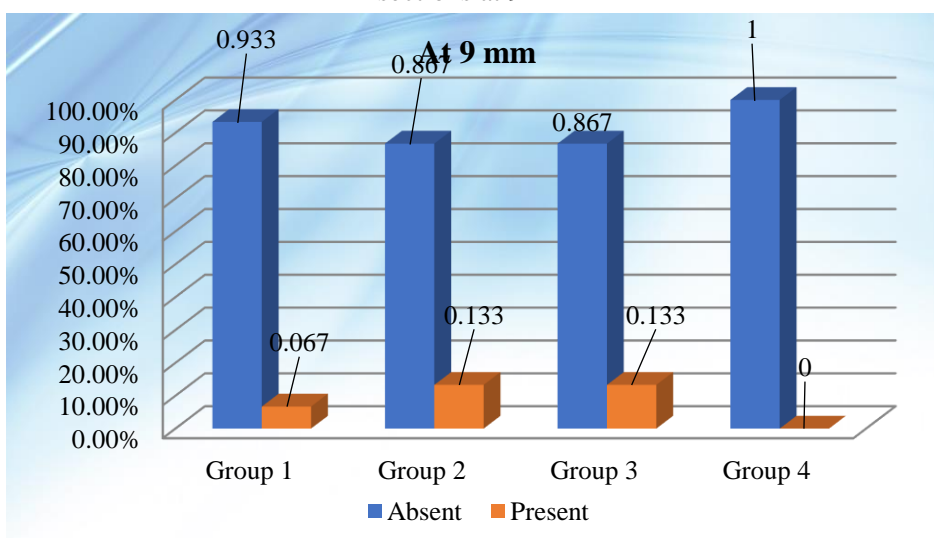


Table 3: Intergroup comparison of the number of teeth in which cracks were observed on the horizontal sections at 9 mm

			At 9 mm		Total
			Absent	Present	
Group	Group 1	N	14	1	15
		%	93.3%	6.7%	100.0%
	Group 2	N	13	2	15
		%	86.7%	13.3%	100.0%
	Group 3	N	13	2	15
		%	86.7%	13.3%	100.0%
	Group 4	N	15	0	15
		%	100.0%	0.0%	100.0%
Total		N	55	5	60
		%	91.7%	8.3%	100.0%
P value			0.494, ns		

Graph 3: Intergroup comparison of the number of teeth in which cracks were observed on the horizontal sections at 9 mm



DISCUSSION

Biomechanical preparation is an important step to achieve success in endodontic treatment. At times, dentine damage can occur during root canal cleaning and shaping. The contact between the instrument and canal walls during preparation creates momentary stress concentrations in the root dentin, which may lead to dentinal defects from which vertical root fracture can originate. Instrument features such as tip design, cross-sectional geometry, taper, pitch design, and flute form may be related to dentinal crack formation.¹⁴

Traditionally, root canal preparation was carried out using stainless steel endodontic files manipulated by hand. In recent years advances in rotary nickel titanium instruments have led to new design and techniques of root canal preparation. but the major drawback associated with rotary NITI instrumentation is the incidence of dentinal defects which further leads to vertical root fracture (VRF).¹⁵ When Ni-Ti rotary instrument are used, a rotational force is applied to the root canal walls. Thus, they can create micro cracks or craze lines in the root dentin. The extent of such a defect formation may be related to the tip design cross section geometry, constant or progressive taper type, constant or variable pitch and flute form.¹⁶

The stresses generated from inside the root canal are transmitted through the root to the surface where they might overcome the bonds holding the dentine together. Fracture occurs when the tensile stress in the canal wall exceeds the ultimate tensile strength of dentine.

Using Ni-Ti engine-driven instruments for root canal preparation has become the fundamental of endodontic treatments. These instruments have many advantages such as less operation time, increased cleanliness of root canal walls and fewer procedural accidents (apical canal transportation, perforations and ledges). These properties mostly stem from the increased flexibility of Ni-Ti alloy which helps in preservation of root canal curvatures.¹⁷

Freshly extracted teeth were used in the study to minimize its interference with crack formation outcome. Teeth were disinfected with 0.1% thymol solution and subsequently stored in distilled water to

prevent dehydration of samples, as dehydration can have impact on crack formation. Acrylic blocks and silicone impression material were used to simulate bone and PDL, respectively. PDL simulation acts as a stress absorber during preparation, thereby better mimicking the clinical situation.

Mandibular first premolars were used in our study due to their smaller dimensions and their thin dentinal walls that are more prone to the stress caused by shaping. This approach was applied since the likelihood of micro crack formation with large tapered files is higher in mandibular premolars than in other teeth.

TruNatomy has an off-centered parallelogram cross-section design with regressive taper. It has been argued that TruNatomy instruments maintain the remaining dentine and tooth intactness due to the instrument design, regressive tapers, and the thinned design, along with the heat treatment of the Ni-Ti alloy. According to manufacturer all files operate at higher speed with less torque:500 rpm and 1.5 Ncm torque run in continuous motion. with just two cutting edges it encounters less applied pressure, ensuring precision with increased ease of use. Thermal treatment provides greater flexibility with improved fatigue resistance.

One Curve (Micro-Mega, Besancon, France) is a heat-treated single-file system that was released in 2018. The heat-treated Ni-Ti alloy is called C. Wire. C. Wire technology is reported to provide a controlled memory and a pre-bending feature to the file for easier access to the root canal. Although One Curve files have a single tip size (size 25) and constant taper (6%), different shape designs are available. The variable cross-sections with a triangular-shaped at the tip of the instrument and S-shaped near the shaft are claimed to allow effective cutting and centered trajectory.¹⁰

HyFlex EDM files are controlled memory instruments, manufactured using a unique process called electrical discharge machining (EDM). Electrical Discharge Machining uses spark erosion to harden the surface of Ni-Ti files in the manufacturing process, which gives the files excellent flexibility and fracture resistance. In addition, thanks to the controlled memory, the risk of root transportation and perforation is reduced by

the files more easily following the root canal anatomy. HEDM uses three different cross sections: quadratic in apical third, trapezoidal in middle third and almost triangular in the coronal third¹⁸.

A crack is defined as the defect originating from the inner root canal space and propagating to the periphery. All other defects that did not originate from the canal wall, as craze lines were not considered as cracks.

In this study determination of the microcracks was investigated by stereomicroscope after teeth sectioning which can be a cause for dentinal defects. However, no cracks have been seen in the control group which means that the microcracks developed due to preparation and not tooth sectioning process. In the present study, all root canal shaping files, produced microcracks in root dentin. These findings are in accordance with Yoldas et al¹³. And Bürklein et al⁴, who found cracks in the root canals prepared by rotary NiTi instruments but not in the root canals instrumented with hand K file.

All tested files have proven to produce microcracks at all levels. The overall results have shown a statistically insignificant difference between the incidence of microcracks between the tested files. The highest incidence of the microcracks were present in the OneCurve group followed by the HEDM and the least with TruNatomy.

This study is in accordance with a study done by Cirakoglu N et al¹⁹, Jaju K et al²⁰, Johnson J et al²¹, Ozlek E et al¹⁸.

Cirakoglu N et al¹⁹ evaluated and compare the microcracks formation in apical root dentin as a result of root canal preparation with protaper next, protaper gold and TruNatomy files in which TruNatomy caused statistically significant fewer cracks than other two other rotary Ni-Ti system.

Jaju K et al²⁰ evaluate and compare the dentinal cracks after root canal preparation with Protaper Gold, TruNatomy, and Profit S3 in which TruNatomy files produced lesser number of cracks than other two files.

Johnson J et al²¹ Compare and evaluated formation of dentinal cracks using three NI-TI rotary files TruNatomy, XP Endoshaper, Protaper Gold, in which TruNatomy showed the statistically significant lowest percentage of cracks(7.8%) as

compared to other two file systems, XP Endoshaper(26.7%), Protaper(15.6%).

Ozlek E et al¹⁸ evaluate and compare the formation of dentinal cracks after root canal preparation with Reciproc Blue, OneCurve and, Hyflex EDM rotary files, in which Hyflex EDM showed statistically significant less dentinal cracks than other file systems used.

Lower dentinal crack incidence in the TruNatomy group may be attributed to the taper differences between TruNatomy and the other groups. It has been argued that TruNatomy instruments are less destructive for root canal system due to the regressive tapers and the heat treatment the Ni-Ti alloy. The slenderized pattern might have caused relatively fewer apical cracks in the TruNatomy system.

TruNatomy revealed the least number of microcracks. This can be referred to the instrument's geometry, i.e., off centered parallelogram cross section, regressive tapers, and slim design. These results comes in agreement with Yoldas et al¹³ who concluded that the number of microcracks formed following instrumentation is dependent on the file design features.

Pedulla E et al²⁴ compared the development of microcracks following root canal preparation using One Shape, F6 SkyTaper, Hyflex EDM, WaveOne, Reciproc, and WaveOne Gold in which less microcracks visible in Hyflex EDM and WaveOne Gold compared to other experimental group.

Different speeds and torques have been used for three-file systems according to manufacturer's instructions. According to Peter et al.⁷ and Capar et al²², the increased rotational speed is related to increased cutting efficiency and lesser crack formation.

Hyflex EDM and One Curve files evaluated in this study produced rotary motion and caused dentinal crack formation at the rate of 15.55% and 22.2%, respectively. Although they have the same kinematics, the reason for crack formation at different rates with these file systems is primarily because the recommended speed of Hyflex EDM is higher than that of One Curve. Therefore, we think that the reason why Hyflex EDM caused less dentinal crack formation compared to One Curve is that it has high cutting efficiency due to high speed¹⁸.

Taper angles of the files used in root canal preparation are effective on dentinal crack formation. Increasing taper angles cause higher stress in the canal wall, and therefore more dentinal cracks are formed. One Curve files (6% constant taper from the apical to the coronal region) produce more dentinal cracks because of being more tapered compared to Hyflex EDM.

Studies have shown that the files manufactured from M-Wire NiTi alloy have higher flexibility and higher cyclic fatigue resistance compared to conventional NiTi files. In addition, controlled memory files have been reported to be more flexible than both M-Wire NiTi alloy and conventional NiTi wire. HyFlex EDM and One Curve are two controlled memory files, one of which is manufactured by electrical discharge machining (EDM) method and the other is manufactured from C. Wire alloy.

In this study, more dentinal cracks were observed in the apical region compared to the middle and coronal regions with all tested files, and no statistically significant difference was found. The 3 mm apical part of the root is considered to be the most critical region in terms of the risk of dentinal crack formation during root canal shaping¹⁸.

In a study by *Sindi et al*²³, the occurrence of microcrack formations in the coronal level (9mm) was 33.3% and the same percentage in both the middle (6mm) and apical level (3mm). Similar to the results of the present study a study by *Pedullà et al*²⁴

showed that all the systems i.e one shape, skytaper, HyflexEDM, Wave one Gold, caused cracks, mainly in the apical section (3 mm). Similar results were reported by *Karatas et al*²⁵ and *Ustun Y et al*²⁶. Contrary to the results of the present study, studies by *Adorno et al*²⁷ and *Liu et al*²⁸ found out that the cracks in the coronal region were more abundant than the cracks in the apical region for all the three groups.

The occurrence of stress due to successive instrumentation, and low capability to the thin and fragile dentin in the apical area to the mechanical stress produced by direct contact with the instrument tip, may cause the formation of cracks. The tip design, cross-sectional geometry, pitch, taper, and flute of rotary instruments could affect the crack formation. Additionally, the use of a single-file system for root canal shaping causes more stress in the apical region, the most fragile part of the root.

Within the limitations of this study, it could be concluded that multiple factors cause dentinal cracks. Instrument features such as tip design, cross-sectional geometry, taper, pitch design, and flute form may be related to dentinal crack formation. The flexibility of NiTi instruments because of heat treatment seems to influence the incidence of microcracks more than other factors.

The possible limitations of our *in vitro* study are the sectioning method, difficulty in identifying internal pre-existing cracks, and the inability to standardize the speed and torque of the rotary files used.

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TITANIUM: A Breakthrough Metal in Dentistry

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Abstract

Titanium is a metallic element which has many attractive characteristics that make it desirable as a material for implants and other biomedical applications. Commercially pure titanium and its alloys are used for dental implants, surface coatings, and recently for crown, partial and complete dentures, and orthodontic wires. They are used in medical speciality for fabrication of prosthetic joints, surgical splints, stents and fasteners. With continuing evolution, Titanium has promising future in Dental and Medical field.

Key words- Titanium, Implant, Biocompatibility, Alloys

INTRODUCTION

Titanium is the ninth most commonly found and fourth most frequently found metal in earth but it is not found freely in nature. It is found mostly in the igneous rocks, its sediments and also from the minerals like ilmenite, leucoxene and rutile.

The ASTM (American Society of Testing and Materials) Standard F1295 Specifies titanium in different grades according to its purity, which is evaluated according to the amount of oxygen. Grade 1-4 refers to Commercially pure- Ti, but with different purity grades. Grade 5 refers to the three

combinations of aluminum (Al) and vanadium (V). Titanium-6Aluminium-4Vanadium (Ti-6Al-4V) and Titanium-6Aluminium-4Vanadium ELI (extra low-interstitial) (Ti-6Al-4V) are the two types of titanium alloys used in dentistry.

Titanium is known for its characteristics such as biocompatibility, excellent corrosion resistance, High rate of osseointegration, good shape memory, flexibility, Highest strength to weight ratio and high mechanical resistance.¹ For more than 25 years, titanium and its alloys have been used for both endosseous and subperiosteal implants and Wrought alloys of Titanium with Nickel and Titanium with Molybdenum are also used for orthodontic wires.

Different Properties of Titanium

Biocompatibility: Titanium highly non-toxic and does not cause any inflammatory or allergic reactions in the human body. This property makes it suitable for use in Dental implants.

Osseointegration: Titanium is one of the best metals that allows for this integration because it is absolutely inert in the human body, immune to attack from body fluids, compatible with the bone growth and strong and flexible.

Strength: Titanium has a tensile strength of between 30,000 and 200,000 lbs. per square inch. Titanium alloy contains roughly six weight percent of aluminium and four weight percent of vanadium, which doubles its tensile strength relative to commercially-pure titanium, but reduces its ductility. The yield strength (170 – 480 MPa) and ultimate strength (240 – 550 MPa) varies depending on the grade of titanium.

Corrosion: The titanium corrosion resistance in the oral cavity is due to the formation of an adherent and highly protective oxide film on its surface which is mainly formed of TiO₂.

Shape memory: The shape memory effect of nickel and titanium, which can be considered a breakthrough. This alloy was named as Nitinol (Nickel-Titanium Naval Ordnance Laboratory). It is widely used in the field of Orthodontics and Endodontics.

Flexibility: Titanium clasps are purported to have greater flexibility than cobalt-chromium cast clasps which should enable them to engage deeper undercut or be used where shorter clasp arms are needed such as on premolar teeth.

Density: The density of Commercially pure Titanium (4.5 g/cm³) which is about half of the value of many of other base metals. Titanium is lighter than the stainless steel (approximately 56% as dense) yet has a yield strength twice and ultimate tensile strength almost 25% higher.

Titanium Bonding: In the the oxidation effects of the porcelain-titanium interface reaction, the conventional degassing procedure is not suitable for porcelain-titanium restorations and that the firing cycle should be below 5000° to minimize the metallic oxide formation on the Ti surface.²

Passivation: Titanium oxidizes on contact with room temperature air and normal tissue fluids. This reactivity is favourable for dental implant devices. This is one important property consideration for the use of titanium in dental implants.³

Material	Nominal Surface Analysis (w/o)	Modulus of Elasticity, GN/m ² (psi μ 10 ⁶)	Ultimate Tensile Strength, MN/m ² (ksi)	Elongation to Fracture (%)	
Titanium oxide	99+Ti	97 (14)	240-550 (25-70)	15	Ti
Titanium oxide	90Ti-6Al-4V	117 (17)	869-896	>12	Ti
aluminum-vanadium					
Cobalt-oxide chromium-molybdenum (casting)	66Co-27Cr-7Mo	235 (34)	655 (95)	>8	Cr
Stainless oxide steel (316L)	70Fe-18Cr-12Ni	193 (28)	480-1000	>30	Cr
Zirconium oxide		97 (14)	552 (80)	20	Zr
Tantalum oxide		—	690 (100)	11	Ta
Gold	99+Au	97 (14)	207-310 (30-45)	>30	Au
Platinum	99+Pt	166 (24)	131 (19)	40	Pt

Table 1: Various types of properties of different materials used for dental implants

APPLICATIONS OF TITANIUM IN DIFFERENT SPECIALITIES OF DENTISTRY

Role In Prosthodontics

There are various applications of commercially pure titanium and it's alloys in different fields of Prosthodontics.⁴

In removable prosthodontics, commercially pure (CP) titanium and titanium alloys (Titanium-6Aluminium-4Vanadium and Ti-6Aluminium-7Neobdinium) have been used for cast partial denture frameworks. It was found in a study that clasps made from Ti alloy are able to maintain more of their retention than Co-Cr clasps and do not show permanent deformation, suggesting that it is a superior material for cast RPD clasps. Titanium has advantage of good retention than clasps made of other metals. Because of flexibility of titanium, it is used in removable prosthodontics.⁵

Titanium is also used in fixed partial denture for the metal ceramic prosthesis. Boening et al used the

milling technique which was combined with spark erosion to fabricate titanium copings. The study concluded that Ti-Ceramic bond passed the Duetshe Industrie Norm test but failed the ISO test. The different studies could not draw conclusion for long term serviceability of titanium in this field. This is due to the Titanium Bonding mechanism property.

