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Effectiveness of Different Irrigation Regimes on the Removal of Smear Layer in the Middle And Apical Thirds of Root Canals – A Scanning Electron Microscopic Study

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Abstract
Aim*: To evaluate the effectiveness of four different irrigation regimes on removal of smear layer and smear plugs at the tubular apertures in the middle and apical thirds of the root canals by using Scanning Electron Microscopy(SEM).*
Settings and Design*: In vitro material science study.*
Materials and methods*: Twenty human mandibular first premolars were used for this study. The teeth were divided into 4 groups-Group I,0.9% physiological saline, Group II ,5.25% alternate use of sodium hypochlorite and 3% hydrogen peroxide, Group III, 5.25% sodium hypochlorite, 3% hydrogen peroxide and ethylene diamine tetraacetic acid (EDTA) and Group IV,5.25% sodium hypochlorite, 3% hydrogen peroxide and ethylene glycol-bis (β-amino ethyl ether) -N'N'N', N-tetraacetic acid (EGTA). The split halves of the root surfaces were examined under a SEM and the images were analyzed for the amount of smear layer using a three score system.*
Results*: There was a significant difference in the effectiveness of different irrigation regimes in removing the smear layer at the middle and apical thirds. Groups III and IV differed significantly from Groups I and II, but did not differ in their ability to remove smear layer, although Group III showed a lower score in the apical third.*
Conclusion*: Though the use of EGTA effectively removed smear layer from the root canals without inducing erosion of the tubules, the most effective irrigation regime was the use of EDTA in combination with NaOCl and H_2O_2, as it completely removed the smear layer from both the middle and the apical thirds.*
Keywords: *EDTA, EGTA, Irrigation, Smear layer, SEM*

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I. Introduction

One of the primary aims of endodontic treatment is to remove all debris from the root canal system prior to placing a root canal filling [1]. Mechanical instrumentation alone will not completely eliminate bacteria from a root canal system [2]. In order to eliminate bacteria predictably from the root canal system, it is necessary to use the supporting action of disinfecting agents such as irrigants [3] and intracanal medication [4]. Cleaning and shaping of root canals using the various root canal instruments creates an amorphous, granular and irregular layer covering the radicular dentin , the smear layer, which was first described by McComb and Smith [5]. Smear layer block the irrigants and sealants from entering the dentinal tubules and thereby prevent disinfection of the dentin walls. It acts as a nutritional source for some intracanal microbes and also promotes post obturation microleakage [6]. As it contains both organic and inorganic components, smear layer removal usually requires a combination of an organic solvent such as sodium hypochlorite and acids such as citric, tannic, polyacrylic, or phosphoric acid, or chelating agents such as Ethylene Diamine Tetraacetic Acid (EDTA) [7]. Alternate use of sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) was also found to have little effect on the removal dentinal debris and the smear layer [3,5]. New irrigating solutions like QMix 2 in 1 (mixture of a bisbiguanide, a polyaminocarboxylic acid, a calcium chelating agent and a surfactant), Biopure MTAD (a combination of an antibiotic-3% doxycycline, a chelating agent-4.25% citric acid and a detergent-0.5% polysorbate 80) [6], 0.2% chitosan solution with high chelating ability (a natural cationic aminopolysaccharide copolymer of glucosamine and N-acetyl glucosamine obtained from chitin, abundant in shells of crustaceans and shrimps) [8], 2% chlorhexidine gluconate, Largal Ultra (17% EDTA+ 0.75%

