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Comparative evaluation of smear layer removal efficacy of conventional endodontic irrigants with fumaric acid along with chlorhexidine and metrogyl – A steromicroscopic study

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Abstract

Aim: The aim of the study is to evaluate and compare the smear layer removal efficacy of fumaric acid along with chlorhexidine and metrogly against Ethylenediamine tetraacetic acid (EDTA), in the coronal, middle and apical third of the canal.

Materials And Method: Forty single rooted human mandibular extracted premolars were decoronated to a standardized length of 14mm. The teeth were shaped up to size F3 (Super Endo, super gold flex files) followed by irrigation with 3% Sodium hypochlorite. Teeth were divided into 4 groups [n = 10]; group 1: EDTA (control group), group 2: fumaric acid, group 3: fumaric acid

with chlorhexidine and group 4: fumaric acid with metrogyl. Each specimen in the group was subjected to the respective solution as the final irrigant. Each tooth was split longitudinally and were then observed using stereomicroscope. The evaluation was made based on the presence of smear layer at the coronal, middle and apical third. Statistical analysis was done by two-way ANOVA test followed by post hoc tukey's test for pairwise comparison.

Result: Smear layer removal was found to be seen significantly more amongst the samples irrigated with fumaric acid with chlorhexidine and fumaric acid with metrogyl, followed by fumaric acid and then EDTA, in the apical one third of the canal.

Conclusion: Within the limitation of our study fumaric acid with metrogyl and fumaric acid with chlorhexidine is better in removing the smear layer from apical third when compared to EDTA and Fumaric acid, whereas in coronal and middle third no statistical difference was found between any groups.

Keywords: Irrigation, Smear Layer, EDTA, Fumaric Acid, Chlorhexidine , Metrogyl.

Introduction

A very essential step for endodontic success is debridement of the smear layer which is created on shaping of root canals that consists of organic and inorganic substances including microorganisms, necrotic materials, and fragments of odontoblastic processes. [1-4] Prevention of penetration of intracanal disinfectants and sealers into the dentinal tubules results in compromise in the seal of the root filling due to the presence of smear layer. [5-7] To reduce residual debris, necrotic tissue and bacteria, formed due to smear layer, many irrigating solutions have been used during mechanical instrumentation of the root canal system. [5,9]

Complete organic and inorganic components of the smear layer cannot be eliminated with the use of a single irrigating solution; hence a combination of different root canal irrigants with root-canal instrumentation should be used. [6]

The most widely used irrigating solution in endodontics is sodium hypochlorite (NaOCl). [10] The adjunctive use of calcium-chelating agent, EDTA, has been recommended for the efficient removal of the smear layer. [4,11] However, it was reported by Ballal *et al.* [12] that 17% EDTA was less efficient in the apical third of the canal when used for 1 min; when used for more than 1 min, it caused erosion of both inter- and peri-tubular dentin reducing the micro hardness of dentin. [13,14] The smear layer was efficiently removed by maleic acid at the apical level when used in combination with NaOCl. [12]

Fumaric acid (Butene-1,4-dioic acid) which is a trans-isomer of maleic acid. [15] It has spectacular properties of being anti-carcinogenic, non-toxic, anti-inflammatory, and has growth modulatory potential. [16] In citric acid cycle during glucose metabolism fumaric acid is a well-known key intermediate product, and its esters have been used successfully for the treatment of psoriasis and multiple sclerosis. [17,18] Hence this new solution was used in the present study to evaluate its efficacy for the smear layer removal from the root canal system. The purpose of this study was to evaluate the efficacy of 0.7% fumaric acid, 0.7% fumaric acid with chlorhexidine and 0.7% of fumaric acid with metrogyl in comparison with 17% EDTA in smear layer removal at coronal, middle, and apical levels of the root canal system by stereomicroscope.

Methodology

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, HKES's S. Nijalingappa Institute of dental sciences and research, Kalaburagi. Forty human permanent single-rooted mandibular premolars were extracted for therapeutic purposes like in case of orthodontic or severe periodontal disease. The extracted human mandibular premolars which were non-carious, single canal with straight roots were selected. The absence of resorption, cracks, fractures, and calcifications was confirmed through radiographs. The calculus and soft tissues were removed with an ultrasonic scaler and kept in 0.1% thymol solution until use. The selected samples were divided randomly into 4 experimental groups (n = 10 each). The samples were decoronated with a straight fissure diamond bur, standardizing the working length at 14 mm [Fig 1-3]. A #10 and #15 K files were utilized to scout the overall length of a canal and create a smooth, reproducible glide path before introducing any rotary instruments.