Titanium is used in the branch of Maxillofacial Prosthesis for fabrication of cranial implants which are used as a mode of retention in eye and ear prosthesis. They are usually manufactured from Grade V pure titanium.

Commercial titanium-based dental implants undergo various surface treatments which are done by applying various methods such as machining, acid etching, anodization, plasma spraying, grit blasting or combination techniques yielding materials with smooth or micro-roughened surfaces. This is done for the Osseointegration of implant with the bone.⁶

• Subtractive treatments	• Additive treatments
Machined	Anodization
• Sandblasted	• Fluoride surface treatment
• Acid-etched surface	• plasma spraying Ti Hydroxyapatite (HA).
• Dual acid-etching	Coating sol-gel
• Sandblasted and acid etched surface (SLA)	• Sputter deposition
• Laser treatment	• Electrophoretic deposition
	• Biomimetic precipitation
	• Drugs incorporated

Table 2: Various types of surface treatments

Titanium is used in fabrication of implant abutments. Zygoma implant abutments are similar to multi-unit abutments which are made of commercially pure titanium (Ti6AL4V).⁷ Temporary abutments can also fabricated using titanium. They are used during the provisional phase, prior to the final restoration and are made of surgical grade titanium alloy (Ti6Al4V). They can be used in situations when temporization is required for a long period (>30 days). Nobel Biocare was the first company to introduce multi-unit abutments in the year 2001 and is designed for the restorations of partially edentulous, completely edentulous arches.⁸ Titanium has played a key role in field of Nanotechnology. Titanium nanostructures have exhibited great potential for better bone integration

and the regeneration properties. Designs of micro/nano-hybrid titanium surfaces have exhibited very superior implant surface properties.⁹

Titanium has been used in implant supported overdenture prosthesis for fabrication of various type of attachments used in it. The example of such is the Locator attachments.¹⁰

Mini dental implants are a type of dental implants with diameters less than 3.0 mm. They have advantages including reduced bleeding, less postoperative discomfort, shortened healing time, and immediate loading. They use titanium in it's fabrication process. In vitro and in vivo studies have shown that Ultrafine graded-Ti can promote adhesion and proliferation of the osteoblasts and it's excellent mechanical properties and

biocompatibility suggest that it is a promising material for mini dental implants.

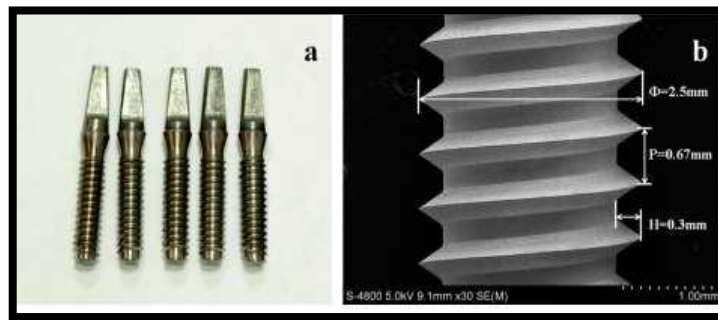


Figure 1: Ultra fine graded-Ti mini implants

Role in Endodontics

Titanium as various applications in the field of Endodontics. Nickel-titanium rotary instruments have become very popular for endodontic treatment because of their lower elastic modulus compared with stainless steel that enables these instruments to negotiate curved root canals with considerably lower likelihood for failure. This is because of shape memory effect of titanium. They have significantly improved resistance to clinical failure and manufactured by some process that minimizes the production of surface cracks, or nickel-titanium alloys with improved fracture toughness must be used.¹¹

Titanium is also used for fabrication of metallic posts that have density similar to gutta-percha.¹²

Role in Orthodontics

The introduction of the beta-titanium alloy in the 1970s provided a wire that had the formability similar to the austenitic stainless steel while delivering much lower biomechanical forces. Beta-titanium wires can also be welded, but they have a tendency to fracture and possess a high coefficient of friction.

Nickel-titanium (NiTi) orthodontic wires are especially well suited for clinical situations that require flexibility and exceptional elastic memory. They have a low stiffness, large working range, and produce very low forces and have limited formability, produce higher frictional forces, and cannot be soldered. The NiTi superelastic wire provides considerable deflection in reduced and relatively constant amounts of force.¹³

Mini implants are widely used in orthodontics nowadays that uses titanium for its fabrication.¹⁴

Titanium brackets have been introduced to overcome the deficiencies and disadvantages of the stainless steel brackets. It consists of pure titanium or a titanium alloy (Titanium-6Aluminium-4Vanadium) and has good biocompatibility and experiences less friction, which would aid in effectiveness of arch-guided tooth movement. The combined use of titanium brackets in combination with the use of acidic fluoride dentrifice and fluoridated foods is completely harmless for the bracket and does not cause corrosion.¹⁵

Role In Oral Surgery

Titanium has the desired mechanical properties to serve as an internal rigid fixation material due to a high degree of biocompatibility. The titanium plates and screws contribute to the internal fixation in maxillofacial osteotomies and fractures. The important properties a material needs to possess in order to fulfil its functions are tensile strength and hardness. Osteosynthesis treatment was improved once the titanium alloys were introduced in the maxillofacial fractures. Ti-6Al-4V (TAV), Ti-GAl-7Nb (TAN) and Ti-6Al-4V ELI (TAV ELI) are the most frequently used titanium alloys in the fabrication of miniplates and the orthopedic devices.¹⁶

Role In Periodontics

A barrier membrane is used primarily in implant, oral and periodontal surgery to prevent the epithelium from growing into an area where bone growth is desired and this method of preventing epithelial migration into a specific area is known as guided tissue regeneration (GTR).

Titanium-reinforced PTFE33 - Titanium reinforcement of PTFE allows shaping of these

membranes and can be used for the socket preservation procedures.

Titanium mesh - Titanium meshes were introduced in 1969 by Boyne and were mainly used to treat continuity defects in the maxilla and mandible. - The rigidity of titanium aids in space maintenance and prevents collapse of the contour. It also prevents graft displacement, permit bending, contouring, and adaptation to any bony defect and limit bacterial contamination due to its smooth surface. Some of the drawbacks of using titanium are: Increased stiffness of titanium sometimes leads to mucosal irritation Adapting a titanium mesh to a defect may require trimming, which may cause exposure of the membrane.

Applications in Medicine

Soft commercially pure titanium is used for cranial surgery where formability is very essential. The hard grades are available for bone plates and screws while the medium strength alloys such as IMI 31 (Ti-6Al-4V) are now the principal titanium materials for total joints such as the knee and the hip. Other areas of medicine where the high strength to weight characteristics of titanium are beneficial are in the manufacture of artificial limbs, in external fixation devices, and in wheel chair production.¹⁷

DISCUSSION

Research is being conducted to improve the properties of the Stainless Steel implants by coating with materials like Titanium Nitride (TiN) and Tungsten Carbide (WC). These methods have demonstrated improved values of wear resistance and hardness, thus improving the properties of the base (Stainless Steel 316L) metal.

The future of surface modification techniques would be to develop the nanoparticle and multifunctional coatings that would combine the advantages of different coatings and provide more favourable method of surface modification.

It has been proved that surface modification has potential to improve the performance of the Titanium implant, thus further research is necessary to develop the novel surface modification techniques which will produce an implant which will have superior biocompatibility, antibacterial property, corrosion and wear resistance.¹⁸

A novel method to rapidly deposit bone apatite-like coatings on titanium implants in simulated body fluid (SBF) has been proposed by Han et al. The processing was composed of two steps: micro-arc oxidation of titanium to form titania (TiO₂) films, and UV-light illumination of the titania-coated titanium in simulated body fluid.¹⁹

CONCLUSION

Due to the several possibilities of using titanium, it seems to be a promising material in modern Dentistry. Because of its physiological inertia, biocompatibility, corrosion resistance, and combination of strength and lightness; it can be considered a versatile and utile biomaterial which will probably increase its importance in dentistry.

A wide use of titanium in dental prosthesis will depend on the technological advancements and more laboratorial and clinical investigations in order to develop more profitable techniques proving its efficiency.

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Gingival Crevicular Blood as a Screening Tool for Diabetic Patient A Randomized Clinical Trial

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Abstract

Objective: To assess and evaluate the authenticity of Gingival Crevicular Blood for screening of diabetes mellitus.

Material & Methods: 53 patients; 27 known diabetic and 26 systemically healthy were enrolled in the present study design. Blood oozing from the gingival crevices of anterior teeth following periodontal probing and finger-prick blood was taken and was analyzed by glucometer.

Results: GCB reading ranged from 78-209 mg/dl with mean \pm s.d (89.2 ± 25.10); while F.P.B value was in the range of 58-190 with mean (123.7 ± 28.3); there was a significant difference obtained between the two values. ($p < 0.5$.)

Conclusion: The present study failed to validate use of GCB for testing blood glucose during routine periodontal examination

Keywords: Diabetes mellitus Gingival crevicular blood, Chronic periodontitis, finger prick blood.

INTRODUCTION

Diabetes mellitus is associated with a wide range of complications, such as retinopathy, nephropathy, neuropathy, micro-vascular and macro-vascular diseases, altered wound healing and periodontitis.¹

Moreover, diabetes and periodontitis seem to interact in a bidirectional manner.² At present, there is strong evidence to suggest that the incidence and severity of periodontitis is influenced in part by diabetes mellitus and the level of blood glucose

control.³ Moreover, periodontal therapy might exert beneficial effects on diabetes control.⁴

Diabetes mellitus is one of the most frequent metabolic disorders with an estimated prevalence of 7% in industrialized countries, of which nearly half the cases are undiagnosed.⁵ Patients with undiagnosed diabetes mellitus are at significantly increased risk for coronary heart disease, stroke and peripheral vascular disease.¹ In addition, recent data indicates that the incidence of the most common type of diabetes mellitus i.e. Type II, maybe increasing by up to 6% per year.⁶

Screening of diabetes at time of periodontal examination provides an additional venue to diagnose and reduce the diabetic burden of the society. Glucometer is routinely used by diabetics for accessing blood sugar levels, recently they have been employed to access the same from gingival crevicular blood.

Periodontal examination as a rule comprises of careful probing of periodontal pockets which result in some amount of bleeding from the gingival sulcus. Instead of swabbing and disposing the gingival crevicular blood this can be employed to assess blood glucose by glucometer.

Number of clinical trials has supported this view while others have refuted the same. Thus the aim of present trial was to evaluate the authenticity of using gingival crevicular blood as a potential site for blood glucose estimation in screening of diabetic individuals.

MATERIALS AND METHOD

53 patients; 27 known diabetic and 26 systemically healthy were enrolled in the present study. Subjects were recruited from outpatient department of Periodontics & Implantology, NIMS Dental College and Hospital, Jaipur after obtaining the clearance from ethical committee.

Inclusion Criteria

1. Diabetic patients with generalized chronic periodontitis diagnosed clinically with presence of periodontal pockets and radiographically with bone loss.
2. Non-diabetic patients with generalized chronic periodontitis diagnosed clinically with presence of periodontal pockets and radiographically with bone loss.

Exclusion Criteria

1. Patients with requirement for antibiotic premedication.
2. Patients with disorder that was accompanied by an abnormally low or high hematocrit. For example, Polycythemia Vera, Anemia, and Dialysis.
3. Patients with intake of substances that interfere with the coagulation system for example, Coumarin derivatives, Non-steroidal anti-inflammatory drugs or Heparin.
4. Patients with severe cardio-vascular, hepatic, immunologic, renal, hematological, or other organ disorders.

Present study was a double blinded randomized controlled trial. First examiner did the periodontal examination and assessed the diabetic history. Second examiner collected gingival crevicular blood glucose readings by collecting blood oozing from the gingival crevices of anterior teeth following periodontal probing with the stick of glucose self monitoring device. Third examiner performed finger prick to access blood glucose level. Glucometer was standardized by known sugar solution after every 10th reading. Patient were informed about the study and written consent was taken from them.

STATISTICAL ANALYSIS

Descriptive data are presented as Mean \pm S.D and range values. The difference between the measurements (Gingival Crevicular blood and finger-prick blood) in the same individual was tested by paired t test. Pearson's product moment correlation coefficient was found to assess the relationship between measurements. Simple linear regression analysis was performed to predict finger prick blood glucose level for any given gingival crevicular blood glucose level. A P-value of 0.05 or less was considered for statistical significance.

RESULTS

54 subjects were divided in two groups, Group 1 (diabetic group) and Group 2, (non- diabetic group). Mean difference between G.C.B and F.P.B reading of diabetic group was 31.8 and for non-diabetic group it was found to be 26.4. Range of 106-212 mg/dl for GCB and 68-190 mg/dl for FPB in diabetic group and a range of 132-78 mg/dl and 58-97 mg/dl respectively for GBB and FPB was found in non-diabetic group.

Mean \pm S.D was 141.5 ± 27.19 and 99.78 ± 29.95 for GCB and FPB respectively in diabetic group. For non-diabetic group Mean \pm S.D was 103.84 ± 12.56 and 77.44 ± 10.36 respectively for GCB and FPB.

A highly significant difference was found between the two groups of diabetic and non- diabetic. $P < 0.001$.

DISCUSSION

Diabetes has emerged as a major health problem in India. According to International Diabetes Federation every fifth diabetic in world would be an Indian by year 2025.⁷ Further the Asian Indian phenotype commonly known as thrifty genotype predisposes Indian population to risk of developing diabetes. It is important to appreciate that these disorders periodontitis do not initiate periodontitis but they may predispose accelerate or otherwise fasten its progression. It has been estimated that about one third of type 2 cases are undiagnosed and screening for undiagnosed type 2 DM is highly recommended.¹⁰

In addition, recent data indicates that the incidence of type 2 DM may be increasing by upto 6% per year.⁶ By this and the close interrelationship between diabetes and periodontitis, it can be assumed that the dental practitioner and especially the periodontists are extremely likely to encounter an increasing number of undiagnosed diabetes patients with periodontitis. The early diagnosis of diabetes however might help to prevent its long-term complications that are responsible for the high morbidity and mortality of diabetic patients.⁸

With regard to the development of painless and noninvasive methods to measure blood glucose, considerable effort has been made in past few years.⁶ However, until now, none are in routine clinical practice.⁸ Since periodontal inflammation with and without complication factor of diabetes is known to produce ample extravasate of blood during diagnostic periodontal examination, no extra procedure, e.g, finger puncture with a sharp lancet is necessary to obtain blood for glucometric analysis.⁹

Even in the case of very low gingival crevicular bleeding, a glucose measurement is possible with the use of self-monitoring device, due to the low amount of blood (μ l) necessary to perform the analysis.¹²

In present study difficulty was reported in collection of blood from gingival crevices, multiple probing was required to collect sufficient amount of blood, also most of the subject preferred finger prick and were comfortable with the same as compared to less gingival crevice blood collection.

In present study there was a significant difference between GCB and F.P.C values indicating both cannot be considered as same. Similar finding was reported in study by Muller 2004 who concluded that there is no usefulness of gingival crevicular blood as bleeding on probing was not sufficient in every third case.

On the contrary 10 examined diabetic patients with unknown periodontal status. and wherein a very strong correlation was observed between gingival crevicular, finger prick capillary and the corrected intravenous blood glucose measurements.¹¹ In another study a strong correlation was observed between GCB and finger stick capillary measured blood glucose when diabetic and non-diabetic patients with moderate to advanced periodontitis were examined.⁵

In the present study, the F.P.B showed consistently lower measurements compared to G.C.B blood glucose. Recently it has been shown that higher glucose level may be detected in gingival crevicular fluid of periodontally diseased site as compared to healthy which could explain the aforementioned difference obtained.

CONCLUSION

Present study failed to prove the authenticity of gingival crevicular blood as a screening tool in diabetes mellitus. The two values had significant difference implying that both cannot be considered as equivalent. Further study on a larger population is desirable to establish the much hyped relation.

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A Survey on Awareness of COVID19 Situation and Associated Practices Measure Among Dental Practitioners in Jaipur City, Rajasthan

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Abstract

Covid 19 is a life-threatening disease which has put complete world in a state of emergency. Thousands of lives are in dangerous and many have lost their lives. The aim of study was to assess the awareness of covid 19 situation and associated practices measure among dental practitioners in Jaipur city, Rajasthan. Total 315 participants were included in this study. A self-designed questionnaire was prepared using google form and that the participants can be reached via online. The questionnaire consisted of demographic statistics (5) and the (13) awareness questions. The statistical evaluation was done using SPSS Version 22.0 software. The values were represented in Number (%) and Mean±SD. Specific variables had been presented with the aid by using frequency and percentage. The present study included 315 subjects out of which 44.7 % were male and female percentage were 55.3%. Percentage of general practices was 89.4%. 85% participant considered wearing disposable masks were not enough to protect patient from transmission. 93.6% participant were doing screening for symptoms related to corona. Participant were using disposable PPE kits. 85.1 % knew about doffing of PPE kits according to biomedical waste. According to 70.2% teledentistry holds future post this pandemic. Majority of participant were affected financially almost 87.2%. The present study concluded that level of

awareness and knowledge of the dentist who are working as a general practices in Jaipur about the covid 19 pandemic and its sign symptoms and precautionary measures.

Keywords: Pandemic, Awareness, Teledentistry, COVID 19, Doffing.