cetrimide), Smear Clear (17% EDTA, cetrimide, and a special surfactant) [9] , Etidronic acid (also known as 1-hydroxyethylidene- 1,1-bisphosphonate or HEBP) a biocompatible chelator [10] that can be used in combination with sodium hypochlorite having adequate calcium chelating capacity have also been introduced and tested as final endodontic irrigants for effective smear layer removal. Maleic acid, a mild organic acid used as an acid conditioner in adhesive dentistry has been found to possess the smear layer removing quality when used as an acid etchant in restorative dentistry [10]. Among the irrigants, NaOCl is considered as the gold standard because of its exceptional qualities as an antiseptic and its tissue dissolving effects, but it has no effect on the inorganic part of smear layer, hence a decalcifying agent should also be used [8]. Scanning electron microscopy (SEM) has been proved as a valuable method for assessing the ability of endodontic procedures to remove debris from root canals. SEM studies have shown that the use of NaOCl and EDTA solutions sequentially between each file, effectively removed both organic and inorganic matter from the root canal lumen. The smear layer was also completely removed, leaving the dentinal tubules open [11,12,7,13]. The most commonly recommended agent is EDTA along with 5.25% NaOCl which is shown to remove smear layer effectively from the coronal and middle thirds, but not completely from the apical thirds [6].

The results of an in vitro study by Semra Calt and Ahmet Serper [14] showed that although EDTA in combination with NaOCl completely removed the smear layer, EDTA caused erosion of the tubules. They suggested that EGTA (Ethylene glycol-bis (β-amino ethyl ether)- $N^{'}$ $N^{'}$ $N^{'}$, $N^{'}$- tetraacetic acid) was an alternative chelator for removing the smear layer, without inducing erosion.

The present study is an attempt to evaluate the effectiveness of the chelating agent, EGTA, in comparison with other commonly used endodontic irrigants, especially with EDTA, on smear layer removal from the middle and apical thirds of the root canal and the potential of EGTA being recommended as an alternative chelating agent to remove smear layer. The four irrigation regimes tested were:

1. Use of 0.9% physiological saline alone (as control)
2. Alternate use of 5.25% NaOCl and 3%H_2O_2
3. Use of 17% EDTA, a chelating agent, as a high volume flush after instrumentation, in combination with NaOCl and H_2O_2, and
4. Use of an alternative chelator, 17% EGTA, as a high volume flush after instrumentation, in combination with NaOCl and H_2O_2

II. Materials And Methods

2.1.Sample preparation

Ethical clearance was obtained from Institutional Ethics Committee, Govt.Medical College, Trivandrum, before starting the study. Twenty human mandibular first premolar teeth of 20 to 22 mm length, with single root canals and closed apices and without calcifications and excessive curvatures, extracted for orthodontic purposes were used for this study. The teeth were decoronated in order to get a stable reference point during measurement and instrumentation. Two opposing longitudinal grooves were made along the entire length of the buccal and lingual surfaces of the roots to facilitate subsequent splitting for SEM examination. Superficial cutting debris was removed from the roots with distilled water.

2.2. Materials

0.9% Physiological saline
5.25% Sodium hypochlorite (NaOCl)
3% Hydrogen peroxide (H_2O_2)
17% Ethlene Diamine Tetracetic Acid (EDTA)
17% Ethylene glycol bis (β- amino ethyl ether)N,N,N', N'- Tetraacetic Acid (EGTA)
Deionized water
Distilled water

2.3. Equipments

Scanning Electron Microscope (S- 2400)
Critical Point Drier (HCP-02)
Ion Sputtering Unit (E-101)

2.4. Instrumentation:

The root canals of all the 20 teeth were instrumented in the following manner:

A size 10K- file was passed into the root canals until visible at the apical foramen. One millimetre was subtracted from the length to establish the working length. A drop of 5.25% NaOCl was placed at the root canal orifices and the pulp tissues were extirpated with a barbed broach. The canals were then sequentially instrumented with K-files up to a size 50 file at the working length. The canals were flared throughout the entire filing process by a circumferential filing technique. During instrumentation, care was taken to maintain an apical

stop; and the specimens were kept moist by holding them in gauze. To simulate the in-vivo apical counter pressure and to prevent any flow-through of the irrigant, the apices were sealed with boxing wax.

2.5. Irrigation:

The amount of irrigant used in each root canal was carefully controlled; the solution being delivered with a hypodermic syringe and a 25-guage needle. After every file size, recapitulation was done, and the canals were flushed with 3ml of the respective irrigant. The treatment time was also carefully monitored so that the total time of chemo mechanical canal preparation was always between 10-13 minutes. Before instrumentation, the teeth were randomly divided into 4 groups of 5 teeth each and different irrigation protocols were used in each group.

