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The effect of three irrigant regimens at different time intervals on the fracture resistance of root canal dentin - An In Vitro study

Dr Gnyani Prasad[1],
Post Graduate
Department of conservative dentistry and Endodontics,
Narayana Dental college and Hospital, Andhra Pradesh, India
Dr Kiranmayi Govula [2],
Associate professor
conservative dentistry and Endodontics,
Narayana Dental college and Hospital, Andhra Pradesh, India
Dr Lavanya Anamula[3],
Head of the Department
conservative dentistry and Endodontics,
Narayana Dental college and Hospital, Andhra Pradesh, India
Dr Kowmudi Maddineni[4],
Senior Lecturer
Department of conservative dentistry and Endodontics,
Narayana Dental college and Hospital, Andhra Pradesh, India
Dr Pavan kumar. Yendluri[5],
Senior Lecturer
Department of conservative dentistry and Endodontics,
Narayana Dental college and Hospital, Andhra Pradesh, India
Dr Venkateswarlu Modem[6],
Senior Lecturer
Department of conservative dentistry and Endodontics,
Narayana Dental college and Hospital, Andhra Pradesh, India
Dr. Swapna Sannapureddy
Reader
Conservative Department and Endodontics
Narayana Dental College & Hospital
Nellore 524002
***CORRESPONDING AUTHORS**: **Dr Kiranmayi.** G*
Associate Professor
Department of conservative dentistry and endodontics
Narayana dental college and hospital
Nellore

ABSTRACT
Objective;*the in-vitro study aims to evaluate the effect of three irrigant regimens at two-time intervals on root dentin.* ***Materials and Methods;*** *Seventy freshly extracted teeth were obtained and sectioned up to 13±1mm from the root apex using a low-speed motor.The root samples were further divided into four groups based on irrigating solutions. Group I 0.9% NaCl Group II 17%EDTA+1% Naocl Group III Qmix Group IV1%Naocl + 18%HEBP.After cleaning and shaping,teeth were placed in the molds and tested for vertical root fracture resistance under a universal testing machine.* ***Results;*** *Data analysis was done using SPSSVersion 21. Basic descriptions of the statistical analysis were presented in the formof mean and standard deviation. The data were assessed statistically using the Shapiro-Wilk test. Group I [0.9% NaCl showed the lowest fracture resistance. Group II (1%NaOCl +EDTA) > Group IV (1%NaOCl+HEBP) > Group III (Q Mix) > Group I [0.9% Nacl].* ***Conclusion:*** *1%NaOCl and 17%EDTA have shown an increase in the RFR [root fracture resistance]. QMix*

and HEBP showed a significant difference in root fracture resistance compared to the control group but less than NaOCl and EDTA. However, both groups have no statistically significant difference.

Keywords: *EDTA, HEBP, Qmix, Root Fracture Resistance, Sodium hypochlorite.*

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I. Introduction

A smear layer formed during root canal instrumentation is an amorphous structure composed of inorganic and organic materials of 1-2 µm thickness. The smear layer holds bacteria or bacterial products and may act as a reservoir for irritants, leading to an unfavorable sealthat would indicate& favor its removal. Various chemical substances are employed in endodontic treatment, capable of removing the smear layer and modifying the root dentin's structural and chemical composition, resulting in changes in its permeability and solubility[1]. A root canal irrigant's desirable properties include dissolving organic, and inorganic tissues, antimicrobial effect, and compatibility with the periapical tissues[2].

Of all the currently used irrigants, NaOCl appears to be the most ideal due to its unique capacity to dissolve necrotic tissues and the organic components of the smear layer. It destroys sessile & spore forms of endodontic pathogens in biofilms and dentinal tubules similar to chlorhexidine(Zehnder *et al*. 2002)[3].

A full-strength 5.25% NaOClsolution significantly reduces the elastic modulus and flexural strength of human dentin compared to saline due to its proteolytic action on dentin's collagen matrix. It can also be unable to prevent the smear layer formation during instrumentation to the full extent. (Zehnder 2005)[4].