INTRODUCTION

The word corona comes from Latin corona, that means "crown" and the morphologically it has spiky protrusion arising from their surfaces as seen in electron microscope. Name of corona virus was coined by June Almeida and David Tyrrell in 1966.¹

But was accepted in 1971 as a genus name by the International Committee for the word of Viruses that is later renamed International Committee on Taxonomy of Viruses². It has four genera:

Sr. No	Genera	Infected In	Origin	Natural Reservoir (Warm Blooded Flying Vertebrae)
1.	Alphacoronavirus	Mammals	2400 BCE	Bat
2.	Betacoronavirus		3300 BCE	
3.	Deltacoronavirus	Birds	3000 BCE	Bird
4.	Gammacoronavirus		2800 BCE	

Till now Seven strains of human coronaviruses are known. Out of that seven strain four strains produces minor symptoms and rest three produces severe symptoms (like Middle East respiratory syndrome-related coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) . COVID-19 is responsible of global pandemic. SARS-CoV-2 (COVID-19) was mainly identified in December 2019 in Wuhan China.

First case of COVID-19 in India was reported in the month of January 2020. Presently India has largest number of confirmed cases in Asia. Second largest number in world after USA³. COVID-19 pandemic has been a part of our daily lives since then. Airborne droplet, saliva or blood are possible mode of transmission of SARS-Cov 2^{4,5}. The dental procedures as well as the close contact with patients increases the risk of COVID-19 transmission^{4,5}. The disease has a prolonged incubation period ranging between 5 days to 14 days. Dentists, dental staff, and patients could be asymptomatic carriers at the time of the dental procedure, which could lead to cross-contamination of the disease.³⁻⁵ Because of this cross transmission there is a significant limitations of clinical practices in the fields of dentistry⁶. That's

why it represents a impactful measure on economy of this sector. Once dental clinic open, they should follow the proper practical protocol guideline which is recommended by CDC and ADA to control the spread of COVID-19. Hence, the current study is aimed to assess the awareness of COVID-19 situation and associated practices measure among dental practitioners in Jaipur city , Rajasthan.

MATERIALS AND METHOD

2.1. Ethical Clearance and Informed Consent

Ethical clearance was obtained from the concerned ethical committee of NIMS University Jaipur Rajasthan. Participates were informed about the questionnaire and written consent was obtained The data were kept confidential and the results did not identify the participates personally.

2.2. Study Population and Sample Size

The Present study was a cross-sectional(questionnaire) study. The study was conducted on dental practitioners as well as academicians in the Jaipur city Rajasthan state. The records regarding the dental practitioner, used to be amassed from IDA. A pilot study was done on 35 subjects for the study validity.

How much sample size is required for the study was calculated by this equation:

$$n = Z^2 t^2 - (\alpha/2) \times s^2 d^2$$

where Z is the standard normal score with 95% confidence interval (CI) ($\alpha = 0.05$), S is the standard deviation of the variable, and d is maximum acceptable error. Taking account of potential errors and sample loss, a final sample size was estimated to be 315.

2.3. Research Instrument and Protocol

A self-designed questionnaire written in English language was once made via a research specialist for the study. The questionnaire was once pre-examined for validity and was revised in accordance to remarks. The final questionnaire consisted of demographic statistics (5) and the (13) awareness questions. The questionnaire used to be made reachable the use of online mode as Google file and the hyperlink was circulated among the participate

using mail Id's and what's app. The principal investigator had access to the study data and the Responses from only those participants who gave consent by answering the questionnaire inside restrict time frame of three week have been covered in the study about.

Total awareness score was calculated primarily based on participants responses. Each negative response was once given "0" and positive response was given "1" and whole score of the participant was once calculated through adding the sum of all responses, that ranged from 1 to 20. The expected maximum total knowledge score was 20 and a minimum score of 0. Based on Bloom's criteria the sum scores, level of knowledge was:

S.No.	Level	Score
1	Low	0-7 (less than 60%)
2	Moderate	8-14 (60–80%)
3	High	15-20 (60–80%)

2.4. Statistical Analysis

The statistical evaluation was using SPSS Version 22.0 software. The values were represented in Number (%) and Mean±SD. Specific variables had been presented with the aid of using by frequency and percentage. ANOVA was used to find the significance of study parameter. The significance level was <0.05.

RESULT

Socio-Demographic and Professional Profile of the Participants

Socio-demographic and professional profile of the participants represented in graph 1. The present study included 315 subjects out of 55.3% of female participant rest were male. Most of participants (66%) are post graduate (MDS). Majority were doing general practice. Percentage of general practices was 89.4%.out of 315 participants only 10.6% doing academics.

Participant Response to Question on General Knowledge of COVID-19

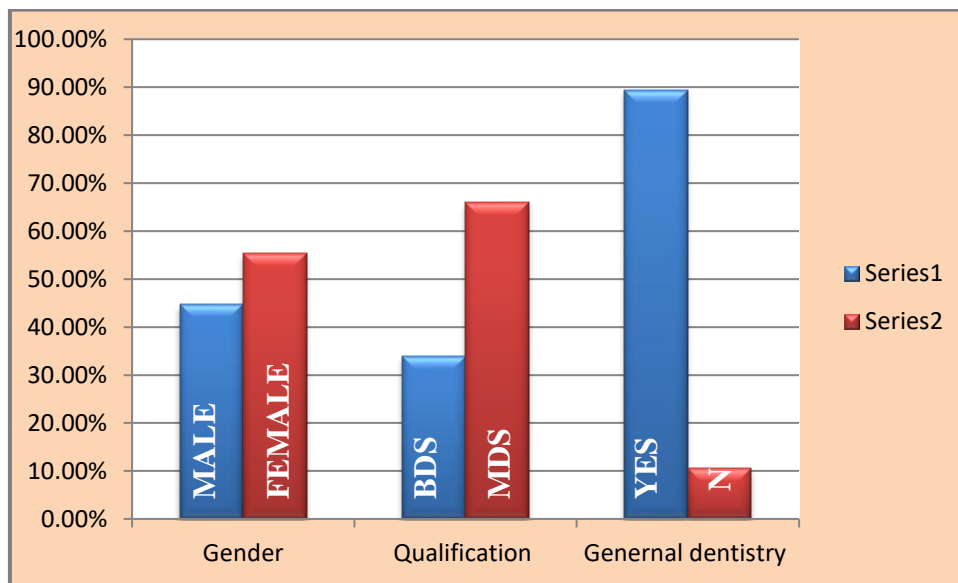
Participant response to question on general knowledge of COVID-19 was depicted in graph 2.

53.2% of participant were not aware about the virus. 95.7 % participants had knowledge about latest protocol of operating at dental clinic post pandemic. Everybody knew about various means of transmission of corona virus and they followed the disinfection protocol after operating procedure on a patient. According to the study consider the wearing 85% participant disposable masks were not enough to protect patient from transmission. 93.6% participant were doing screening for symptoms related to corona by using screening which provided by government. 89.4% of participant were using disposable PPE kits. Percentage of participant who were using separate PPE kits for different patients was 80.9%. 85.1 % knew how to about doffing of PPE kits according to biomedical waste. Everybody knew about hand hygiene protocol.

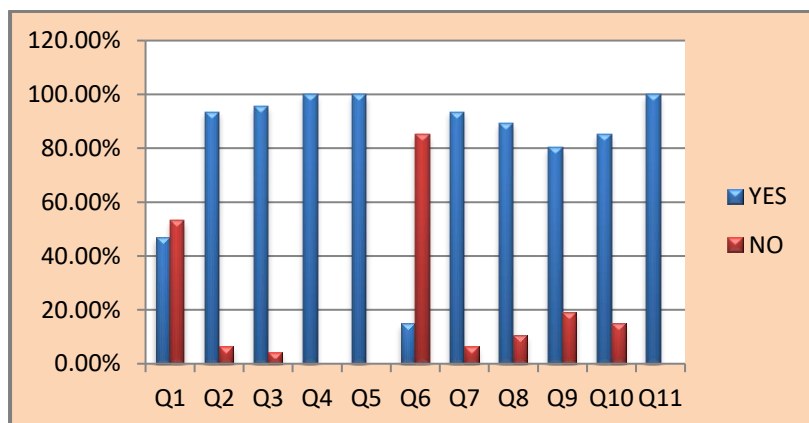
Participant response to question on future aspects of COVID-19

Participant response to question on future aspects of COVID-19 were depicted in graph 3.

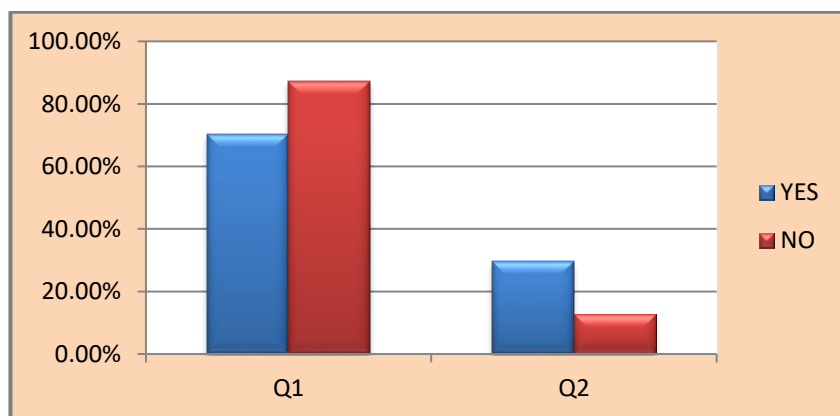
According to 70.2% teledentistry holds future post COVID -19 pandemic. Almost 87.2% participant were affected financially, whereas 12.8% did not get affected because of this COVID- 19 pandemic.



Graph 1: Socio-Demographic and Professional Profile of the Participants



Graph 2: Participant Response to Question on General Knowledge of COVID-19



Graph 3: Participant Response to Question on Future Aspects of COVID-19

Table 1 Knowledge Scores of Participants Regarding COVID-19 (Bloom's Criteria)

Knowledge Score	Number of Participant	Percentage	95% CI
HIGH	207	65.7	75.16-82.64
MEDIUM	105	33.3	32.25-37.17
LOW	03	9.5	05.87-12.12
TOTAL	315		

Knowledge scores of participants regarding COVID-19 (Bloom's criteria) Table 1 - 65.5 % of participant reported high knowledge regarding COVID-19. Only 9.5% participant did not know regarding COVID-19.

DISCUSSION

COVID-19 which is discovered from China has caused a serious problem for every individual on the earth. Due to this WHO declared it a global pandemic. This global pandemic has rampant spread. To stop this pandemic lockdown is only solution. Because of this lockdown many problem raises in the Indian society⁷. The aim of study was to assess the awareness of COVID-19 situation and associated practice measures among dental practitioners in Jaipur city , Rajasthan. This is a first study in the state examining the awareness and measure among the dental practitioners with a large sample size of 315. In this study we used a close ended questionnaire in order to get quick response from the participant . In our study female ratio (53%) is more then the male ratio .reason for more female dentist is because of less working hour and favorable condition⁸. Similar to our results Khade et al⁹. but according to bhagavawthala shows a equal male and female ratio¹⁰.

In our study percentage of MDS(66%) is more than the BDS but most of participant were general practitioners (89.4%) only (10.6%) doing specialty practices. Recently a study concluded that asymptomatic patients and who are in the incubation period are also carrier of the corona virus. Which can be easily direct transmitted. More than 90% of dentist aware of clinical sign and symptoms occurring due to corona. Everyone follows the hand

hygiene protocol after every procedure. This result similar to khander et al⁹.

Most of the dentists participating in this study reported that surgical masks would not provide sufficient protection (88.8%). Similar to the results obtained from this study, Ahmed et al¹¹. stated that 85% of the participants believe that surgical mask will not provide adequate protection. Surgical face masks provide approximately 80% filtration rate when worn correctly. The aerosolized particle sizes of COVID-19 are between 3-100 nm. Using an FFP3 mask provides a 99% filtration rate of all particles down to 0.6 µm in size.^{12,13}

In addition, everyone aware of recent protocols which is issued by CDC,ADA in the prevention of disease transmission. However, there are some loopholes in the knowledge with regard to the use of Personal protective Equipment (PPE) kits and the prophylactic medications and their toxicity. ONLY 85.1% participant know how to doffing PPE kit according to biomedical waste protocol. We are in a learning phase, and new information's are being updated every second. Poor knowledge has led to the dependence primarily on the clinical indicators to diagnose COVID19. These findings are similar to that reported in previous research carried out among dental practitioner by **Ahmed MA et al**¹¹. In our study 65.5 % participant knew regarding COVID-19, only 9.5% participant did not know about COVID-19 reason may be they are academicians. Almost all the participated in this study had the correct information about the incubation period of the virus, the cardinal signs of the disease (dry cough, dyspnea and fever), and the great importance of hand hygiene in the prevention of the disease. These results were similar to the results of the

Khader et al.⁹, Kamate et al.^{14,17} and Kinariwala et al.¹⁵

The government of India as well as state has suggested that lockdown is a only precautionary measure to prevent the infection. This lockdown is also includes the dental practice. Most of the dentists depend upon the clinical source of income. In addition to, once the clinic open there may be a decrease in the patients flow, ultimately affecting the monthly income. 87.2%% of the participants felt pandemic effect the financial growth in the dental practices. According to participant this pandemic holds a future for the teledentistry post this pandemic.

There are few issues in our study, cross-sectional nature of the study and restrained time frame of data collection. This could result in sampling error and therefore, our results might not have accurately reflected the true levels of awareness of dental practitioner across Rajasthan state.

CONCLUSION

The present study concluded that level of awareness and knowledge of the dentist who are working as a general practices in Jaipur about the COVID-19 pandemic fairly good and its sign symptoms and precautionary measures. However further studies required with larger sample size across the country.

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Conventional and Microwave Histo-processing of Soft Tissue Specimens: A Comparative Study

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Abstract

Background: Microwave-accelerated tissue processing is believed to have brought a revolutionary improvement in the field of histopathology and histochemistry. This technique shortens the time for tissue processing from days to minutes, allowing even more rapid histopathologic diagnosis. The aim of the study is to compare the macroscopic and microscopic quality of the microwave histoprocessing with that of conventional method and to determine its impact on turnaround time. Materials and Methods: There was nine types of normal and pathological soft tissue specimens. Each type of tissue was divided into seven sets and were further, cut into a size of 0.5x0.5x0.5cms. All the Sixty three specimens were processed by conventional method only and by domestic microwave in combination with conventional methods according to six protocols. Results obtained showed that the macroscopic and microscopic features of microwave processed tissue were similar to conventionally processed tissue and the correlation coefficient 'r' value was 0.764. Microwave assisted tissue processing reduced the total time for preparing tissue blocks to about an hour without compromising the overall quality of the histologic sections. Conclusion: Microwave stimulated processing provides an attractive alternative over traditional conventional processing.

Keywords: Microwave; histoprocessing; histologic quality; turnaround time.

INTRODUCTION

In order to preserve the structure of any tissue and impregnate them with a suitable media, they have to be adequately fixed and processed, so that thin sections can be made for staining and microscopic evaluation.⁽¹⁾ Examination of tissues under a

microscope requires a slice of tissue that is thin enough to transmit light, and the preparation of such thin slices is called section cutting or microtomy. The soft tissues must undergo preparatory treatment before being sectioned, which involves impregnation in a suitable

embedding medium to provide support and a suitable consistency for microtomy. This preparatory treatment is known as tissue processing.⁽²⁾

Tissue processing in histology is a physical process that involves chemical solutions reacting with biological specimens.⁽³⁾ Conventional tissue processing is as old as 100 years and still remains the gold standard against which all new technologies and methods need to be assessed. Although routine use of formalin fixation, an overnight dehydration, paraffin infiltration, manual embedding, and sectioning have served well in producing relatively good-quality tissue sections, it is the major bottle neck in the workflow of histopathological laboratories.⁽⁴⁾ It includes the aforementioned steps and is completed in 21-24hr. Advantages are its reliability and inexpensive nature. The disadvantages are that it is time consuming and the need to work with noxious chemicals.⁽⁵⁾ However this too means delay in report generation for one day or longer leading to delay in planning or institution of the treatment which is crucial in critically ill patients. Thus to reduce this turnaround time, various technology has been introduced in the field of histoprocessing.⁽⁶⁾

Rapid processing of histopathologic material is becoming increasingly desirable to fulfill the needs of clinicians treating acutely ill patients.⁽⁷⁾ As we moved into 21st century, the standard practice is now increasingly challenging because of the inability to meet the support required by current clinical demands. Because the routine manual histoprocessing remains laborious, time consuming, and requires toxic chemicals, alternative methods such as microwave⁽⁸⁾ tissue processing are the "future ray of hope".⁽⁹⁾

Microwave which invented by Percy Spencer in 1945 are becoming an integral part of our lives.⁽¹⁰⁾ Although used in food processing, chemical, pharmaceutical and many other industries for many years, it was Kok and Boon from Netherland and Antony Leong from Australia who advocated microwave heating for fixation and processing of the tissue in the late 1980's.⁽⁴⁾ In this process, the penetrative properties of the microwave and the conversion of this incident energy into heat, is made use of, the advantages include shorter

processing times, eliminating noxious chemicals like xylene and lesser degree of denaturation of nucleic acids.⁽⁵⁾ Thus, a novel histoprocessing method for paraffin section was developed and fast processing was possible due to stimulated diffusion of the heated reagent.⁽⁴⁾

Microwave causes heating within a material by exciting molecules to rotate. The rotation produces energy in the form of heat. Heat reduces the viscosity of liquids, thereby increasing the rate of diffusion of reagents into and out of the tissue. Unlike conventional heating, the effect occurs simultaneously throughout the whole material being microwaved ('internal heating'). This resulted in substantial reduction in each of the basic steps of histoprocessing, thereby reducing turnaround times and permitting same day diagnosis for a variety of types of tissue biopsy specimens.⁽¹¹⁾

Hence the present study was carried out to document the usefulness of microwave-assisted tissue processing and to compare the turnaround time and histologic quality with that of the conventional method.