Group I (0.9% physiological saline)

The irrigant used during instrumentation was 0.9% physiological saline solution. After instrumentation, the canals were given a final flush with 3 ml of saline solution for 1 minute. Saline was evaluated as a standard for comparison with each of the three test groups because of its lack of any chemical effect.

Group II (NaOCl-H_2O_2-NaOCl) The root canals were irrigated with 3ml each of 5.25% NaOCl and 3% H_2O_2 alternately between each file, followed by irrigation with 5 ml of saline [15]. Finally, irrigation with 3 ml of NaOCl solution for one minute, followed by a final rinse with 3 ml of deionized water to terminate any solvent action of the irrigants and also to avoid the development of NaCl crystals.

Group III (NaOCl-H_2O_2-NaOCl and EDTA-NaOCl)

The same sequence of irrigation was used as for Group II, except that after the canals had been mechanically prepared using NaOCl-H_2O_2-NaOCl as the basic irrigation regime alternately between each file size, the root canals were irrigated for 3 minutes with 10ml of 17% EDTA solution as a final flush at the end of instrumentation, after which the canals were irrigated with 3 ml of 5.25% NaOCl followed by a final rinse with 3 ml of deionized water to halt the chemical activity of the irrigants. The 17% EDTA solution was prepared by dissolving 17gm of Disodium salt of EDTA, 17gm of 5N NaOH and Distilled water up to 100ml. (As recommended by Nyggar- Ostby) [16]. Group IV (NaOCl-H_2O_2-NaOCl and EGTA-NaOCl) The same sequence was used as for Group III, except, that a high volume final flush with 10ml of 17% EGTA solution was used at the end of instrumentation. Same technique as for preparing EDTA was followed, with disodium salt of EGTA.

2.6. SEM Examination And Smear Layer Rating

The canals were then dried with sterile absorbent paper points and the teeth were air dried for 3 days. Then the boxing was removed and the teeth were split carefully along the longitudinal grooves, with a hammer and chisel. Specimens were conserved, coded and prepared for SEM examination. All were examined under a Scanning Electron Microscope (S-2400) to evaluate the presence or absence of smear layer in the dentinal surfaces and visualization of the entrance to dentinal tubules of the middle and apical thirds of each root canal to compare the effectiveness of each irrigant regime. The coronal third of the canals were not evaluated because cutting debris from root amputation could have affected the amount of superficial debris in this area. Photomicrographs of representative areas on each of the middle and apical thirds were taken with a magnification of x 1000, x 2000, and x 5000 magnification in order to determine the effectiveness of the irrigants being tested at both the levels. The root canal cleanliness was qualitatively evaluated at the middle, and apical regions of each root half of each specimen by a faculty, who was blind to the irrigation regimen employed for each group. The scores were attributed using a graded scale from 0 to 2 to assess the quality of smear layer removal according to Abbot et al [1] [TABLE -1].

Table 1: The smear layer rating system

Score	Contents
0	No smear layer on the surface of root canals (all the tubules clean and open with no debris)
1	Partial smear layer on the surface of root canals (some tubules open and some tubules partially occluded with scattered debris)
2	Total smear layer covering the surface of root canals (very few or no open tubules and tubules occluded with debris)