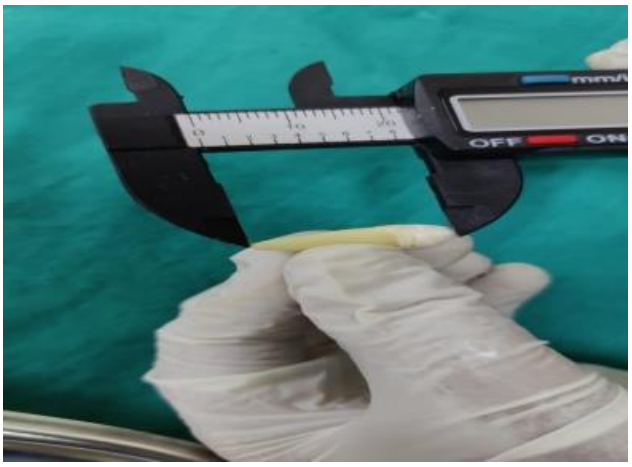


Fig 1: Measuring with vernier callipers.

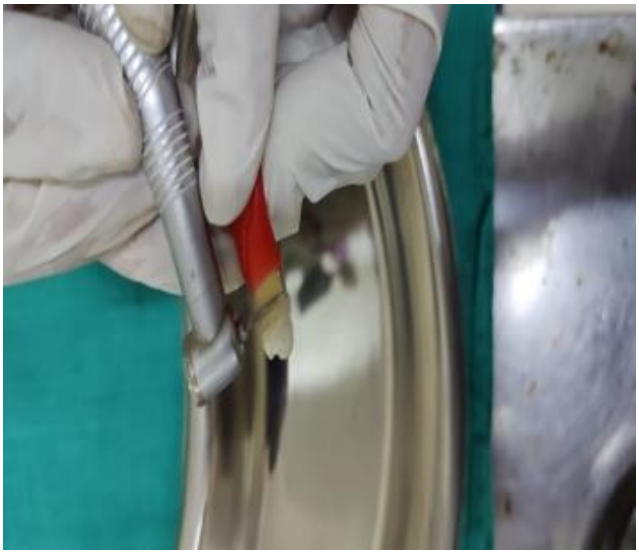


Fig 2: Decoronation of the tooth.

Fig 3: Decoronated samples.

Fig 4: RVG images.

Canals were then prepared up to an apical preparation of size #F3 according to manufacturer's instructions [Fig 6]. 1 ml of 3% sodium hypochlorite as irrigant was used between each subsequent file in all teeth specimens [Fig 7].

Fig 5: Irrigating solutions

Fig 6: Cleaning and shaping with rotary file.

Fig 7: Irrigating with syringe

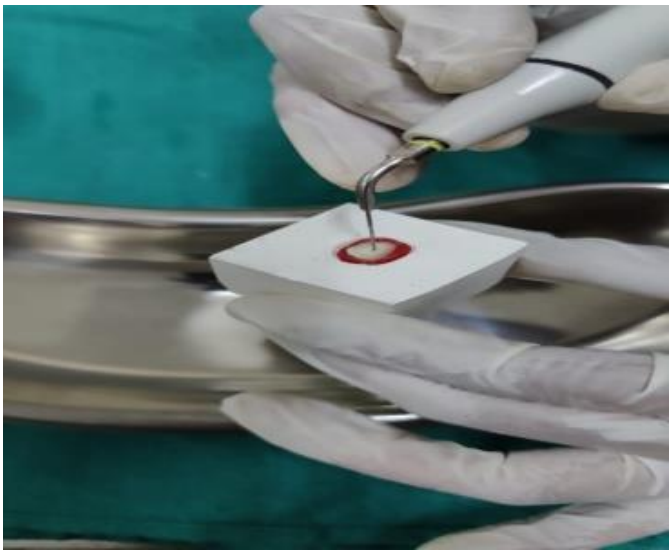


Fig 8: ultrasonic activation.

Fig 9: Longitudinal grooves.

All the teeth were randomly divided into 4 groups accordingly,

GROUP 1: 5 ml of 17 %EDTA

GROUP 2: 5ml of 0.7 %FUMARIC ACID

GROUP 3: 5ml of 0.7 % FUMARIC ACID WITH CHLORHEXIDINE.