MATERIALS AND METHOD

The soft tissue specimens were selected from the Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Bareilly, (UP). Normal and pathological soft tissue specimens were included and hard tissue specimens were excluded from the study. A total of sixty three normal and pathological soft tissue specimens from which seven each of salivary gland, muscle, lymph node, adipose tissue, stratified squamous epithelium & connective tissue, Odontogenic cyst, odontogenic myxoma, oral squamous cell carcinoma and fibroma were included in the study.

Procedure: All the tissue specimens were cut into a standard size of 0.5×0.5×0.5 cm and each specimen was measured before and after tissue processing using a graph & metric scale in order to identify the shrinkage. Fixation of all the tissue specimens was carried out with 10% buffered formalin.⁽⁸⁾ The 200ml of chemical reagents were used for processing of all the tissue specimens. The tissue specimens was processed using only conventional, only microwave and the combination of conventional and microwave processing

techniques. Later the formalin fixed paraffin embedded tissue block were prepared and microtomy was carried out followed by hematoxylin & eosin staining,⁽¹²⁾ (Figure 1). The slides were then histopathologically evaluated for quality of tissue sections, tissue architecture, staining quality, nucleus and cytoplasmic differentiation and for overall quality of diagnosis.⁽³⁾ (Figure 3-10). A review of turnaround times for tissues processed under different protocols were also evaluated.

The Conventional / Manual Tissue Processing:

The tissue specimens were processed by conventional method according to the criteria of Bancroft JD.⁽¹²⁾ All the processing was done at room temperature, except for the impregnation and embedding, which were carried out at 56°C followed by section cutting and H & E staining. (Table 1)

Microwave Tissue Processing: A domestic microwave (INALSA: EG8021TP-AN) which had all programs fully loaded to run at appropriate time and predetermined power was used. In order to absorb the excess heat generated by the microwave, a second beaker containing a water load of 200ml was used in all the procedures next to the beaker with the tissue where the temperature was in the range of 45°C-58°C. According to Klump et al.⁽¹³⁾ and Prasad GK et al.,⁽¹⁴⁾ fixation in microwave was carried out with 10% buffered formalin for 15 min at power of 30%. Dehydration was carried out with 100% Iso propyl alcohol for 15 min at power of 50%. Xylene was used as clearing agent for 15 min at power of 50%. The paraffin impregnation took 15 min at power of 50%. The tissue specimens were then embedded in paraffin, cut with rotary microtome of 4-6µm thick and stained with haematoxylin and eosin.⁽¹²⁾ According to Mathai AM, et al.,⁽¹¹⁾ a combination of conventional and microwave tissue processing was adapted using various protocols. (Table 2)

Histopathological Evaluation: All the slides were microscopically evaluated and scored using a customized evaluation form under the microscopic parameters listed below:

- **Quality of Tissue Sections:** According to the modified criteria of Prasad GK, et al.,⁽⁴⁾ the cellular morphology was classified as

Interpretable (score 2) on the basis of greater eosinophilia of cytoplasm producing enhancement of the nuclear cytoplasmic contrast, good stroma, whether secretory products are appreciable, absence of red blood cell lysis, and whether differentiation can be made between inflammatory cells.⁽¹⁵⁾ If there was granularity of cytoplasm,⁽¹⁵⁾ focal condensation of stroma, cellular outline blurred,⁽¹⁶⁾ mucin was not seen, red blood cells lysed (focal or generalized),⁽¹⁵⁾ and no differentiation could be made between inflammatory cells then it was classified as **Intermediate** (score 1). If none of the features were present, then it was convinced as **uninterpretable** tissue section (score 0).

- **Tissue Architecture:** All the tissue sections was assessed using modified criteria by Babu M, et al.,⁽⁷⁾ and Patil S, et al.,⁽¹⁾ for the parameters like cellular clarity, cytoplasmic details, nuclear details, color intensity and interface of epithelium & connective tissue under the grading of **Not maintained** (score 0), **Intermediate** (score 1) and **Well maintained** (score 2) tissue architecture.
- **Quality of Staining:** According to the modified criteria of Prasad GK, et al.⁽⁴⁾ staining of tissues was evaluated as poor, non-uniform, and uniform. **Poor** (score 0) indicates that the tissue failed to take up stain adequately, stained unevenly or had artifacts in processing or staining. **Non-uniform** (score 1) indicates that details were not visualized up to the mark, but slide was suitable to give diagnosis. **Uniform** (score 2) means good contrast between the nucleus and cytoplasm, and visibility of details along with brilliance of staining.
- **Nucleus and Cytoplasmic Differentiation:** According to the modified criteria of Prasad GK, et al.,⁽⁴⁾ slides were evaluated on the basis of chromatin condensation, prominent nuclear membrane, and crisp staining of the nucleus and mitotic activity, if appreciable. It was graded as **Good** (score 3) if all features were appreciated, as **Average** (score 2) if smudging and pyknosis of nuclei were seen,⁽¹⁷⁾ as **Poor** (score 1) in case of indistinct nuclei and as **Not**

seen (score 0) when the nuclei are not appreciated.

- **Overall Quality of Diagnosis:** The scoring was done according to the modified criteria described by Boon et al.,⁽¹⁸⁾ and Ayala et al.,⁽¹⁹⁾ as **Poor** (score 0) if the tissue was not clearly demonstrated (Not good for microscopy), **Average** (score 1) if tissues not very well demonstrated, but can be used for microscopy and as **Good** (score 2) if the tissue was clearly demonstrated.

RESULTS

The comparison was carried out on different parameters like tissue shrinkage, histopathological evaluation and turnaround time.

Tissue Shrinkage: The Pre- and post processing soft tissue specimen shrinkage ranged from minimum of 0.0mm to a maximum of 0.2mm among all the study groups except in protocol IV which was 0.1mm. The mean and standard deviation values of tissue shrinkage in pre-processed tissues was 0.5 ± 0.0 in all the study groups, whereas post-processed tissue in protocol I showed 0.467 ± 0.071 , in protocol II was 0.400 ± 0.087 , in protocol III was 0.422 ± 0.083 , in protocol IV was 0.478 ± 0.044 , in protocol V was 0.467 ± 0.071 , in protocol VI was 0.456 ± 0.088 , and in conventional group was 0.444 ± 0.073 . Upon statistical evaluation Protocol II, III and conventional group showed a statistical significance with the p value of 0.008, 0.023 & 0.050 respectively, whereas protocol I, IV, V and VI showed a non-significance results with p value being 0.195, 0.169, 0.195 and 0.169 respectively. (Figure 2)(Table 3; Graph 1)

Histopathological Evaluation: (Figure: 2 & 3) The microscopic assessment between Conventional and Microwave Tissue Processing under Protocol I showed a non-significant p value of 0.159 for quality of tissue, 0.331 for tissue architecture, 0.094 for quality of staining, 0.360 for nucleus and cytoplasmic differentiation and 0.427 for overall quality of diagnosis. Whereas under Protocol II a non-significant p value of 0.331 for quality of tissue, 0.056 for tissue architecture, 0.396 for quality of staining, 0.360 for nucleus and cytoplasmic differentiation and 0.556 for overall quality of diagnosis was noted. (Table 4)

The microscopic assessment between Conventional and Microwave Tissue Processing under Protocol III showed a non-significant p value of 0.535 for quality of tissue & tissue architecture, 0.113 for quality of staining, 0.427 for nucleus and cytoplasmic differentiation and 0.696 for overall quality of diagnosis. Whereas under Protocol IV, a non-significant p value of 0.331 for quality of tissue, 0.159 for tissue architecture, 0.499 for quality of staining, 0.193 for nucleus and cytoplasmic differentiation and 0.311 for overall quality of diagnosis was noted. (Table 4)

The microscopic assessment between Conventional and Microwave Tissue Processing under Protocol V showed a non-significant p value of 0.136 for quality of tissue & tissue architecture, 0.936 for quality of staining, 0.185 for nucleus and cytoplasmic differentiation and 0.427 for overall quality of diagnosis. Whereas under Protocol VI showed a non-significant p value of 0.539 for quality of tissue, 0.609 for tissue architecture, 0.455 for quality of staining, 0.100 for nucleus and cytoplasmic differentiation and overall quality of diagnosis. (Table 4)

Correlation Coefficient for protocols I, II, III, IV, V, & VI of microwave with conventional tissue processing was 0.542, 0.389, 0.732, -0.142, 0.123 & 0.764 respectively. (Table 5)

Turnover Time: The turnover time for various protocols of I, II, III, IV, V, VI and conventional tissue processing were 7hrs 15mins, 13hrs 30mins, 18hrs 15mins, 1hr 45mins 12hr 45mins, 1hr and 19hrs respectively. (Table 6)

DISCUSSION

For decades, instrumentation used in tissue processing remained relatively unchanged. A recent addition in the list of techniques involved for rapid processing of tissues is the use of microwaves, which has revolutionized histotechniques.⁽¹⁰⁾ Microwaves are the electromagnetic wave that can penetrate various types of materials. Their penetration depth is dependent on the electric conductivity of the medium. Upon penetration into tissues, the energy is absorbed by the molecules.⁽²⁰⁾ The usage of microwave provides with a shorter processing time, and lesser degree of denaturation of nucleic acids. Also, domestic microwave are readily available, affordable and have provided

appreciable results in previous studies.^(2, 4, 7, 10, 18, 21-23) The present study was carried out to compare and evaluate, if various protocols using domestic microwave was useful and better alternative to conventional tissue processing.

In the present study, the tissue shrinkage noted in both conventional as well as in microwave technique was almost similar. The results were in concordance with that of Kok, et al.,⁽²⁰⁾ and Panja P, et al.⁽⁵⁾ However, contrasting results were observed by Kayser K, et al.,⁽²⁴⁾ who identified tissue processed by microwave showed excess shrinkage as compared to conventional processing. This could be due to the heat generated by microwave oven⁽²⁴⁾ or the concentration gradient between the fluids inside & outside the tissue, diffusion current crossing the cell membrane during the fluid exchange increases the possibility of tissue shrinkage.⁽¹²⁾

In the present study, upon microscopic assessment, when quality of tissues was statistically compared between conventional and microwave processing methods, it showed a non-significant difference. It was similar to the studies conducted by Morales et al.,(2002 & 2004)^(22, 25) and Mathai AK, et al.⁽¹¹⁾ Thus microwave processed tissue sections had similar nuclear cytoplasmic contrast, with good erythrocyte integrity and lymphocyte appearance as that of conventional method. The contrasting results were observed in the study performed by Patil S, et al.,⁽¹⁾ and Babu T, et al.⁽⁷⁾ The microwaves stimulate the polar molecules causing collision with the adjacent molecules which causes part of the rotational energy to be transferred through them producing heat. This effect occurs simultaneously throughout the whole material being microwaved.⁽²⁶⁾

In the present study, upon microscopic assessment, when tissue architecture was statistically compared between conventional and microwave processing methods it showed a non-significant difference. It was similar to the studies conducted by Kango GS, et al.,⁽⁴⁾ Boon et al.,⁽¹⁸⁾ Morales et al.,(2002 & 2004)^(22, 25) and Chaudhari et al.,⁽¹⁶⁾ who found that stroma, secretory products, as well as cellular and nuclear morphology were identical between conventionally and microwave processed tissue. As the mechanism of microwave heating depends on

oscillating or exciting polar or charged molecules in the tissue. Alternating electromagnetic fields are produced which cause polar molecules of proteins to rotate through 180° at 2.45 billion cycles per second. This result in generation of instantaneous heat that is proportional to the energy flux and continues until the radiation ceases.⁽²⁶⁾

The quality of staining in the microwave and conventionally processed tissue did not show any significant variation in the present study. This was in consonance with the findings of Boon et al.⁽¹⁸⁾ Chaudhari et al.,⁽¹⁶⁾ Morales et al.,(2002 & 2004)^(22, 25) Panja et al.,⁽⁵⁾ Zenobia et al.,⁽²⁷⁾ Galvez et al.,⁽²⁸⁾ Suri et al.,⁽²⁹⁾ Leong et al.,⁽³⁰⁾, Rohr et al.,⁽²³⁾ and Kok et al.,(1988 & 1990).^(20, 31) This can be attributed to uniform distribution of heat, chemical reagents and effective dehydration seen in the microwave technique.⁽⁷⁾

In the present study, the nuclear and cytoplasmic differentiation was similarly observed in tissues when processed by microwave and with conventional processed tissue. Contrastingly, Patil S et al.,⁽¹⁾ Boon et al.,⁽¹⁸⁾ Panja P et al.⁽⁵⁾ and Babu, TM et al.,⁽⁷⁾ in their study found better quality of cellular and nuclear details when processed by microwave technique. In this process, the penetrative properties of the microwave converts the incident energy into heat, there by creating a uniform environment for the chemical reagents to perform the work, and enhances the results, observed in microwave tissue processing.⁽⁷⁾

In the present study, the overall quality of diagnosis for tissue processed with conventional and microwave method under protocol VI were indistinguishable. This was similar to the findings of Mathai AK et al.,⁽¹¹⁾ whereas contrasting results were observed by Babu, TM et al.,⁽⁷⁾ in their study, where overall quality of microwave-processed tissue appeared slightly better than routinely processed and routinely stained slides.

In the present study, when the microwave tissue processing method by various protocols were intercompared, it was observed that in Protocol VI, where all the steps was carried out only in microwave was best among them. Similar findings were observed by Sivadas P et al.,⁽³²⁾ and Mathai AM et al.⁽¹¹⁾ In this process, the penetrative properties of the microwave and the conversion of

this incident energy into heat is used. The increased rate of processing is ascribed to the increased rate of diffusion. Diffusion is a key factor in histoprocessing, permitting chemicals to infuse into the tissue faster. Increased temperatures decrease the viscosity of the processing fluids and thereby facilitate diffusion.^(2, 22)

The turnover time estimated was minimum in microwave protocol VI method of just one hour as compared to maximum of 19 hours in conventional processing. Various studies like Babu et al.,⁽⁷⁾ Panja et al.,⁽⁵⁾ Mathai AM et al.,⁽¹¹⁾ Shashidhara et al.⁽²¹⁾ and Kongoet al.,⁽⁴⁾ have also used a similar methodology with consistent results. For routine purposes, it is often desirable to obtain the paraffin sections in a few hours, but this is not possible with the conventional method. With the help of microwave it is theoretically possible to speed up tissue processing through the use of heat and it was possible to run several short cycles of about 1hr each, during the working day so that stained sections were available on the same day as the specimens were received.⁽²⁵⁾

We believe that rapid microwave assisted tissue processing is an optimal method for substantially reducing the turnaround time and permitting the

histopathology laboratory to provide same day diagnosis for a variety of tissue biopsy specimens.

The merits of microwave histoprocessing have surpassed the routine conventional protocol in many ways like: Being less labor intensive and facilitating rapid diagnosis. Tissue processing using a microwave is cheaper and using the domestic microwave instead of a highly expensive commercially available microwave further reduces the cost. By this innovative method, pathologists can now offer an early final diagnosis which eventually results in a more efficient and better management of patients. Since the only equipment required for this method in histopathology is a microwave oven, the technique is considered highly suitable for hospital laboratories as well as research laboratories where histological materials are routinely processed.

We strongly believe in a famous quote “A stitch in time saves nine” and hence we have made an attempt toward faster, reliable, cost-effective diagnosis and timely institution of treatment for better health care.