2.7. Statistical Analysis

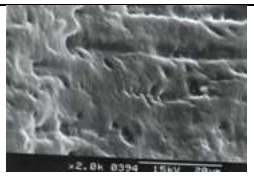
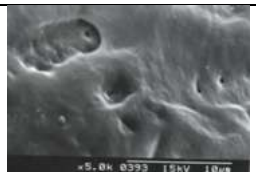
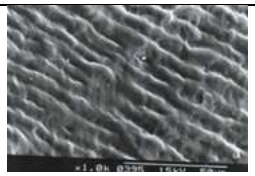
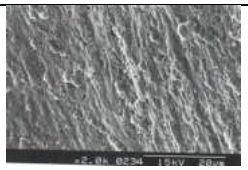
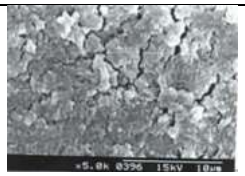
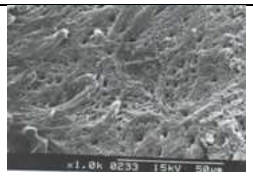
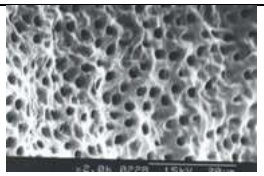
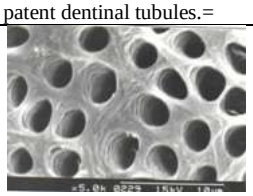
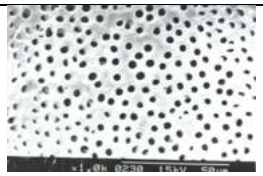
The results were tabulated and data were analyzed using nonparametric statistical analysis techniques using SPSS software (SPSS Inc, Chicago). Nonparametric data of smear layer scores were presented as a percentage distribution and their mean and median scores were calculated for each group at both middle and apical thirds. Kruskal-Wallis test was used to determine if there were significant differences between the four

groups. The Mann Whitney U-test was carried out for intergroup comparisons for middle and apical thirds separately to confirm the significant differences between pairs of groups. The significance level was set at $p \leq 0.05$, which was corrected by bonferroni's method.

III. Results

The observations made in this study indicated that there was a significant difference in the effectiveness of different irrigation regimes in removing the smear layer at the middle and apical thirds. (TABLE-2) Group I specimens treated with 0.9% physiological saline showed a heavy smear layer, in the middle and apicals that covered whole of the root canal surface; occasionally the location of some tubules were apparent. (TABLE 2). When only NaOCl and H_2O_2 were used as the irrigant solutions (Group II), in the middle third, most of the dentinal tubules openings were occluded by the smear layer, while in the apical third total smear layer with no open dentinal tubules were observed. Occasionally the locations of some tubules were apparent, as a crack over the apertures of the tubules. Group III specimens irrigated alternately with 5.25% NaOCl and 3% H_2O_2 followed by 17% EDTA as the final flush had no smear layer in the middle third of any of the specimens. The apical third of one specimen had a partial smear layer, while all the other canals had apparently open dentinal tubules which were regularly distributed, but EDTA caused erosion of the tubules. The combination of EGTA and NaOCl-H_2O_2 irrigation (Group IV) was found to be effective in removing the smear layer from the dentin walls. In these specimens, tubules were not obscured by the smear layer in the middle third and were completely open to the canal surface. EGTA did not cause erosion of the intertubular and peritubular dentin. However, in the apical third, only two specimens had smear free root canal surface; whilst remaining three specimens had partial smear layer with clogged dentinal tubular orifices. The effects of the three test irrigation regimes were also compared on longitudinal sections (TABLE 3). In Group I (saline) specimens heavy smear layer was observed on the canal walls with obliterated tubular apertures and smear plugs extending into the tubules where as in Group III (EDTA) and Group IV (EGTA) specimens, tubular openings seemed to be unplugged in both. But EDTA specimens displayed widening of the tubules and erosion at the site of openings as compared to EGTA treated specimens. TABLE 4 shows the individual smear scores of all the groups. Thus it was observed that the three test groups displayed more variations among them, than within a single group, except for EGTA in the apical third. It is clear from the tables that, Group III (17% EDTA) scored lowest of scores among all the three test groups; at both the middle and apical thirds.