In combination with sodium hypochlorite, EDTA can also enhance the smear layer removal, promoting a progressive dissolution at the cost of erosion in peritubular and intertubular dentin. In addition to cleaning ability, chelators detach biofilms adhering to root canal walls. The EDTA may be used to perform as a finalflush or alternately with NaOCl during the root canal preparation (Soares *et al.* 2010)[4]. Among all the combinations of irrigants,the combination of hypo and 17 %, EDTA has been preferred to remove organic and inorganic contents of the smear layer present in the root canal system.[5]

Recently, etidronate solution (HEBP) emerged as a substitute for the commonly used chelators because it can be combined with NaOCl, without interfering with its antimicrobial properties in the short term (Zehnder *et al.* 2005)[6]. It was also suggested that when the mixture is used during biomechanical preparation, the smear layer is not formed (Lottanti *et al*. 2009)[7.] Besides, hard-tissue debris accumulation in the isthmus area is reduced (Paqu e *et al.*, 2012)[8].The HEBP is a weak chelator; therefore, it is less aggressive than EDTA on dentine. However, if it is used for a final flush, these solutions need 300 sec to remove the smear layer (De-Deus *et al*. 2008)[9] altogether. It seems likely that if NaOCl and HEBPmixture is used during biomechanical preparation, it can dissolve pulp debris and eliminate microorganisms while preventing smear layer formation.[10].

Q mix was introduced for the dual effect on smear layer removal, which consists of polyaminocarboxylic acid chelating agent bibisguanide antimicrobial agent [2% CHX] surfactant and deionized water. Q mix was efficient for smear layer removal when used for three minutes[11].

Oversized root canal therapy, pressure transmission to canal walls during shaping, various chemicals used during cleaning and shaping, obturation of canals, presence of post or screw, isthmus, and occlusal overload by masticatory forces often lead to the development of vertical root fractures[12]

Previous studies have reported that microhardness (Slutzky-Goldberg *et al.* 2004)[13], flexural strength (Zhang et al. 2010)[14], elasticity (Grigoratos *et al*. 2001)[15], erosion (Sen *et al*. 2009)[16], and fracture resistance of roots might be negatively affected by the chemical properties and the concentration of endodontic irrigants. They were therefore leading to the risk of vertical root fracture.

Hence this study focuses on various combinations of irrigants and their effect on root fracture resistance.

MATERIALS:-

PREPARATION OF SOLUTIONS

- The irrigant solutions used in the study were 0.9% normal saline,17%EDTA (ENDOCARE,V-Dentcare limited,Khanpur,Ahmedabad,India), 18% HEBP (TWIN KLEEN, MAARC DENTAL, Phalgar,Maharastra,India), and 1% sodium hypochlorite (RANIKEM Laboratory reagent,India) and Qmix (Dentsply,Johnson City,USA)
- A 1% sodium hypochlorite solution was prepared by careful dilution of 5% sodium hypochlorite (Ranikem) using distilled water at a ratio of 1:8.
- 17%EDTA liquid provided directly by the manufacturer (V Dentcare Endocare India) was used in the study.

- A fresh mixture of 18% HEBP and 1 % sodium hypochlorite was prepared by mixing two capsules, each containing 9% HEBP (Twinkleen, MarcDental India), in a 1% sodium hypochlorite solution immediately before the experiment.
- All the solutions were stored at 5^0C between the experiments. Before use, the irrigants were removed from the refrigerator and equilibrated to room temperature before the methodology.

II. Methodology:

Seventy freshly extracted teeth were collected and carefully stored in 0.9% NaCl and tissue remnants were removed using Gracey curettes. Teeth with root caries, cracks, and resorption were excluded. Teeth were sectioned up to 13±1mm from the root apex to obtain a specimen with 2mm dentine thickness. The sectioning was done using diamond discs mounted on a low-speed motor (Marathon Lab Micromotor).The root samples were further divided into four groups based on irrigating solutions.

The apical foramen of each specimen was sealed using modeling wax. A Protaper Next rotary file system was used to prepare each tooth. Initial exploration of the canal was done by using a 10-k file.The sequence of instrumentation used was10-15-X_1-X_2-X_3-F_1 along with 2ml of irrigating solution at each instrument change for 3 minutes. Hence each tooth received a total of 12ml initial irrigant solution for 18 minutes. A final irrigant of 17%EDTA was used in group II of 2ml per 5 minutes.

	Initial irrigation	Final Irrigation
TIME	18 minutes	5 minutes
Group I	0.9% NaCl	0.9% NaCl
Group II	1% NaOCl	17%EDTA
Group III	Qmix	Qmix
Group IV	1% NaOCl + 18%HEBP	

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PREPARATION OF MOLDS FOR UTM

After the canal preparation, the roots of the samples were covered with 0.2-0.3mm utility wax-up to CEJ to simulate the periodontal ligament space, and 1-2mm below the CEJ line was left free. Cylindrical-shaped molds (3mm x 3mm) were taken, and petroleum jelly was applied to prevent auto-polymerizing resin from adhering to the mold by acting as a separating medium. Teeth samples were vertically mounted in the molds with the help of cotton pliers. An auto-polymerizing resin was placed layer by layer with adequate proportions of powder-to-liquid ratio. The samples were allowed to polymerize for 24hours.