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Processing steps	Reagents	Time
Fixation	10% buffered formalin	Over night 720 mins (12hrs)
Dehydration	Isopropyl Alcohol 70%	60 mins (1hr)
	Isopropyl Alcohol 80%	60 mins (1hr)
	Isopropyl Alcohol 90%	60 mins (1hr)
	Isopropyl Alcohol 100%	60 mins (1hr)
Clearing	Xylene	60 mins (1hr)
	Xylene	60 mins (1hr)
Impregnation	Paraffin wax	30 mins(1/2hr)
	Paraffin wax	30 mins (1/2hr)
Total duration	1140 minutes (19 hours)	

Table 1: The conventional method of tissue processing

Steps	Protocol I	Protocol II	Protocol III	Protocol IV	Protocol V	Protocol VI
Fixation	Microwave	Conventional	Conventional	Microwave	Conventional	Microwave
	15 min	Over night	Over night	15min	Over night	15min
Dehydration	Conventional	Microwave	Conventional	Microwave	Microwave	Microwave
	Iso propyl alcohol 70%, 80%, 90%, & 100%	Iso propyl alcohol 100%	Iso propyl alcohol 70%, 80%, 90% & 100%	Iso propyl alcohol 100%	Iso propyl alcohol 100%	Iso propyl alcohol 100%
	1 hour each	15mins	1 hour each	15mins	15mins	15mins
Clearing	Conventional	Microwave	Conventional	Microwave	Microwave	Microwave
	Xylene I & II	Xylene	Xylene I & II	Xylene	Xylene	Xylene
	1 hour each	15mins	1 hour each	15mins	15mins	15mins
Wax Impregnation	Conventional	Conventional	Microwave	Conventional	Microwave	Microwave
	Paraffin I & II	Paraffin I & II	Paraffin	Paraffin I & II	Paraffin	Paraffin
	30min each	30min each	15min	30min each	15min	15min
Duration	7hr 15min	13hr 30min	18hr 15min	1hr 45min	12hr 45min	1 hour

Table 2: The Microwave tissue processing protocols

Study Groups	Pre-Processing	Post- Processing	p Value
	Mean±Std. Deviation	Mean±Std. Deviation	
Protocol I	0.50±0.0	0.467±0.071	0.195
Protocol II	0.50±0.0	0.400±0.087	0.008
Protocol III	0.50±0.0	0.422±0.083	0.023
Protocol IV	0.50±0.0	0.478±0.044	0.169
Protocol V	0.50±0.0	0.467±0.071	0.195
Protocol VI	0.50±0.0	0.456±0.088	0.169
Conventional	0.50±0.0	0.444±0.073	0.050

Table 3: The mean±standard deviation shrinkage values of pre- & Post-processed tissues

Microscopic Assessment	p Value of Conventional Vs Different Protocols					
	I	II	III	IV	V	VI
Quality of Tissue	0.159	0.331	0.535	0.331	0.136	0.539
Tissue Architecture	0.331	0.056	0.535	0.159	0.136	0.609
Quality of Staining	0.094	0.396	0.113	0.499	0.936	0.455
Nucleus and Cytoplasmic Differentiation	0.360	0.360	0.427	0.193	0.185	0.1000
Overall Quality of Diagnosis	0.427	0.556	0.696	0.311	0.427	0.1000

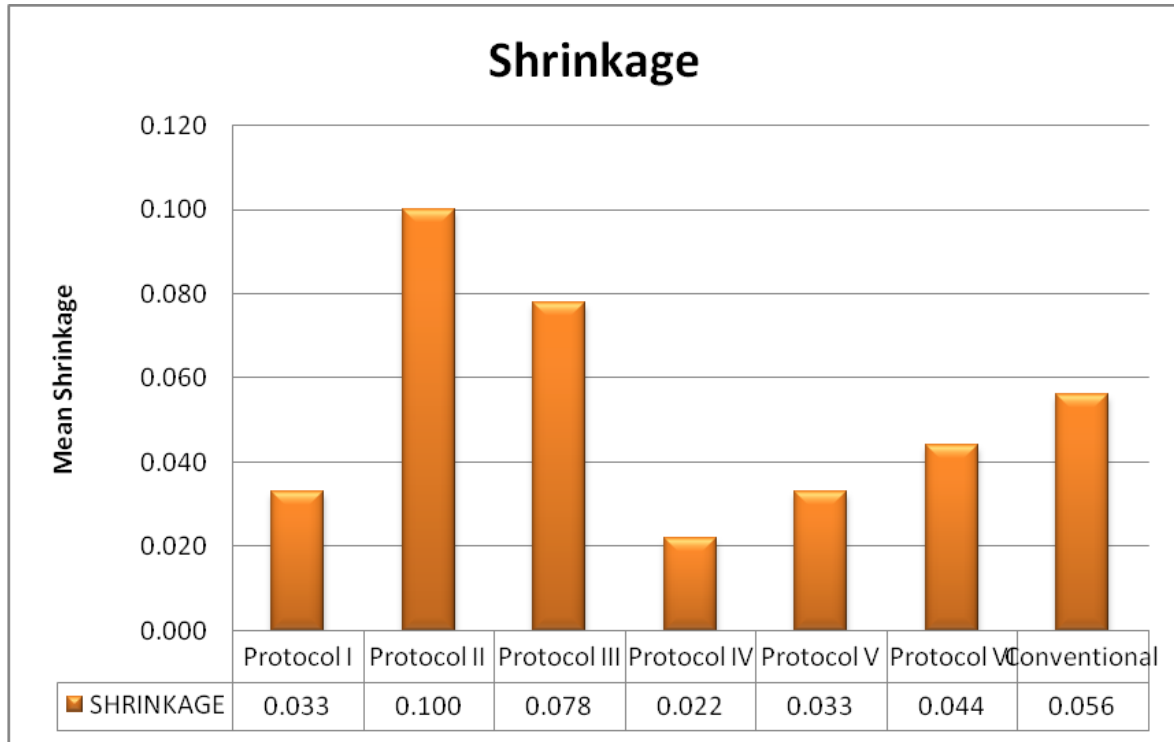
Table 4: Statistical analysis of microscopic assessment of tissue processed by conventional when compared to various protocols processed by microwave

Study Groups	Correlation Coefficient 'r'
Protocol I	0.542
Protocol II	0.389
Protocol III	0.732
Protocol IV	-0.142
Protocol V	0.123
Protocol VI	0.764

Table 5: Correlation Coefficient for various protocols of microwave with conventional tissue processing

Study Groups	Turnover Time
Protocol I	7 hr 15 Min
Protocol II	13 hr 30 Min
Protocol III	18 hr 15 Min
Protocol IV	1 hr 45 Min
Protocol V	12 hr 45 Min
Protocol VI	1 hr
Conventional	19 hr

Table 6: The turnover time for various protocols of microwave and conventional tissue processing



Graph 1: The mean±standard deviation values of tissue shrinkage among the study groups

Figure Legends

Figure 1: The pre and post processed tissue specimens being evaluated for its shrinkage

Figure 2: The photomicrograph showing the normal salivary glands under microwave different protocol tissue processing (H&E X10)

Figure 3: The photomicrograph showing the squamous cell carcinoma under microwave different protocol tissue processing (H&E X10)

Caries Progression and Prevention Strategies: Demineralization vs Remineralization' - A Review Article

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Abstract

Dental caries involves interactions between the tooth structure, the microbial biofilm formed on the tooth surface and sugars, as well as salivary and genetic influences. The dynamic caries process consists of quickly alternating phases of tooth demineralization and remineralization, which culminates in the beginning of particular caries lesions at certain anatomical predilection spots on the teeth if net demineralization happens over a long period of time.

The remineralization process is a natural healing mechanism that returns minerals to the hydroxyapatite (HAP) crystal lattice in ionic form. The caries process is a continuous series of demineralization and remineralization cycles. We highlight and briefly discuss some of the most recent advancements in remineralization therapy in this article. Most modern medicines strive to boost fluoride's effectiveness by including additional potentially active substances such calcium, phosphate, stannous, xylitol, and arginine in the formulation. Other remineralization approaches have focused on forming remineralizing scaffolds inside the lesions (e.g., self-assembling peptides), while some innovative remineralization methods like use of lasers and biofilm modifiers etc., have made substantial development in recent years, the data for the majority of them is still insufficient to determine their genuine therapeutic value.

Keywords: Demineralization, Remineralization, Nanoparticles, Fluoride releasing agents, CPP-ACP, CPP-ACPF.

INTRODUCTION

Dental caries is a complex, dynamic disease caused by biofilms that causes phasic demineralization and remineralization of dental hard tissues. Caries can affect the tooth crown and, later in life, exposed root surfaces, and can develop in both primary and permanent dentitions. Caries start and development are influenced by the balance of pathogenic and protective factors. Pathological variables change the balance in the direction of dental caries and disease development, whereas protective factors promote remineralization and lesion arrest.^{1,2}

The process of introducing minerals from the surrounding environment (saliva, biofilm) into partially demineralized tooth structures is known as dental remineralization. Remineralization can cause amorphous mineral precipitates in the intercrystal and interdod gaps, or it can replace minerals in partly demineralized enamel and dentin (Cochrane NJ, 2010). Remineralization might take either spontaneously or as a result of therapy. Fluoride – based treatments have the greatest degree of supporting evidence among the various therapies (Amaechi and van Loveren 2013; Fontana 2016), and widespread use of fluorides is often regarded as the primary cause of dental caries reduction in most populations. However, currently available F treatments have limited efficacy in some people, and the benefit of F in lowering dental caries prevalence has reached a plateau at the community level (Haugejorden and Magne Birkeland 2006). As a result, researchers have been working on novel remineralization treatments to bridge the efficacy gap. The majority of novel techniques have been developed to complement rather than replace established F therapies, although those that do not use F might offer an alternative for patients who are hesitant to utilise F.³

Remineralizing treatments now include the following:

1. Mineral saturation-increasing compounds, such as fluorides, CPP-ACP, CPP-ACPF, TCP, CSP; bioactive glass containing Calcium Sodium Phosphosilicate (Novamin), Sodium Trimetaphosphate
2. Biofilm modifiers, such as arginine, triclosan, and xylitol, as well as herbal substances.⁴

The pathophysiology of dental caries, as well as some of the most recent breakthroughs in remineralization therapy have been considered and reviewed in this article.

Pathophysiology of dental caries

Cariou lesion dynamics are dependent predominantly on the availability of fermentable sugars, other environmental conditions, bacteria, and host factors. Specific environmental circumstances, such as the availability of fermentable dietary carbohydrates and the absence of oxygen, function as boosters for microbial activity. According to Reisine S, Litt M, a current perspective of caries involves consideration of how behavioural, social, and psychological variables, as well as biological elements, are involved. According to Nigel B. Pitts et al., dental caries is best defined as a complicated biofilm-mediated illness caused by frequent eating of fermentable carbohydrate (sugars such as glucose, fructose, sucrose, and maltose) and poor oral hygiene in conjunction with insufficient fluoride exposure.

Every cavity may have its unique demineralizing consortium of active organisms and genes when taken together, however the following basic principles are universal:

- 1) The presence of acidogenic-aciduric microorganisms, as well as their capacity to bind to the pellicle-coated tooth surface, either directly (as pioneered by MS) or indirectly (beneficiaries such as bifidobacteria and lactobacilli)
- 2) Conditions that favour the growth and metabolism of such species, including as availability to low-molecular carbohydrates, particularly sucrose, and a low redox potential. Fermentation and acid generation are accelerated when sugar levels are high and oxygen levels are low.

The resting pH of saliva tends to predict the caries experience of the individual and also is an indicator of salivary buffering capacity. Individuals with a resting salivary pH of around 7.0 had little caries activity or none at all, whereas those with a resting pH of 5.5 have a lot of caries. Caries activity is less severe in those with pH levels between 5.5 and 7.0. When subjected to a refined carbohydrate challenge, a lower resting salivary pH value predicts a

noticeably low pH and the preservation of this low pH for a longer amount of time before recovering to the baseline resting pH level.⁵

The caries process is slow, requiring repeated episodes of prolonged acidic exposure persistently below the essential pH for enamel disintegration (pH 5.5, demineralization) with periods of restoration to the plaque's resting pH. (pH 7.0, remineralization period). The dynamic equilibrium between demineralization and remineralization will be tipped towards demineralization with the development of clinically detectable white spot lesions in the face of failure to remove plaque from retentive tooth areas, a diet high in refined carbohydrates, and frequent carbohydrate ingestion.

Prevention strategies of demineralization

Demineralization is the removal of mineral ions from hard tissue HA crystals such as enamel, dentin, cementum, and bone. Remineralization is the process of returning these mineral ions to the HA crystals. Because demineralization is a reversible process, partly demineralized HA crystals in teeth can remineralize if exposed to oral conditions that promote remineralization. The recent rise in the frequency of dental caries among young children has emphasised the need for a novel strategy to caries prevention in children under the age of six. Children should visit the dentist for the first time at the age of one or when their first tooth erupts, according to new disease preventive management models.⁶

Since the pathogenesis of dental caries is well understood, strategies to prevent caries development would seem to be relatively simple. Either the attacking forces can be reduced (dental plaque/dental biofilm removal, alteration of dental plaque so that it is less able to convert dietary sugars to acids, neutralisation of acids within plaque, removal or reduction of dietary sugars) or the host resistance can be enhanced (reduce enamel's solubility in acid, the potential for remineralization of demineralized enamel, covering enamel surfaces to create a barrier between dental plaque and enamel).⁷

Fluoride (since 1990), a well-known anti-caries agent, has demonstrated its potential to promote remineralization while inhibiting demineralization. Overexposure to fluoride, however, has been linked to the development of fluorosis. Although it is

commonly established that fluoride is safe for the average person when used appropriately, it has been proposed that fluoride exposure be limited to specific individuals. As a result, various anti-caries measures are being developed.⁸

Professional application of fluoride has a long history. The benefits include the low frequency of applications (two to four per year) and the knowledge that the application has been completed. The cost is significant since professional time is consumed, and effort is required to attend the appointment. As a result, professional fluoride treatments are usually directed towards people who are most in need.^{9,10}

Caries Management Tools for The Future

Several technical breakthroughs are on the verge of becoming clinical realities, and if they prove to be effective, they will be adopted.

Assessment of bacterial challenge by chairside molecular probes.

Caries risk assessment will require the use of chairside bacterial probes to determine a patient's cariogenic bacterial challenge.¹¹

Caries immunization using dental caries vaccine. In the view of vaccine development, research focus is mainly on the incorporation of purified bacterial antigens into mucosal immune systems and delivery to mucosal IgA inductive sites. The three main types of *S. mutans* antigen that are involved in pathogenesis of dental caries and for which specific sIgAs have been found are antigen I/II, GTFs and glucan-binding proteins (GBPs). Accordingly, the mechanisms of action of these specific antibodies are:

- Clearance of bacteria in saliva by antibody-mediated aggregation,
- Inhibition of the adherence of bacteria by blocking bacterial-surface receptors and

Modification of metabolic functions of bacterial enzymes.¹²

Early caries detection and intervention

Early caries detection can be used to encourage remineralization by salivary augmentation, topical fluoride and chlorhexidine use, and diligent dental cleanliness. Furthermore, when new approaches for early caries intervention become available, many patients may no longer require restorations,

maintaining tooth structure and preventing or reversing the course of dental caries.¹³

Caries prevention by laser treatment

The use of lasers in caries prevention was first encountered in 1972 by Stern and Sognaes [15 diode lasers] using ruby Laser since then many investigations demonstrated the use of lasers in preventive dentistry and has been proposed that the lasers can be used as an adjunct to conventional fluoride therapy in remineralizing the tooth structure. The United States Food and Drug Administration (FDA) approved the use of an erbium: yttrium aluminum-garnet, or Er:YAG, laser on teeth in May 1997. This was the first time that a laser was approved for use on dental hard tissues. This FDA approval was for the use of this laser in the treatment of dental cavities and the cutting of healthy tissue prior to the implantation of restorations. For almost two decades, Kantorowitz and colleagues and Featherstone and colleagues have examined the impact of lasers on hard tissues. Fried and colleagues have released a study on a novel CO₂ laser for removing carious tissue that is effective.¹⁴

Compounds Increasing Mineral Saturation

Numerous types of remineralizing agents and remineralizing techniques have been researched and many of them are being used clinically, with significantly predictable positive results.

Requirements of an ideal remineralization material are as follows:

- Diffuses into the subsurface or delivers calcium and phosphate into the subsurface
- Does not deliver an excess of calcium
- Does not favor calculus formation
- Works at an acidic pH
- Works in xerostomic patients
- Boosts the remineralizing properties of saliva⁷

Classification

Remineralizing agents have been broadly classified into the following:

- Fluorides
- Nonfluoride remineralizing agents
 - Alpha tricalcium phosphate (TCP) and beta TCP (β -TCP)
 - Amorphous calcium phosphate
 - CPP-ACP

- Sodium calcium phosphosilicate (bioactive glass)
- Xylitol
- Dicalcium phosphate dehydrate (DCPD)
- Nanoparticles for remineralization
 - Calcium fluoride nanoparticles
 - Calcium phosphate-based nanomaterials.
 - NanoHAP particles
 - ACP nanoparticles
 - Nanobioactive glass materials
- Polydopamine
- PA
- Oligopeptides
- Theobromine
- Arginine
- Self-assembling peptides
- Electric field-induced remineralization

The process of introducing minerals from the surrounding environment (saliva, biofilm) into partially demineralized tooth structures is known as dental remineralization. Remineralization can cause amorphous mineral precipitates in the intercrystal and interrod gaps, or it can replace minerals in partly demineralized enamel and dentin (Cochrane NJ et al. 2010). Remineralization might take either spontaneously or as a result of therapy. Fluoride – based treatments have the greatest degree of supporting evidence among the various therapies (Amaechi and van Loveren 2013; Fontana 2016), and widespread use of fluorides is often regarded as the primary cause of dental caries reduction in most populations. However, currently available F treatments have limited efficacy in some people, and the benefit of F in lowering dental caries prevalence has reached a plateau at the community level (Haugejorden and Magne Birkeland 2006). Consequently, investigators have been developing new remineralization therapies to close this gap in efficacy.

Calcium and phosphate are essential minerals for remineralization, while F plays a crucial role in enhancing the process. Because remineralization can be hampered by limited bioavailability of calcium and phosphate, new product formulations designed to ensure a constant supply of calcium and phosphate have been developed (Cochrane et al.

2010).¹⁵ The following remineralization therapies were designed to directly increase mineral concentration in the environment around caries lesions (in saliva or biofilm).