Table 2: SEM image of root canal walls at the middle and apical thirds

Irrigation regime	**Middle third**		**Apical third**
	x 2000	**x 5000**	**x 1000**
Group I **Physiological Saline**	 **Score=2** Root canal wall showing total coverage by smear layer, with minute openings indicating dentinal tubular orifices.	 **Score=2** Typical amorphous smear layer, with few dentinal tubules.	 **Score=2** No visible tubular orifices.Undissolved heavy layer appears as an irregular surface.
Group II **NaOCl-H_2O_2-NaOCl**	 **Score=2** Tubular apertures are totally obliterated by an adherent smear layer.	 **Score=2** Smear layer not removed, surface appeared cracked, no patent dentinal tubules.=	 Heavy smear layer, position of some tubular orifices apparent.
Group III **NaOCl-H_2O_2-NaOCl and EDTA-NaOCl**	 **Score=0** Smear layer completely removed, dentinal tubules are open to canal surface.	 **Score=0** Root canal walls appear clean without smear layer, dentinal tubules are wide open, with	 **Score=0** Apparent and regularly distributed opened tubular orifices, smear layer has been effectively

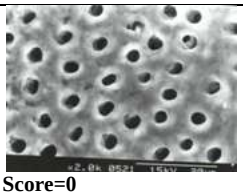
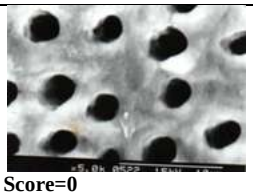
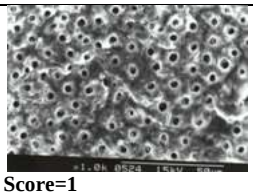
		peritubular and intertubular erosion.	dissolved.
Group IV **$NaOCl$-H_2O_2-NaOCl and EGTA-NaOCl**	 **Score=0** Clean, smoothly planed appearance of the canal wall, with the smear layer completely removed.	 **Score=0** Clean instrumented root canal, with no smear layer and sharply defined dentinal tubules orifices.	 **Score=1** Scattered open tubular orifices, some are blocked by smear layer; partially dissolved smear layer .

Table 3: Effect of different irrigation regimes on the radicular dentine in longitudinal section

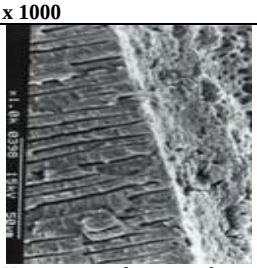
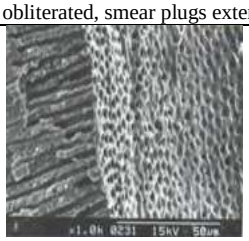
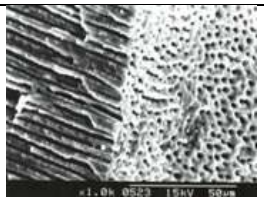
Irrigation regime	**x 1000**
Group II **$NaOCl$-H_2O_2-NaOCl**	 Heavy smear layer on the canal walls, tubular apertures are totally obliterated, smear plugs extending into the tubules.
Group III **EDTA**	 The superficial smear layer and smear plugs completely removed, and dentinal tubular orifices are open and enlarged.
Group IV **EGTA**	 The superficial smear layer and smear plugs completely removed, and dentinal tubular orifices are open but not enlarged.

Table 4: Smear layer ratings in all groups at middle and apical thirds

Group	**Middle third**							**Apical third**						
	Specimens					**Mean(SD)**	**Median(IQR)**	**Specimens**					**Mean(SD)**	**Median(IQR)**
	1	**2**	**3**	**4**	**5**			**1**	**2**	**3**	**4**	**5**		
GroupI Saline	2	2	2	2	2	2(0)	2(0)	2	2	2	2	2	2(0)	2(0)
GroupII $NaOCl - H_2O_2$	2	2	2	2	2	2(0)	2(0)	2	2	2	2	2	2(0)	2(0)
GroupIII EDTA	0	0	0	0	0	0(0)	0(0)	0	0	0	1	0	0.2(0.447)	0(0.5)
GroupIV EGTA	0	0	0	0	0	0(0)	0(0)	0	1	0	1	1	0.6(0.55)	1(1)

Statistical analysis of the observations made in this study indicated that there was a significant difference in the effectiveness of different irrigation regimes in removing the smear layer at the middle and apical thirds. (For middle –third, Kruskal-Wallis χ^2=19.0, p=0.0001; For apical third; Kruskal-Wallis χ^2=17.3, p=0.001) TABLES 5 and 6 show that there are highly significant differences between the groups which are illustrated in Fig. 1 & 2.