Molds were coated with a varnish to prevent moisture evaporation and also for group identification. Samples were stored at 24^0c in normal physiological saline until the test was performed.

The test cylinders were mounted on the base of the UTM to assess the fracture resistance (FIE Universal Testing Machine).A 4mm spherical-shaped stainless steel tip was directed toward the long axis of the teeth. The displacement was set at 5cms, and Vertical loads were applied with a force load of 600N at a speed of 1mm per minute.

The software [ASTM] was set to record the maximum force applied on the tooth beforefracturing inthe form of values. Load at the point of fracture was recorded in Newton.

STATISTICAL ANALYSIS

Data analysis was done using a statistical package for social sciences (SPSS Version 21). Basic descriptions of the statistical analysis were presented in the formof mean and standard deviation. The data were assessed statistically using theShapiro-Wilk test. ANOVA was used to analyze the overall group difference forfracture resistance among all the groups, and Turkey Post Hocs were used to assess pair-wise comparison. The level of significance was $p<0.005$.

III. Results:

Table 1 represents the values of the Mean and Standard Deviation of all four groups, which illustrates that Group II (1% NaOCl + EDTA), has the highest fracture resistance, and Group I [0.9% NaCl] showed the lowest fracture resistance.

Group II (1%NaOCl +EDTA) > Group IV (1%NaOCl+HEBP) > Group III (Q Mix) > Group I [0.9% Nacl].

Table 2 displays ANOVA test results of the overall comparison of fracture resistance of 4 groups, showing a significant mean difference and the standard deviation.($P<o.o5$).

Table 3 represents a pairwise comparison among four groups using Turkey post hoc tests. Compared to Group I NaOCl, Group II and Group III showed a statistically significant mean difference. When Group II compared to

Group III and IV, Group III showed significant statistical differences. Group III also showed a significant statistical difference fromGroup IV.

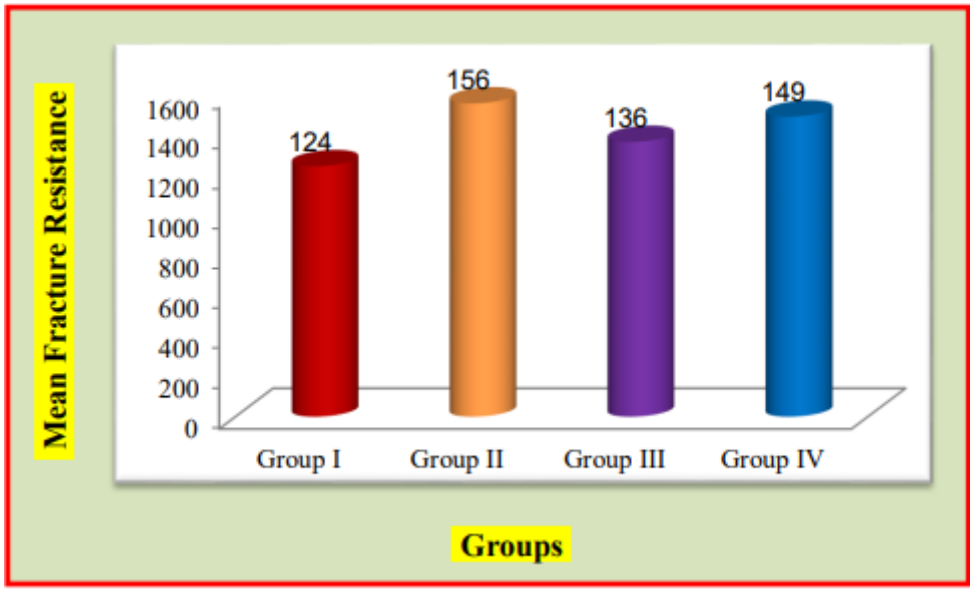
TABLE 1: Overall Comparison of Fracture Resistance using ANOVA Test

Groups	Mean ± SD	Standard error	F Value	P value
Group I	1248.00 ± 72.54	22.93	14.359	<0.001*
Group II	1560.00 ± 127.48	28.50		
Group III	1369.00 ± 122.68	27.43		
Group IV	1494.00 ± 178.77	39.97		

TABLE 2: Turkey Post Hoc test showing the pairwise comparison

Groups		Mean Difference	Standard error	P value
Group I	Group II	312.00	53.28	<0.001*
	Group III	121.00	53.28	0.115**
	Group IV	246.00	53.28	<0.001*
Group II	Group III	191.00	43.50	<0.001*
	Group IV	66.00	43.50	0.433**
Group III	Group IV	125.00	43.50	0.027*