Fluorides. For remineralization of caries lesions, F treatment is still the gold standard (Amaechi and van Loveren 2013; Fontana 2016). Various F treatments have been proved to be clinically beneficial in halting caries lesions, and F toothpastes have shown to have a dosage response (Walsh et al. 2010). Toothpastes with a fluoride content of 5000 ppm F are more effective in remineralizing root caries lesions than those with a fluoride content of 1000 to 1500 ppm F (Wierichs and Meyer-Lueckel 2015); nevertheless, evidence for their better effectiveness in remineralizing enamel lesions is still limited. As a result, they should not be recommended to all patients with active lesions, and their increased cost and restricted availability should also be considered.¹⁶ F products at high concentration, such as varnishes, provide effective remineralizing therapy for noncavitated enamel lesions in primary and permanent dentition. Although F mouth rinses and high-concentration F gels have been demonstrated to be successful in reducing lesion development, there is little evidence that they can remineralize teeth. This paucity of data on remineralization may be due to previous research that concentrated mostly on F's caries-preventive qualities, which was the most common hypothesised mode of action at the time.¹⁷

Combination of some metals with F could enhance the antimicrobial effect of the therapy. Silver diamine F (SDF), for example, has been demonstrated to be very successful at remineralizing caries lesions in both coronal (Gao et al. 2016) and root surface (Wierichs and Meyer-Lueckel 2015) dentin. SDF has lately been investigated for its ability to remineralize early enamel lesions on the approximal and occlusal surfaces (MattosSilveira et al. 2014). Silver nitrate (AgNO₃) has been recommended as a replacement to SDF because it is not accessible in some regions. In a recent RCT, the effectiveness of 25% AgNO₃ followed by 5% NaF varnish in stopping early children caries lesions was compared (Chu et al. 2015). The most major disadvantage is that both SDF and AgNO₃ will

colour the remineralized tissue black or dark brown.¹⁰

Stannous F (SnF₂), which has the ability to interfere with biofilm development while also affecting the demineralization/remineralization process, is another example of metal association with F that has rekindled interest in dental caries control. Tin ions have been shown to exhibit antibacterial properties in several laboratory investigations (partially reviewed by Fernández et al. 2016). In the presence of cariogenic biofilms, recent laboratory studies imply that current SnF₂ formulations have a superior anticariogenic impact (by inhibiting enamel demineralization).¹⁸ The exact mechanism of action of SnF₂ is currently unknown. The most relevant calcium and phosphate-based systems accessible today are shown below.

CPP-ACP: Casein Phosphopeptide–Amorphous Calcium Phosphate (Recaldent). CPP-ACP is a calcium and phosphate-stabilized system that has been studied intensively over the past 20 years. Several literature and systematic reviews have been conducted on its anticaries impact (Azarpazhooh and Limeback 2008; Reynolds 2009; Zero 2009; Li et al. 2014). While the bulk of the studies indicated a significant remineralizing effect, the reviews expressed concerns about the lack of independent studies and the usage of short-term in situ models, which have limited practical applicability. Furthermore, the bulk of remineralization studies have been conducted on incipient lesions related with orthodontics, limiting the results' applicability to other areas. Other studies have recently shown that CPP-ACP remineralization may be achieved on non-orthodontic lesions ranging in severity from moderate to severe.¹⁹ F varnish or a placebo were employed as the control in these 6-month studies. However, a 1-year clinical research found no advantage of CPP-ACP over placebo tooth mousse, most likely due to both study groups using daily F toothpaste. The conclusions indicated in recent studies (Li et al. 2014; Fontana 2016) are still valid due to inconsistent results, the short duration of the trials, and the dominance of solely fluorescence changes in lesions after orthodontics as the outcome. More clinical proof is still needed to demonstrate that the CPP-ACP therapy is not inferior to F therapies.²⁰

CPP-ACPF: Casein Phosphopeptide–Amorphous Calcium Phosphate Fluoride. To improve remineralization effectiveness, F has been added to the CCP-ACP formulation. According to a recent systematic evaluation, this change in the formula has not been proved to be more beneficial than CPP-ACP alone. The most recent reports have produced mixed findings. CPP-ACPF did not give any further advantage in the remineralization of postorthodontic white-spot lesions, according to a recent study. CPP-ACPF was not proven to be more effective than conventional home care therapy in another short-term clinical research (Huang et al. 2013). CPP-ACPF appeared to have a particular remineralizing impact on smooth surface caries lesions but not on pit and fissure lesions, while another research found that CPP-ACPF was more effective than a placebo in treating pit and fissure lesions.²¹

fTCP: Functionalized β -Tricalcium Phosphate. TCP is a crystalline structure that has been functionalized to yield β -tricalcium phosphate (fTCP). This fTCP was created to prevent calcium from reacting prematurely with ionic F, allowing it to function as a low-dose delivery mechanism (Karlinsky and Pfarrer 2012). There were no current clinical data on its remineralization potential that could be discovered. However, clinical trials including fTCP in varnish and high-concentration F toothpastes are now being conducted. CSPS stands for Calcium Sodium Phosphosilicate Bioactive Glass (NovaMin). Wefel (2009) and Burwell et al. (2009) reviewed the potential for this bioactive glass material for remineralization a few years ago, both stating that the technique was promising but that additional study was needed. RCTs were being conducted at the time the reviews were published. For this current review, no published clinical data were found for remineralization, and the level of evidence of in vitro and in situ data is weak and controversial. In vitro, NovaMin outperformed CPP-ACP mousse in boosting enamel remineralization, however in situ, adding 5% CSPS to sodium monofluorophosphate toothpaste did not increase remineralization of enamel specimens. TMP stands for Trimetaphosphate of Sodium. TMP is a cyclic polyphosphate salt that binds to the surface of the enamel. It was examined clinically in the 1990s and

is presently being evaluated for its ability to boost the action of F. TMP addition of F varnishes (Manarelli et al. 2015) and gels (Danelon et al. 2013) leads to increased remineralization of simulated caries lesions, according to in situ data. Finally, TMP added to a toothpaste with a low F concentration demonstrated remineralization ability comparable to that of a toothpaste with a standard F concentration in situ. In situ data has clearly demonstrated the technology's promise; nevertheless, clinical application should only be recommended when these results have been verified in clinical trials. Overall, calcium phosphate–based systems have not consistently outperformed F products, and therefore should not be suggested to replace F products on a regular basis. Patients who are hesitant to use F products or young children who are at risk of dental fluorosis may benefit from calcium phosphate–based systems that are devoid of fluoride.²²

Biofilm Modifiers

The following therapies were designed to enhance the remineralizing effect of F by affecting the amount, composition, and metabolic activity of the dental biofilm around caries lesions.

Arginine. The amino acid arginine has been proven in several investigations to impact the pH and ecology of dental biofilms. As a result, it was added to toothpaste (1.5 percent arginine) with insoluble calcium and 1,450 ppm F (as sodium monofluorophosphate) to help prevent cavities by increasing remineralization. This possible influence on biofilm pH might assist maintain biofilm equilibrium with the host, in addition to mechanical plaque management (Cummins 2016). Several randomised clinical investigations (5 for remineralization and 3 for caries lesions prevention) have shown that the arginine+F+Ca formulation has consistently superior effects when compared to nonarginine formulations. Three remineralization investigations were carried out in naturally occurring enamel caries lesions on the buccal surfaces of maxillary incisors, and the results were assessed using quantitative light-induced fluorescence (QLF; Srisilapanan et al. 2013; Yin, Hu, Fan). The other two trials (Hu et al. 2013; Souza et al. 2013) looked at the toothpaste's capacity to stop and reverse root caries lesions, using hardness

as a clinical sign of lesion activity. According to a recent systematic review and meta-analysis, this formulation may have a better anticaries effect (Li et al. 2015). Because of the substantial risk of commercial bias in the research studied, a separate systematic review found that the current data is insufficient to establish the greater anticaries effectiveness of arginine-containing toothpaste.²³

Triclosan. Triclosan is an antibiotic that may influence biofilm acid production, resulting in increased saturation and so remineralization. In the 1990s and 2000s, large studies revealed that adding triclosan to dentifrice formulations can result in small but statistically significant decreases in the incidence of coronal and root caries in children and adults. It was also indicated in a 2004 in situ research that it could have an influence on remineralization. triclosan-loaded dendrimers have recently been investigated as possible remineralization agents (Zhou et al. 2014), however no clinical data on this novel technology is now available.²⁴

Xylitol. The use of chewing gum carrying xylitol increases salivary flow rate, helps in remineralization and enhances the protective properties of saliva. This is because the concentration of bicarbonate and phosphate is higher in stimulated saliva, and the resultant increase in plaque pH and salivary buffering capacity prevents demineralization of tooth surfaces.

Xylitol in oral syrups commercially available as: Mematrix Xylitol syrup.²⁵

Herbal Compounds

In vitro studies of herbal or other natural chemicals as remineralization therapy have been conducted. They might impact mineral saturation and precipitation, operate as antimicrobials, or stabilize collagen, which could act as a scaffold for mineral deposition, depending on the component (Chandna et al. 2016). When remineralizing fake root caries lesions in vitro, proanthocyanidins have been found to have a synergistic impact with calcium phosphate-based drugs. While they appear to be a potential choice, more in situ and in vivo research is needed to corroborate the in vitro results.²⁶

Self-assembling Peptides

Because minerals from saliva or other treatments require nucleation sites for precipitation and remineralization, a biomimetic technique was

developed approximately a decade ago to speed up the process (Kirkham et al. 2007). In lab investigations, monomeric low-viscosity peptide solutions were shown to spontaneously generate scaffolds capable of hydroxyapatite nucleation, which facilitates remineralization in enamel lesions (Kirkham et al. 2007). Recent in vitro data confirmed the technology's remineralization capability, as evidenced by visual examination and scanning electron microscopy, QLF, photothermal radiometry and luminescence, microhardness, energy-dispersive x-ray spectroscopy analysis, and confocal laser scanning microscopy results. According to laboratory findings, the self-assembling peptide P11-4 diffuses deeply into the body of the caries lesion, enabling remineralization by promoting de novo hydroxyapatite nucleation (Kind et al. 2017). This treatment has been shown to be safe for human usage in a recent RCT (Alkilzy et al. 2017). The results of this 6-month single-blind trial show that P11-4 in conjunction with F varnish (22,600 ppm F) promotes remineralization of early carious lesions better than F varnish alone (Alkilzy et al. 2017).

CONCLUSION

In recent years, several remineralization methods have made substantial progress. The majority of these treatments work by establishing stable systems capable of delivering bioavailable calcium, phosphate, and F directly to the lesion or surrounding biofilm. One of the most difficult aspects of remineralizing treatments is providing the proper concentration of minerals at the right time; increased mineral concentration at the wrong moment might cause undesirable surface precipitation, reducing the therapy's efficacy. Mineral delivery that is slow and extended may promote subsurface mineral gain. Other treatments aim to change the environment around lesions (for example, biofilm modifiers) or the shape of the lesions' body (e.g., scaffolds). Finally, during the 2017 ICNARA meeting, a novel iontophoresis device developed to expedite the flow of mineral ions into deeper regions of demineralized enamel was introduced (International Conference on Novel Anticaries and Remineralizing Agents). Early results have been promising (Pitts 2017), although it is still too early to assess its remineralization potential.

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Role of Nanotechnology in Dental Decay: A Review

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Abstract

Dental caries (tooth decay) is probably the most common chronic diseases in the world. Lack of anti-caries properties in traditional caries filling materials can result in secondary caries and treatment failure. The invention of nanotechnology in dental caries is an effective strategy for caries management because the bacterial metabolism gets interfered, the formation of biofilm gets inhibited, demineralization gets reduced, and remineralization gets promoted, which is the reason why it is in the pioneer role. In this review article we have tried covering all the aspects and types of nanomaterials that will be useful for our budding and experienced dentists worldwide in anticaries management.

Keywords: Metal nanoparticle, QAS-PEI, Remineralized Nano Anticaries Material, Nanostructures Forms and Shapes, Nanodrug delivery system, Self-Assembling Biomimetic Enamel Repair

INTRODUCTION

Dental Caries is an infectious microbiologic disease of the teeth which causes the calcified tissues to undergo dissolution and destruction. Collection of microorganisms where the cells adhere to each other

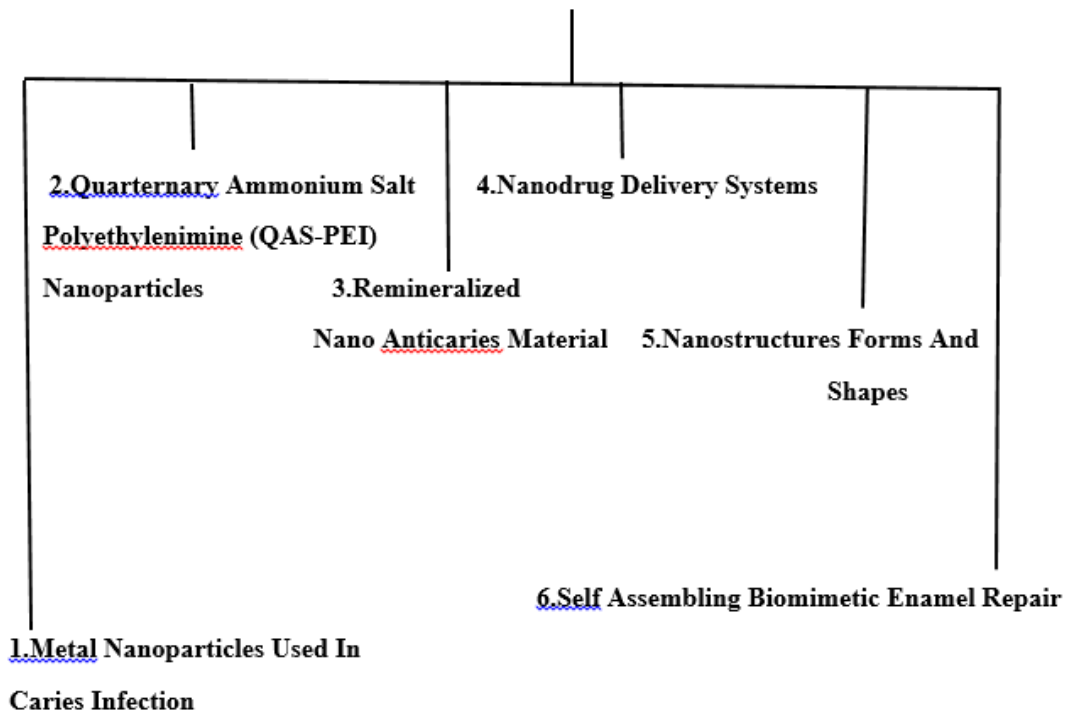
as well as to the tooth surface¹ is called as biofilm. These biofilm of oral bacteria and yeast leads to several localized disease in the oral cavity including dental caries, periodontal diseases, candidiasis and

endodontic, orthodontic and implant infections¹.Complex interaction between acid producing bacteria and host factors including teeth and saliva² leads to caries formation. Based on modern theory of caries etiology, imbalance of oral flora could result in acid accumulation and lead to tooth demineralization³.The initial colonizers at first sticks to the acquired pellicle which is a salivary-dietary- derived proteinaceous layer present on the tooth surface that subsequently influences the further colonization of microbial organisms¹. As a result, dental plaque is formed and its maturation is characterized by bacterial interactions such as **Co-aggregation** and **Quarum Sensing** and increasingly diverse bacterial populations².Acids as by products are being produced by these bacterial species from the metabolism of fermentable carbohydrates resulting in demineralization below the tooth surface.

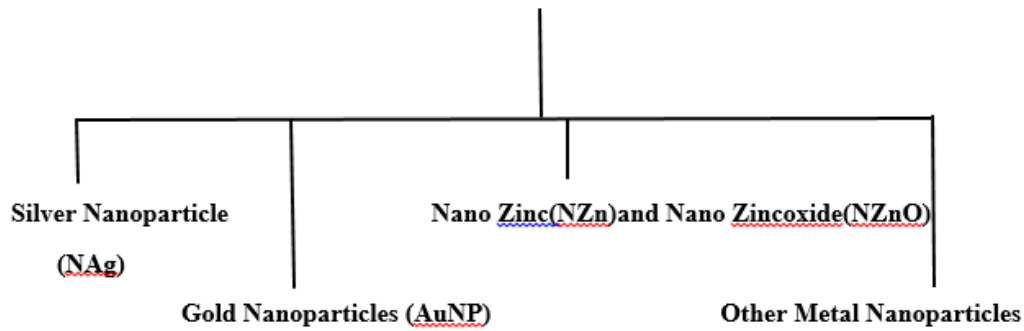
Richard Feynman first proposed nanotechnology. It is a technology that is based on the properties and application of materials in the range of 1-

100nm.Their physical/chemical advantages for eg: volume effect, surface effect, quantum size, quantum tunnel and dielectric confinement³makes them better than the traditional materials. The diameter of nanoparticles is inversely proportional to its specific surface area, mechanical properties and antibacterial effect. In simpler words more smaller the diameter of nanoparticles greater will be its specific surface area and stronger will be its mechanical properties and antibacterial effect³.Nanoparticles can be used as the carrier of antibacterial drugs since they have the properties of targeting bacteria with least side effects on the host. To say about the antibacterial mechanism of nanoparticles, it includes metal ion release, oxidative stress and non oxidative mechanism³.The positively charged nanoparticle comes in contact with negatively charged bacterial cell membrane by static electricity which changes the permeability of cell wall leading to rapture of cell membrane and organelle leakage.

ANTICARIES NANOMATERIAL



1. Metal Nanoparticles Used In Caries Infection



4. Nano Drug Delivery Systems

- i) Mesoporous Silica Nanoparticle(MSN)
- ii) Aluminosilicate Clay nanotubes
- iii) Reactive nanogel Adhesives
- iv) Liposomes
- v) Polyamidoamine (PAMAM)
- vi) Halloysite NanoTube (HNT)
- vii) Dental Caries Vaccine

1. Metal Nanoparticles Used in Caries Infection

Metals has been used for centuries as antimicrobial agents. Silver, Copper, Gold, Titanium, and zinc have attracted particular attention, each having different properties and spectra of activity.

With respect to nanoparticles, the antimicrobial properties of silver (Sondi and Salopek-Sondi,2004) and copper (Cioffi et al, 2005) have received the most attention. Both of these have been coated onto or incorporated into various materials (Li et

al,2006), including PMMA (Boldryeva et al,2005) and hydrogels (lee and Tsao,2006).An inverse relationship between nanoparticle size and antimicrobial activity has been demonstrated, where nanoparticles in the size range of 1 -10nm have been shown to have the greatest biocidal activity against bacteria (Morone et al,2005;Verran et al, 2007).Nanoparticles also offers advantages to the biomedical field because of their improved biocompatibility (Kim et al, 2007)due to their small size. Also, bacteria acquires more resistance against conventional and narrow-target antibiotics (Pal et al, 2007) than metal nanoparticles. This is thought to occur because metals may act on a broad range of microbial targets, and many mutations would have to occur for microorganisms to resist their antimicrobial activity.