Table 5: K-W one way ANOVA for overall comparison between the four groups- at middle third

Groups	Mean	Median	SD	IQR	H	p-value	Significance
I Saline	2	2	0	0	19.00	0.0001	HS
II $NaOCl-H_2O_2$	2	2	0	0			
III EDTA	0	0	0	0			
IV EGTA	0	0	0	0			

$p < 0.05$. HS: Highly Significant

Table 6: K-W one way ANOVA for overall comparison between the four groups- at apical third

Group	Mean	Median	SD	IQR	H	p-value	Significance
GroupI Saline	2	2	0	0	17.30	0.001	HS
GroupII $NaOCl-H_2O_2$	2	2	0	0			
GroupIII EDTA	0	0	0	0			
GroupIV EGTA	0	0	0	0			

$p < 0.05$. HS: Highly Significant

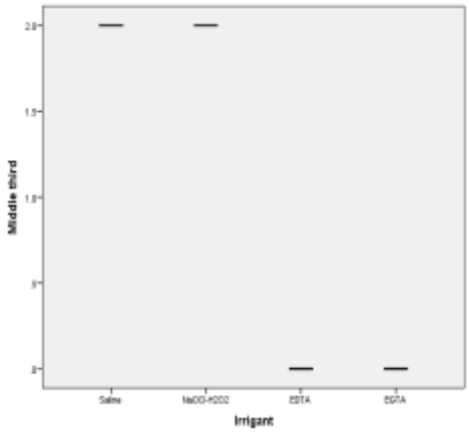


Fig 1: Box plot showing significant differences between the groups in their smear layer rating scores at middle third. Groups I and II having significantly higher scores than Group III and IV.

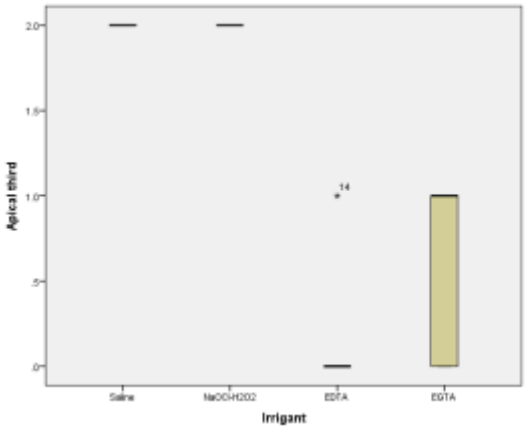


Fig 2: Box plot showing significant differences between the groups in their smear layer rating scores at apical third. Groups I and II having significantly higher scores than Group III and IV. Group III having lowest scores (a single outlier) but Group IV shows greater variability within the group.

Mann Whitney U-test was used to compare between groups at both the middle and apical thirds and the results revealed that Group I (saline), differed significantly from Group III (EDTA) and Group IV (EGTA) in its effectiveness on removing smear layer, in the middle and apical thirds ($p<0.01$). But there was no significant difference in the debridement ability between Group I (saline) and Group II ($NaOCl-H_2O_2$), either in the middle or in the apical thirds ($p>0.05$). Comparing with Group II ($NaOCl-H_2O_2$), Group III (EDTA) and Group IV (EGTA) showed highly significant results for both the middle third and the apical third ($p<0.01$). But on comparing Group III (EDTA) and Group IV (EGTA) there was actually no statistically significant difference in their ability to remove smear layer in the middle and apical thirds ($p>0.05$), but Group III (EDTA) showed a lower score than Group IV (EGTA) in the apical third ($p>0.05$) (TABLES 7 & 8)