GRAPH 1 SHOWS THE MEAN FRACTURE RESISTANCE



IV. Discussion.

During root canal shaping, loss of moisture occurs due to the absence of pulp tissue, and the preparation of canals can alter the mechanical properties and integrity of endodontically treated teeth, consequently reducing root fracture resistance. Also added deleterious effects like dentin dehydration, altered dentin microhardness, flexure strength & modulus of elasticity, and erosion caused by the irrigating solution can

directly lead to fracture of endodontically treated teeth impairing their longevity. Various combination of irrigating solutions along with the irrigating time plays a vital role in reducing root fracture resistance. In this study, three irrigant regimens along with two-time intervals were tested [17].

Many studies have proved that Sodium hypochlorite is the most effective irrigating solution as it inhibits bacterial cell metabolism. A combination of Irrigant solutions like sodium hypochlorite, EDTA, and chlorhexidine is essential for the disinfection of the root canals. It may, in turn, lead to decreased vertical root fracture resistance by altering flexural strength, elastic modulus, and microhardness. The deleterious effects of sodium hypochlorite on the proteoglycan matrix might cause dentine contraction, increase stress concentration, and crack propagation. Continuous hypochlorite irrigation throughout instrumentation at least 1-2 minutes after each instrument shaping will serve the purpose. In this study, hypochlorite was used at 2 ml for 3 minutes to gain its maximum effect[18]

EDTA is a chelator that is used after sodium hypochlorite as the final irrigant. It is slightly alkaline or neutral and gets precipitated at acidic Ph. A 17% or 15% EDTA solution is preferred, and the recommended time for smear layer removal is around 1-2 minutes[19]. However, depending on conditions like infected canals, predentine,bacteria, ground dentine, and pulp remnants might require even more extended periods of exposure.EDTA affects the inorganic portion of the root dentine and smear layer; therefore, complete removal of the smear layer can be achieved using sodium hypochlorite before the final rinse with EDTA[20]. Using EDTA along with sodium hypochlorite leads to the effective removal of the smear layer with good antibacterial properties.

The chelator containing irrigating solutions stays in contact with the canal walls and has been reported to vary from 30 sec to 10 mins. The previous studies by calt*et al*. and serpe*et al*.2002, have shown that EDTA irrigation for one minute effectively removed the smear layer. However, a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion[21].

To date, no single irrigating solution could alone remove both the organic and inorganic components of the smear layer. Hence, several studies suggested the use of a combination of irrigants ethylenediaminetetraacetic acid (EDTA), sodium hypochlorite (NaOCl).7% percent maleic acid (MA) is a chelating agent found to possess better smear layer removal capability than 17% EDTA. It is also less cytotoxic than 17% EDTA[22].

Qmix was introduced for the dual effect of EDTA and chlorhexidine for smear layer removal. It is a constituent of bisbiguanide (Chlorhexidine 2%) and surfactant and deionized water. It has high antimicrobial properties against Enterococcus faecalis compared to chlorhexidine and is found similar to EDTA in terms of smear layer removal[23].

In this study, Qmix showed less Vertical root fracture resistance than the EDTA group. Because of the dual nature of Qmix, it can be assumed to have mineral changes more than EDTA because of the known effects of detergent on root microhardness (Ballal NV *et al* 2016)[24]. According to study results by Chia MSY*et al*. in 2020[25,] Qmix exerted the same mineral changes as EDTA; this can be explained by the addition of detergent to EDTA does not increase the removal of calcium ions from dentine compared to sterile EDTA solution. According to Gonzalez Lopez 2006[26]the addition of chlorhexidine to chelate, solutions have no decalcifying effect.

In this study, total irrigation of HEBP was done for 23minutes, of which 5minutes is for final irrigation. The mixture of NaOCl with chelating solutions, such as etidronic acid (HEBP) and tetrasodium EDTA (Na4EDTA), has been suggested to obtain better cleanliness and disinfection of the root canal system in a reduced amount of time due to the continuous chelation during instrumentation.

Based on the calcium-binding capacity and stability constant of the HEBP-calcium complex, the use of 7% HEBP solution was found significantly less useful in debriding root canals than 10% citric acid. Further experiments showed that HEBP- calcium chelation from root canals is dependent on the concentration of the chelator in the solution. With a 20% HEBP solution, the number of calcium ions eluted from the root canals was similar to 17% EDTA or 10% citric acid. HEBP can be mixed with NaOCl and can be used as a single irrigant during and after the instrumentation without short-term loss of either compound's desired properties so that a smear layer is never created[27]. Hence in this study,18%Hebp was preferred along with 1% sodium hypochlorite.