The Anti - Caries Mechanism Of Metal Nanoparticles

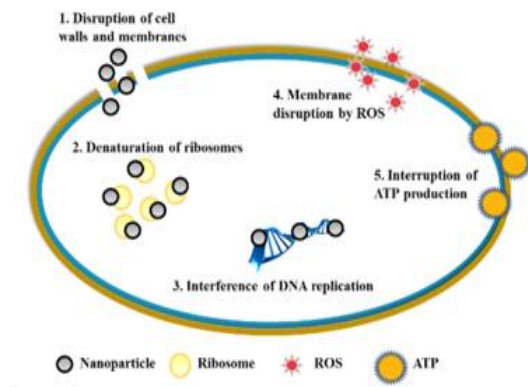


Fig1: The antibacterial mechanism of metal nanoparticle

A. Silver nanoparticle

Silver has a broad spectrum of antibacterial properties, which can inactivate enzymes and prevent DNA replication in bacteria. Surface area ratio is further increased by NAg, which makes the silver particles smaller and antibacterial effects better. According to some study, the antibacterial effect of NAg increases in a dose dependent manner from 0.05%-0.1% without any effect on bonding strength or color. Self-etching adhesive⁴ containing 0.05%-0.1% NAg can affect the bonding strength at pH 2.7 according to Elkaas DW et al. Based on a study done by Cheng et al⁵ 0%- 0.175% of NAg composite resin can remarkably diminish biofilm growth and metabolic activity, in which antibacterial activity shoots up in a dose - dependent manner. But,

the nano- silver mass fraction was more than 0.175%⁶ when the flexural strength of modified composite resin decreased significantly

Antibacterial Activity of Silver nanoparticle

Nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance⁷. Silver has always been used against various diseases; in the past it has been used as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria due to its low cytotoxicity⁸. AgNPs were considered, in recent years, particularly attractive for the production of a new class of antimicrobials, opening up a completely new way to combat a wide range of bacterial pathogens.

Details of AgNPs and their mechanisms of action against Bacteria and biofilm

Bacteria	Mechanism Of Action
<u>Acinetobacter baumannii</u>	Alteration of cell wall and cytoplasm.
Escherichia coli	Alteration of membrane permeability and respiration
<u>Enterococcus faecalis</u>	Alteration of cell wall and cytoplasm
<u>Klebsiella pneumoniae</u>	Alteration of membrane
<u>Listeria monocytogenes</u>	Morphological changes, separation of the cytoplasmic membrane from the cell wall, plasmolysis
<u>Micrococcus luteus</u>	Alteration of membrane
Nitrifying bacteria	Inhibits respiratory activity
<u>Pseudomonas aeruginosa</u>	Irreversible damage on bacterial cells; Alteration of membrane permeability and respiration
Proteus mirabilis	Alteration of cell wall and cytoplasm.
<u>Staphylococcus aureus</u>	Irreversible damage on bacterial cells
<u>Staphylococcus epidermidis</u>	Inhibition of bacterial DNA replication, bacterial cytoplasm membranes damage, modification of intracellular ATP levels
<u>Salmonella typhi</u>	Inhibition of bacterial DNA replication, bacterial cytoplasm membranes damage, modification of intracellular ATP levels
Vibrio cholera	Alteration of membrane permeability and respiration

B. Gold Nanoparticle (AuNP)

Gold shows a weak antimicrobial effect in comparison with silver and copper⁹. However, gold nanoparticles are employed in multiple applications involving biological systems. The binding properties of gold are exceptional and this makes it

particularly suitable for attaching ligands to enhance biomolecular interactions. Gold nanoparticles also exhibit an intense colour in the visible range and contrast strongly for imaging by electron microscopy¹⁰.

C. NanoZinc (NZn) And NanoZincoxide (NZnO)

The antibacterial ability of NZn results from the quantum size effect of dissolving and releasing zinc nanoparticles¹¹ and that is why it has a wide antibacterial spectrum. NZn has the property of reducing the expression of MMPs and increase the lifespan of adhesive¹². Based on a study the addition

of Zn^{2+} to total-etch adhesive can result in inhibition of MMP activity, reduction in the decomposition of dentin collagen bundle, and protection of mineral crystal formation at the eosin-tooth interface, which will result in improvement of the nano-mechanical properties¹³.

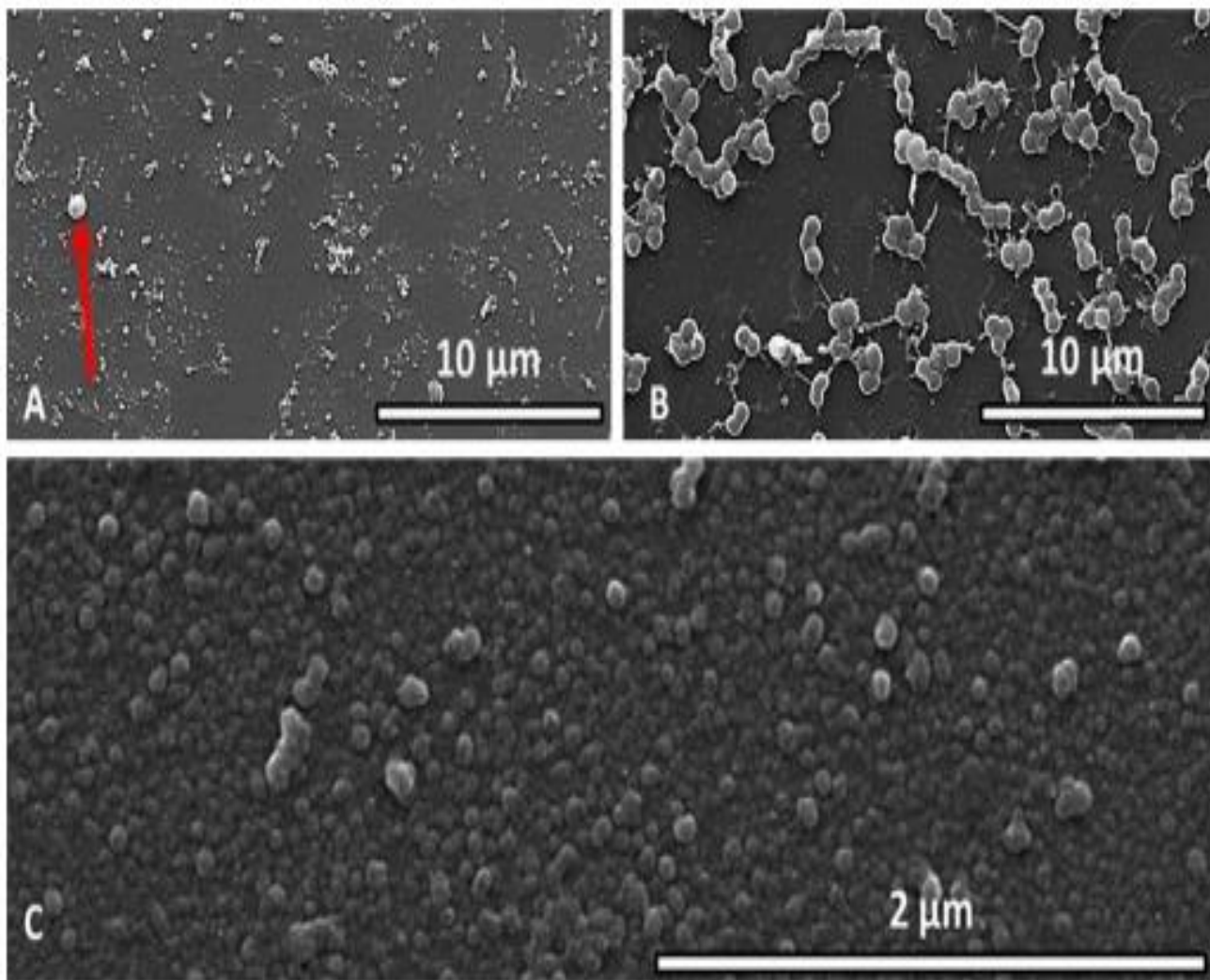


Fig 2: Assessment of the bactericidal effect of nanoparticulate ZnO-coated glass substrates. (A) Arrow indicates an individual *Staphylococcus aureus* cell present on the coated surface. Debris present is likely to be remnants of dead bacteria. (B) A population of *S. aureus* present on an untreated surface. (C) High-resolution image to highlight the uniformity of the coated surface

2. Quarternary Ammonium Salt Polyethylenimine (QAS-PEI) Nanoparticles

QAS is a highly active cationic agent with a wide antibacterial spectrum¹⁴. Based on the polyethylenimine cross-linked structure, QAS-PEI nanoparticles were prepared making the modified composite bear the property of high chemical

stability and antibacterial properties under different oxidants and storage conditions, without the oral micro ecological balance³ getting effected. QAS-PEI has the antibacterial mechanism based on the electrostatic interaction between QAS-PEI which is positively charged and bacterial cell walls¹⁵ which is negatively charged.

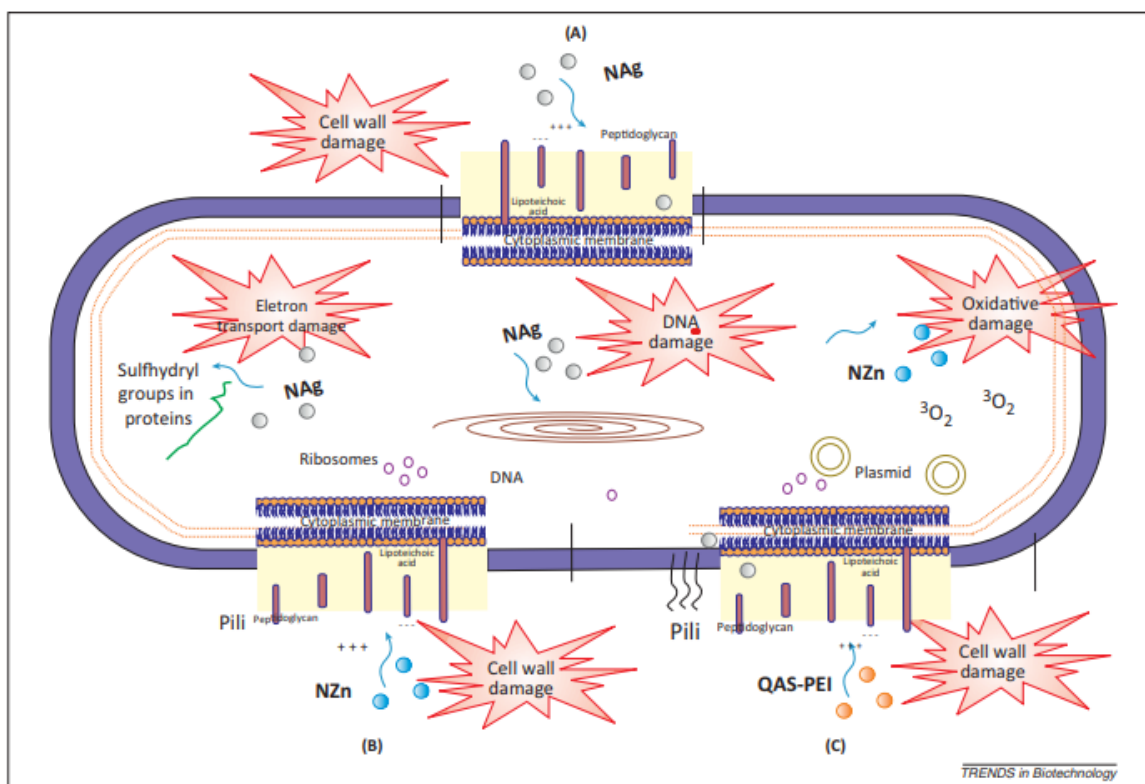


Fig 3: Schematic representation of a bacterial cell showing the components targeted by different antibacterial agents incorporated in dental materials. (A) Silver nanoparticles (NAg): NAg have been incorporated in restorative materials to combat the cariogenic bacteria colonization in the marginal gaps and on their surfaces. (B) Zinc oxide nanoparticles (NZn): the primary cause of the antibacterial function of NZn is credited to the disruption of cell membrane activity. (C) Quaternary ammonium polyethylenimine (QAS-PEI) nanoparticles: the mechanism of action may be related to absorption of positively charged polymers onto negatively charged cell surfaces of the bacteria. This process is thought to be responsible for the increase of cell permeability and may disrupt the cell membranes.

3. Remineralized Nano Anticaries Material

Multiple innovative applications of nanotechnology have been postulated in the aim of attaining net remineralization of early caries lesions as a non-invasive approach for dental caries management⁶. The remineralization procedure confers the provision of calcium and phosphorous ions from an external source to the dental structures in order to enhance ion precipitation into the demineralized surface to earn lucid remineralization and furnish anti-cariogenic effect.

4. Nano Drug Delivery Systems

i) Mesoporous Silica Nanoparticle (MSNs):

The surface of MSN can be modified by functional groups resulting in MSN being compatible with various solutions and it can also be stored as different types of molecules³. Other than that MSN is easy to adhere to the dentin surface because of its high affinity. MSN can also

release calcium and phosphate slowly leading to the improvement of the bonding durability and remineralization effect.

ii) Aluminosilicae Clay nanotubes:

Aluminosilicate ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$) clay nanotubes (Halloysite, HNT) have many advantages (e.g., biocompatibility, hydrophilicity, and high mechanical strength) that make them a good candidate to be used as a reinforcing agent for improving resin-based dental adhesive properties⁶. Besides, these clay nanotubes can also behave as biologically safe reservoirs leading to the encapsulation and controlled delivery of a wide variety of therapeutics. In adhesive dentistry, these nanotubes can be a carrier of MMP inhibitors, that would ultimately result in reducing and/or eliminating resin–dentin bond degradation.

iii) Reactive nanogel Adhesives

Nano-gels are 10- to 100-nm cross-linked globular particles synthesized through a versatile route allowing for adjustable hydrophobic character and level of methacrylate

functionalization⁶. Nano-gels can be swollen by and dispersed in monomers such as BisGMA, HEMA and solvents, which, in dentin bonding, are anticipated to carry the nanoparticles into demineralized dentin.

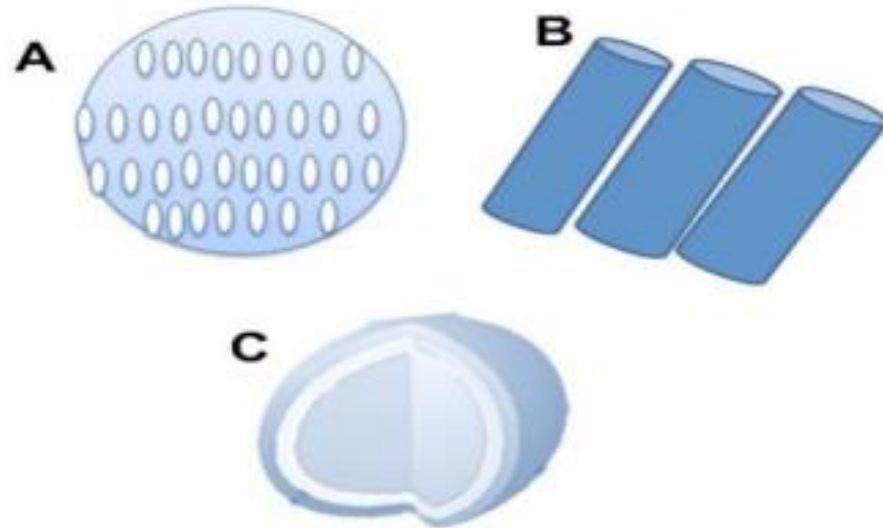


Fig 4: Schematic diagram representing different patterns of drug carriers nanoparticles; A: Mesoporous spherical nanoparticle, B: Nanotube, C: polymeric Nanocarrier.

iv) Liposomes

A liposome is a microvesicle formed by encapsulating drugs in the lipid bilayer³. Liposomes were used as a drug carriers for the first time by Gregoriadis et al. Lipophilic or water-soluble drugs can be encapsulated by liposomes which possesses the properties of anti-cancer, antibacterial, anti-inflammatory drug carriers along with gene delivery because it has an additional advantage of targeting, slow-

released and tissue affinity after encapsulating drugs. The anticaries role of liposomes is mainly described by liposome drug delivery system which is actually the source of calcium, phosphorus, and other minerals being precipitated on the surface of tooth hard tissue promoting mineralization. Liposomes containing fat-soluble or water-soluble antimicrobial agents can diminish plaque biofilms as well.