Table 7: Intergroup comparison using Mann –Whitney U test - at middle third

Groups	Mean	Median	SD	IQR	M-W U	p-value	Significance
Saline $NaOCl – H_2O_2$	2 2	2 2	0 0	0 0	12.5	1	NS
Saline EDTA	2 0	2 0	0 0	0 0	0.0	0.003	HS
Saline EGTA	2 0	2 0	0 0	0 0	0.0	0.003	HS
$NaOCl – H_2O_2$ EDTA	2 0	2 0	0 0	0 0	0.0	0.003	HS
NaOCl EGTA	2 0	2 0	0 0	0 0	0.0	0.003	HS
EDTA EGTA	0 0	0 0	0 0	0 0	12.5	1	NS

$p < 0.05$. HS: Highly Significant , NS: Not significant
p values were adjusted by bonferroni's correction

Table 8: Intergroup comparison using Mann –Whitney U test - at apical third

Groups	Mean	Median	SD	IQR	M-W U	p-value	Significance
Saline $NaOCl – H_2O_2$	2 2	2 2	0 0	0 0	12.5	1	NS
Saline EDTA	2 0	2 0	0 0	0 0	0.0	0.004	HS
Saline EGTA	2 0	2 0	0 0	0 0	0.0	0.005	HS
$NaOCl – H_2O_2$ EDTA	2 0	2 0	0 0	0 0	0.0	0.004	HS
NaOCl EGTA	2 0	2 0	0 0	0 0	0.0	0.005	HS
EDTA EGTA	0 0	0 0	0 0	0 0	7.5	0.2	NS

$p < 0.05$. HS: Highly Significant , NS: Not significant
p values were adjusted by bonferroni's correction.

IV. Discussion

Mc Comb and Smith, the first researchers to describe the smear layer on the surface of instrumented root canals [5] suggested that the smear layer consisted not only of dentin as in the coronal smear layer, but also the remnants of odontoblastic processes, pulp tissue, and bacteria. Mader et al reported that the smear layer thickness was generally 1-2 μm [17] and it consisted of a superficial smear layer and material packed into the dentinal tubules to a depth of 40 μm. Though smear layer removal is still a controversy, most of the research has focused on its removal. The purpose of this study was to the evaluate effectiveness of different irrigation regimes in removing dentin smear layer in roots of permanent teeth. SEM was opted in this study as the tool for evaluating the smear layer because it is commonly available. Other techniques like digital image analysis can overcome the possible evaluator bias, takes less time, and can also measure other parameters of interest like density and average diameter of dentinal tubules [10]. In Group I (control group) and Group II, where normal physiological saline and NaOCl- H_2O_2- NaOCl regime respectively were used as the endodontic irrigants, the dentinal tubules were completely covered by smear layer in the middle and apical thirds under SEM. These findings were in agreement with the results of many other studies which have also reported that NaOCl leaves the prepared canal wall entirely covered with a smear layer which even a final flushing with the irrigant cannot remove [5,18-20]. Though NaOCl solution is still the best known irrigant because of its low toxicity, its good antibacterial and excellent tissue dissolving effects [22] it would be unlikely to remove the smear layer consisting of inorganic, calcified debris [22,23].

The alternating use of H_2O_2 and NaOCl solutions was often advocated in the past [7]. The results of this study confirm previous reports, that this combination was not more effective in removing smear layer than NaOCl alone as evidenced by the total coverage of root canal walls by the typical amorphous smear layer in both the middle and apical thirds of Group III specimens (NaOCl- H_2O_2). In the present study, the use of 17% EDTA for 3 minutes and a final rinse with 3 ml of NaOCl at the end of instrumentation (Group III), effectively removed the smear layer and opened the dentinal tubules in both the middle and apical thirds. Patent dentinal tubules were clearly seen which indicated absence of smear layer and tubular orifices were not plugged with

debris. The observation that smear layer was removed by chelating agents was reported by Goldman LB [19] and Yamada [13]. The chelating solutions removed the smear layer and NaOCl the superficial debris, but none of these agents used alone were able to remove the debris completely. Bystrom et al, Blackler S and Baumgartner J C et al have also reported that alternating NaOCl and a chelating agent, such as EDTA was extremely effective and superior to the use of NaOCl or EDTA alone in cleaning the canal system [4,19,24]. Pranav Khaord et al [25] also recommended the alternate use of NaOCl and EDTA for the efficient removal of smear layer.