In the present study, 18% HEBP was used as its chelating efficiency was found better than 9% HEBP because of a higher concentration, which followingDinesh Kumar *et al*.[28] study, 2012, where it was proved to be more efficient than 9% HEBP in removing calcium from the root canal but relatively weaker than SmearClear and BioPure MTAD due to their capability of diffusing into dentinal tubules as they lack surfactants. Consequently, a less aggressive calcium complexing agent such as 7–10% HEBP could be administered during root canal preparation to prevent erosive dentinal changes.

Previous studies proved that 1% NaOCl, when used in larger volumes and with increased irrigation times, possesses good bactericidal activity.According to a study by Marending *et al*[29,] the immersion of root dentine bars in 5 mL of 1% NaOCl at 37 C for one hour did not cause a significant drop in their elastic modulus,

flexural strength compared to the corresponding values obtained with specimens immersed in water. In contrast, 5% and 9% hypochlorite reduced the samples' elastic modulus and flexural strength by 50%. Both carbon and nitrogen content of the specimens were significantly ($P < 0.05$) reduced by 5% and 9% NaOCl, while 1% NaOCl did not significantly reduce these values against the water control[31]. Hence to minimize the possible effects on root fracture resistance, 1% sodium hypochlorite was included in this study[30].

Based on the ANOVA test, in this study,all three test groups showed a significant difference in increasing the vertical root fracture resistance of the roots compared to the control group. There is no or little significant difference between Group I (control) and Group III (Qmix). The reason can be attributed to the sole effect of chlorhexidine, the presence of EDTA,and the absence of hypochlorite in the Qmix group having less effect on organic matrix removal.

Group II (EDTA) showed a significant difference from Group III (Qmix). The results are per the recent studies of Ballal *et al*. (2016) showing the superior effect of QMix in removing the smear layer in the apical third than that of EDTA.[31]

The reason could be partially why EDTA, with higher surface tension, was the least effective in smear layer removal from the apical third (Bhandari*et al* 2017)[32]. The lower smear layer capability of EDTA in the apical third can also be attributed to the fact that its chelate activity is based not only on the removal of calcium ions from inorganic dentine but also on the elimination of calcium from the organic components of dentine, such as water-soluble non-collagenous proteins. Because non-collagenous proteins are present in lower concentrations in the apical region, the degree of EDTA decalcification is diminished. Tubular sclerosis of root dentine in the apical root region may further affect EDTA efficacy. However, apart from EDTA in the apical third, the Qmix was significantly superior, affecting the smear layer. However, in contrast to EDTA and MTAD, no significant difference was observed among all thirds of root canals after QMix treatment. This result indicates that QMix penetration in the apical third of the root canal was as good as in other thirds which might have decreased the root fracture resistance.

The obtained results are consistent with previous studies showing that irrigation solutions and Syringe needle irrigation are the least effective in the apical third. (UlusoyGörgül*et al*, 2013.[33]

Group II showed no significant difference from group IV(HEBP). The irrigation regimens that employed HEBP as a chelating agent resulted in a significant decrease in hardness, but the hardness values were lower than those resulting from protocols that include EDTA ($P< 0.001$). It shows that the decrease in hardness is directly proportional to the chelating power of the irrigant material. The above findings confirm that HEBP is a weak calcium-complexing agent that causes less dentin change than other chelating agents. According to another study by C. Emre Erick *et al*. 2019 [34],HEBP is a less destructive agent than EDTA when used as the final irrigation solution. A previous scanning electron microscopy study reported that 18% HEBP in conjunction with NaOCl completely removed the smear layer (Lottanti *et al*., 2009) and that exposure to HEBP concentrations of 9% and 18% for 3 min caused mild demineralization of the dentinal structure.

Group III (Qmix) showed a significant difference from Group IV(HEBP). More comparative studies have to be done between these groups in the future.

V. Conclusion

Within the limitations of this study, 1%Naocl and 17%EDTA have shown an increase in the RFR [root fracture resistance]. QMix and HEBP showed a significant difference in root fracture resistance compared to the control group but less than NaOCl and EDTA. However, both groups have no statistically significant difference. Further research in the form of clinical studies is needed to find out the further effect of HEBP on root dentin.

Even though an effective final regimen of irrigants was used, the time required for irrigation should be adequate to remove the smear layer and increase fracture resistance.

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