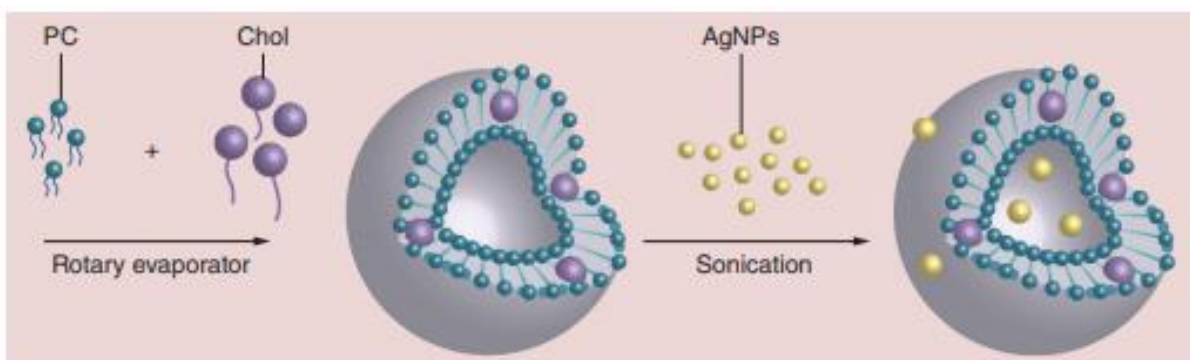


Fig 5: Synthesis of liposome silver nanoparticles

v) Halloysite Nano-Tube (HNT)

Halloysite is a kind of natural silicate mineral, which mainly exists in nature in the form of nanotubes³. The main role of HNT is that it acts as a carrier to regulate the delivery of therapeutic agents along with excellent biocompatibility, hydrophilicity, and high mechanical strength.

vi) Polyamidoamine(PAMAM)

A dendrimer is a novel type of high branched nano polymer with a cavity in the molecule and easy to modify surface groups³. It possesses the property of excellent biocompatibility, reduced toxicity, and non-immunogenicity. Modification of PAMAM terminal groups into various functional groups as drug delivery carriers can result in controlling release of the drug and act as both antibacterial and remineralization in caries.

vii) Dental Caries Vaccine

It is an effective strategy in caries prevention to induce oral mucosal immune systems through the nasal tract³. Vaccination carries the following advantages of greater patient compliance, induces systemic immunity, and administration is convenient.

5) Nanostructures Forms and Shapes

Nanostructures comes in different forms and shape having dimensions in the range between 0.1-100 nm⁶. They are made up of different compositions, and resulting in greater variety of modified properties to enable innovative applications in dentistry

6) Self Assembling Biomimetic Enamel Repair

Dental enamel represents a unique hard biological tissue yet, incapable to repair when subjected to acid dissolution under the effect of caries activity followed by ulterior collapse of its organic construction yielding cavity formation. The non-regenerative phenomenon arises as a result of being devoid of vital cellular component lost soon after tooth emergence into the oral cavity⁶. Biomimicry approaches for enamel repair rely on bio-mineralization to enhance artificial enamel formation in attempt to reform surface damage

CONCLUSION

With the development of caries diagnosis and prevention, nanotechnology will significantly improve medical treatment. The application of nanoparticles against caries infection is very important because it has antibacterial, remineralization, and drug loading capacity. This review article highlights that nanoparticles have innumerable role in the prevention and treatment of dental caries. However, their disadvantages and potential cytotoxicity and environmental effects cannot be neglected. In the coming days, we should focus on discovering better and modern technology to develop highly effective anti-caries nanoparticles along with highest safety for patients. Further studies about nanotechnology in dental caries prevention and management are needed.

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Evaluation of Homogeneity and Voids of Two Obturation Techniques using CBCT: An invitro study

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Abstract

Aims and objectives: to evaluate the voids and homogeneity of obturation of the root canals using two different techniques namely the lateral compaction technique, and single cone obturation.

Material and methods: 60 maxillary and mandibular first premolars which were freshly extracted for orthodontic purpose were collected and used for this study. The 30 samples in group 1 were obturated using Lateral Compaction technique. Another 30 samples in group 2 were obturated using single cone obturation technique

Results: The lateral compaction method is considered as better due to higher total volume percentage (82.25±6.56 at coronal third, 92.38±8.48 at middle third and 91.48±6.68at apical third) as compared to single cone obturation wherein total volume percentage (77.31±9.89 at coronal third, 83.53±8.27 at middle third and 87.01±8.79 at apical third)

Conclusion: Lateral Condensation technique had a lesser number of voids as compared to single cone obturation technique. Lateral condensation showed better homogeneity and quality of obturation than by single cone obturation

INTRODUCTION

The most important goal of root canal treatment is the three dimensional obturation of canal space after removal of pulpal tissue.¹ Three dimensional obturation of the root canal space helps to remove the cause of microleakage from the oral cavity and also from the peri radicular tissues into the root canal system.²

Microleakage in the root canal space occurs through various ways one of them is through the oral cavity into the tooth via the junction between the dentin and the obturation material or the root canal sealer. It can also occur due to the lack of adequate coronal as well as apical seal. When a perfect coronal as well as apical seal is achieved, the microorganisms present in the root canal are deprived of the essential nutrients needed for their growth and survival. Hence, one of the most important role of obturation is to provide a three dimensional hermetic/ fluid tight seal for the root canal space from both coronal and apical leakage.³ It has been reported in endodontic literature that in earlier times, root canals have been filled with a large variety of materials but all these materials which were used in the earlier times were not considered as the ideal root canal filling materials due to certain deficiencies in their properties as well as handling characteristics. Later, gutta percha gained acceptance and is now considered as ideal for this purpose.

Various modifications of gutta-percha have been made available. The form of gutta-percha which is used in the Lateral Compaction Technique is beta (β) form due to its improved properties such as stability, hardness and reduction in stickiness. The alpha (α) phase of gutta-percha is used in the thermoplasticized obturation techniques due to its low viscosity and flow properties resulting in a more homogenous root canal filling.⁴

There are various number of obturation techniques which are used such as Cold Lateral Condensation technique which is considered as the gold standard. Lateral condensation (compaction) of gutta-percha has been shown to be a successful technique if the adequate spreader depth of 1 mm is achieved apically. One of the potential shortcomings associated with Lateral Compaction is the relative poor replication of the root canal walls and the

tendency to form voids as well as the creation of spreader tracts between the filled gutta-percha points.⁵ Certain defects such as voids, spreader tracts, incomplete fusion of the gutta-percha cones and the lack of surface adaptation have been reported with all the obturation techniques.⁶ All these defects can lead to failure of root canal treatment.

So to avoid the failure of procedure, quality of the root fillings can be assessed through different experimental approaches such as the acid dissolution of roots, electrochemical method, fluid filtration, dye penetration, radiovisiography, the SEM analysis of the interface between the filling material and the canal wall, radioisotope bacterial leakage, microscopic analysis, Cone beam computed tomography (CBCT) and Micro- Computed Tomography (μ -CT).^{7,8,9}

As we all know Radiovisiography (RVG) is an important and most available tool to access the quality of root canal space obturation. With paralleling technique and mesial distal angulation (SLOB/ MBD) techniques one can easily evaluate the presence of voids and adaptability of gutta percha in root canal space. Computed Tomography currently considered as the leading diagnostic tools for endodontic research. Thus CBCT has been successfully utilized in endodontics for the three dimensional reconstruction of the affected teeth.¹⁰ So aim of this in vitro study is to evaluate the voids and homogeneity of obturation of the root canals obturated by using two different obturation techniques namely the lateral compaction technique, and single cone obturation.

MATERIALS & METHOD COLLECTION OF SAMPLES

60 maxillary and mandibular first premolars which were freshly extracted for orthodontic purpose were collected and used for this study.

Exclusion Criteria: Teeth which had caries, restoration, apical resorption or had undergone previous endodontic treatment were excluded from this study. In order to standardize the samples for homogeneity, teeth which had acute curvatures, root canal anomalies and calcifications were excluded.

Preparation of the Samples

All the specimens were washed in running tap water for two minutes and were then immersed in 5.25% sodium hypochlorite solution for a period of 24 hours in order to remove the organic debris.

In Group I, Root canals were prepared using ISO size 15-30 No. K-Files with step back technique. All the standard protocols followed till the completion of cleaning & shaping of all the samples.

In Group II, Root canals were prepared using ISO size 15-20 no K-File with Step- Back technique. After thorough irrigation with 5.25% sodium hypochlorite (Prime Dental products Pvt Ltd, Mumbai, India) Canals were prepared using Protaper Gold Rotary File System (Dentsply, Maillefer, Ballaigues, Switzerland) upto size F3 with Step Down Technique. All the standard protocols followed till the completion of cleaning & shaping of all the samples.

Group 1 - Cold Lateral condensation

The 30 samples in group I were obturated using Lateral Compaction technique. This technique was done using a 30 size (0.02 taper) gutta percha (Dentsply, Maillefer, Ballaigues, Switzerland) as the master cone. The master cone was placed into the canal till the working length with tug back. Then 25, 20 and 15 sized finger spreaders (Dentsply, Maillefer, Ballaigues, Switzerland) were used to create spaces for Lateral Compaction of guttapercha and the root canal was filled with the appropriately sized accessory cones. The excess gutta percha at the orifice of the root canal was sheared off by using Buchanan plugger.

Group 2 – Single cone obturation

Another 30 samples in group 2 were obturated using single cone obturation technique as recommended by manufacturers instructions till Protaper-F3 gutta percha. After satisfactory coating of canals with sealers, the greater taper gutta percha points were inserted and vertically compacted with the help of hand pluggers.

After obturation, all the access cavities were filled with Cavitemp and then stored at 37°C and at 95% humidity for about 72 hours to aid in the complete setting of the sealers.

First RVG radiographs were done in order to evaluate the homogenous obturations. This was followed by CBCT scans in order to determine the volume of the obturated material at the coronal, middle and apical third of the obturated root canals. Approximately 15mm of the root length was taken into consideration for the CBCT analysis. This length of 15 mm was further divided into equal coronal, middle and apical segments. To detect the presence or absence of space or voids each segment was cut in slice thickness of 0.5 mm. and each tooth was then scanned for a section of 30 slices.

The volume of the root canal in each slice was calculated by multiplying the root canal area by the slice thickness (0.5mm) during the CBCT analysis (R). Thus the total volume of the predetermined (15mm) root length was calculated by adding up the values obtained for the total 30 slices. In the study specimens where void was seen, the inner area of the void was calculated using the linear measurements obtained using the Vatech pax-I 3D software and this value was multiplied by the slice thickness in order to calculate the Volume of the Void (V). The volume of the obturated material was calculated using the formula (R-V).

The Volume Percentage of the voids in the filled root canal was calculated by using the formula, $(R-V) \times 100/R$ where, R is the volume of the root canal space and V is the volume of the void space. The homogeneity of obturation was evaluated by estimating the number of the spaces or voids at different level of coronal, middle and apical segments of the obturated root canals.

Statistical Analysis

Descriptive data are presented as Mean \pm SD. One-way ANOVA was used for multiple group comparisons followed by Post Hoc Tukey's HSD Test for differences among mean. Level of significance was <0.05.

RESULT

The present in vitro study was designed to evaluate the quality of two different obturation techniques; single cone and lateral condensation. The total volume percentage of obturation was recorded using CBCT scanner for both the techniques.

Table 1:- An Intergroup Comparison of Mean Total Volume Percentage (TVP) and standard deviation values of obturation techniques

TVP (Total volume percentage)		Groups	
		Lateral Compaction	Single Cone
Coronal	Mean	82.2557	77.3175
	SD	±6.56	±9.89
Middle	Mean	92.3896	83.5354
	SD	±8.48	±8.27
Apical	Mean	91.4842	87.0111
	SD	±6.68	±8.79

The lateral compaction method is considered as better due to higher total volume percentage (82.25±6.56 at coronal third, 92.38±8.48 at middle third and 91.48±6.68 at apical third) as compared to single cone obturation total volume percentage (77.31±9.89 at coronal third, 83.53±8.27 at middle third and 87.01±8.79 at apical third)

Table-2: An Intragroup comparison of Mean Total Volume Percentage (TVP) using unpaired t test

Coronal TVP	Mean	Unpaired t test		Unpaired t test	
		t value	p value	Lateral Compaction	Single Cone
Lateral Compaction	82.2557	459.26	<.0001	<.0001	<.0001
Single Cone	77.3175			—	<.0001
Middle TVP				Lateral Compaction	Single Cone
Lateral Compaction	92.3896	2944.6	<.0001	<.0001	<.0001
Single Cone	83.5354			—	<.0001
Apical TVP				Lateral Compaction	Single Cone
Lateral Compaction	91.4842	2375.6	<.0001	<.0001	<.0001
Single Cone	87.0111			—	<.0001

Lateral Compaction and single cone obturation technique showed statistically significant results.

Table 3: An Intergroup comparison of Mean Total Volume Percentage (TVP) using one way ANOVA and pair wise comparison using post Hoc Tukey's HSD test

Lateral Compaction	Mean	ANOVA		Post Hoc Tukey HSD		
		F value	p value	Coronal	Middle	Apical
Coronal	82.2557	459.259	<.0001	<.0001	<.0001	<.0001
Middle	92.3896			—	<.0001	<.0001
Apical	91.4842			—	—	<.0001
Single Cone				Coronal	Middle	Apical
Coronal	77.3175	2741.9	<.0001	<.0001	<.0001	<.0001
Middle	83.5354			—	<.0001	<.0001
Apical	87.0111			—	—	<.0001

Among lateral compaction and single cone obturation, coronal, apical and middle area showed statistically significant results.

DISCUSSION

Endodontic treatment failures mainly occur due to the inadequate obturation of the root canal space. Hence, the quality of the obturation determines the long term success of the endodontically treated tooth.

In this study cleaning and shaping was performed by step back technique (group 1) using conventional K-files 15 – 30 size, and samples of group II were prepared with crown down technique using the protaper gold rotary system as per manufacturer's instructions.

During and after instrumentation, the irrigants facilitate the removal of the microorganisms, tissue remnants and dentin chips from the root canal space through a flushing mechanism. Irrigants also prevents the packing of the hard and soft tissues in the apical root canal and also the extrusion of infected material into the periapical area. The most widely used endodontic irrigant is 0.5% to 6.0% sodium hypochlorite (NaOCl). It has a strong bactericidal activity and it also aids in dissolving vital and necrotic organic tissues.^{11,12}

Smear layer can serve as an avenue for the ingress of microorganisms and also it can act as a source for the growth and activity of viable bacteria, which remain entrapped in the dentinal tubules.¹³ Hence, in order to remove the smear layer, the root canals were irrigated with 5.25% sodium hypochlorite and 17% EDTA solution intermittently in this study.

In the present study, Sealapex was used primarily as a root canal sealer to produce rigid and strong cross-linked polymer with dentin collagens.¹⁴

A preoperative CBCT analysis was done to evaluate the volume of the entire root canal space of all the study samples. CBCT is used as the diagnostic tool in this study because it is a non-invasive diagnostic method in which the entire root canal space can be evaluated in a single scan. CBCT is the best way to observe the homogeneity of the endodontic obturation.^{15, 16, 17}

Both the obturation techniques, were evaluated for the homogeneity and the quality of the obturation using CBCT. Homogeneity means that the root canal filling is a well compacted, uniform mass, without the presence of voids. The obtained results (Table 3) demonstrated that in the inter group comparison for the presence of voids it was seen

that the Lateral Condensation technique had significantly lesser number of voids (459.259) as compared to single cone obturation technique (2741.9) with significant p value <.0001.

(Table 3). Mean TVP in the coronal, middle and apical segment for both the techniques were statistically significant with the p value of <.0001.

The incidence of voids within root fillings may be affected by many factors such as the anatomical configuration of the canal system, the quality of canal preparation, the consistency and volume of sealer, the operator's expertise, and the technique used.¹⁸

The Cold Lateral Condensation technique showed a significantly higher percentage of volume filled when compared with the Single cone obturation technique. This could be due to a mismatch in the shape of the master cone and the anatomy of the root canal.¹⁹ Thus, achieving the fluid-tight seal in this technique is dependent on the usage of a sufficient amount of sealant. Poor adaptation of the single cone at the middle and coronal thirds of the root canal is another disadvantage due to the shape mismatch.¹⁹

In our study, the apical section of single cone obturation showed a lesser area of voids when compared to middle and coronal sections. This could be due to the close and maximal fit of the gutta percha master cone apically more than coronal.

A Kumar and ARV Pai et al concluded that a greater taper of the master cone with close fit can generate more hydraulic forces while seating it and further increase the flow of sealer apically by means of shear thinning mechanism. This may probably cause compression or collapse of air entrapped bubbles under pressure, and/or filling of voids by the enhanced flow of the sealer and could result in a lack of or lesser voids in the apical sections.²⁰

These results are supported by Obeid et al, who concluded that single cone obturation had a good density in the apical portion when compared to the coronal portion of the canal.²¹

In intra group (Lateral compaction) mean total volume percentage (TVP) was higher in middle of the root canal space followed by apical and coronal (Table 2). In Lateral compaction group, it may be

explained because root canals tend toward a rounder cross section at the apical segment (Wu et al. 2002), favouring the adaptation of the master cone, limiting the spreader penetration and, consequently, preventing accessory cones being inserted up to the working length.²²

In the present study, although none of the obturation techniques were able to get 100% homogeneity, the lateral compaction method exhibited best due to higher total volume percentage (82.25±6.56 at coronal third, 92.38±8.48 at middle third and 91.48±6.68 at apical third) as compared to single cone obturation total volume percentage (77.31±9.89 at coronal third, 83.53±8.27 at middle third and 87.01±8.79 at apical third) (Table 1).

CONCLUSION

Within the limitations of this study, it can be concluded that voids were present in all the

groups. In the inter group comparison it was seen that the Lateral Condensation technique had a lesser number of voids as compared to single cone obturation technique with statistically significant p value <.0001. In intra group (Lateral compaction) mean total volume percentage (TVP) was higher in middle of the root canal space followed by apical and coronal with statistically significant p value <.0001 for all segments. The lateral condensation group had the maximum overall volume percentage of the obturated material. Lateral condensation showed better homogeneity and quality of obturation followed by single cone obturation.

Therefore, further studies are necessary to evaluate the prognosis of the treatments performed with this technique, mainly in root canals presenting complex anatomy.

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