The association between NaOCl and EDTA has been shown to produce a stronger bactericidal action than NaOCl alone [4]. However, the combination of NaOCl and EDTA produced additional effects on the instrumented root canal walls. Removal of calcium ions from the dentin by the chelator (EDTA), softened the dentinal tissue, especially the hydroxyapatite–rich peritubular dentin and increased the diameter of exposed dentinal tubules [26]. Baumgartner and Mader [19] reported that the combination of NaOCl and EDTA caused a progressive dissolution of the dentin at the expense of peritubular and intertubular areas so that the diameters of tubular orifices on the instrumented canal walls were enlarged to 2.5 to 4µm. This is confirmed in this study, as the diameter of the tubular orifices appeared to be enlarged in the specimens treated with EDTA.

The operational sequence used for Group III (i.e. final irrigation with EDTA, and then NaOCl was aimed at encouraging as much as possible the action of NaOCl in depth [27]. A high volume of EDTA (10ml) removed the smear layer and a concentration as high as 17% was necessary, because it was to be expected that it would be diluted by the NaOCl already present in the canal. The time of 3 minutes administration of EDTA was selected for this study, as a clinical compromise: on the one hand to allow the chelating agent to develop its effect without causing severe erosion of the dentinal tubules [28] and on the other hand to make only a small addition to the treatment time [20]. It was in agreement with the findings of Von der Fehr and Ostby [28] and Ciucchi S et al [20].

Many research works were carried out to find an agent which effectively removes smear layer without causing demineralization of dentin. Thus another chelating agent, EGTA, (Ethylene glycol-bis (β-amino ethyl ether) N',N',N', N' tetraacetic acid, which is widely used in Molecular Biology labs for thrombolysis and platelet segregation, has been introduced to the field of endodontics. EGTA is reported to bind Ca^{2+}, more specifically [14]. So its effect on removal of calcium rich smear layer has been compared to that of EDTA under the same experimental conditions in this study.

In Group IV, it was observed that after instrumentation, a final high volume flush with 17% EGTA and then NaOCl effectively removed the smear layer and opened the dentinal tubules in the middle third of the root canal, without inducing erosion and conjugation of dentinal tubules as seen with 17% EDTA administration, but EGTA was not as effective in smear layer removal as EDTA, as the apical portion of the root canal was approached. The advantage of EGTA was that, diameter of the tubular orifices in the specimens treated with EGTA were less enlarged. This supports the report of Semra Calt and Ahmed Serper [14]. But whether EGTA could be used as an alternative chelator to EDTA needs further investigation.

These results show that EDTA action is stronger than that of EGTA, as EDTA has effectively removed smear layer from both the middle and apical thirds of the root canals and it is not clear that the erosion and joining of orifices from the action of EDTA is deleterious [14].

Conclusion

Within the limitations of this study, it can be concluded that:

1. The use of saline as the only irrigant left a typical amorphous smear layer on the root canal walls.
2. The alternate use of NaOCl and H_2O_2 failed to remove smear layer from the middle and apical thirds of the root canals surface.
3. The use of EGTA in combination with NaOCl and H_2O_2 effectively removed smear layer from the middle third of the root canals, without inducing peritubular and intertubular erosion.
4. There was a decline in the debridement ability of EGTA as the apical end of the canal was approached.
5. Although the diameters of the exposed dentinal tubules were enlarged by the combination of NaOCl and EDTA, the most effective irrigation regime was the use of EDTA as a high volume flush at the end of instrumentation, as it completely removed the smear layer from both the middle and the apical thirds.

Smear layer removal is controversial and certainly, not the only factor affecting root canal therapy. From this perspective, it is important to consider the performance of irrigating solutions under several different biological and chemical conditions and in relation to different treatment techniques. Finally, it should be emphasized that, as with most in vitro studies, the findings of this study remains to be confirmed clinically.

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