GROUP 4: 5ml of 0.7 % FUMARIC ACID WITH METROGYL

Root canal irrigants i.e, 17% EDTA, 0.7% Fumaric acid, 0.7% fumaric acid with CHX and 0.7% fumaric acid with metrogyl were delivered with 5 ml syringe of 26-gauge needle, keeping the needle 1 -2 mm short of working length.

In all the groups, final irrigation was done using 5 ml of 3% sodium hypochlorite (which was activated by using passive ultrasonic [Fig 8]) for 1 min followed by 5 ml of saline for 1 min, to remove any precipitate generated during the cleaning and shaping process and then they were dried with sterile paper points.

The access cavity was closed with sterile cotton pellet; longitudinal grooves were prepared on buccal and lingual surface of each root with slow speed silicon carbide disc without penetrating into root canal, each tooth was slit into 2 halves using chisel and mallet and were stored in distilled water [Fig 9].

Samples were dehydrated using ethyl alcohol and both halves of each split tooth were divided into cervical, middle, and apical thirds measuring the CEJ to the terminus of apical preparation using an indelible pencil. All sections were stained with 1% methylene blue for 2 mins and were allowed to bench dry. The stained sections were then attached to individual glass slides using beading wax, coded and arranged for evaluation. Scoring for the presence of smear layer for both halves of the split teeth were carried out visually at cervical, middle and apical thirds using a light stereomicroscope at 40X and 70X magnification.

Stereomicroscopic Evaluation

Canal cleanliness was evaluated by photographs taken at apical, middle and coronal thirds at a 1000X magnification. Stereomicroscopic grading for the specimen's samples were graded according to the rating scale from 1-3.

Scale 1 - indicating a clean canal with no or very little debris.

Scale 2 - for debris present in less than half the evaluated canal.

Scale 3 - for debris occupying more than half of the evaluated canal.

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Descriptive was reported for each variable. Descriptive statistics such as and standard deviation mean for continuous variables and frequency with percentages of categorical variables were calculated.

Summarized data was presented using Tables and Graphs. Shapiro Wilk test was used to check which all variables were following normal distribution. As Data was found to be normally distributed (p-value was more than 0.05) bivariate analyses was performed using Two-way ANOVA test followed by post hoc Tukey's test for pair wise comparison. Level of statistical significance was set at p-value less than 0.05.

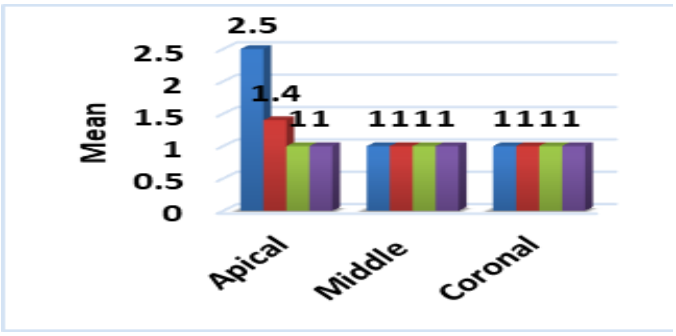
Results

In intergroup comparison, on apical end, smear layer removal was found to be seen significantly more among samples irrigated with fumaric acid with CHX and fumaric acid with metrogyl followed by fumaric acid and then EDTA [Graph 1].

At middle and coronal end no differences were observed in smear removal efficacy of four study irrigants. In Intragroup comparison, smear layer removal efficacy was found to be more effective in coronal and middle than in apical third in group 1 and 2 [Table 1].

Table 1: Statistical significance at p<0.05

		GROUP1 (EDTA)	GROUP 2 (FUMARIC ACID)	GROUP 3	GROUP 4 (FUMARIC ACID WITH	P value (Intergroup)	Post hoc
Apical	Mean	2.5000	1.4000	1.0000	1.0000	0.0001	Group 1>2>3,4
	N	10	10	10	10		
	Std. Deviation	.52705	.51640	.00000	.00000		
Middle	Mean	1.0000	1.0000	1.0000	1.0000	-	
	N	10	10	10	10		
	Std. Deviation	.00000	.00000	.00000	.00000		
Coronal	Mean	1.0000	1.0000	1.0000	1.0000	-	
	N	10	10	10	10		
	Std. Deviation	.00000	.00000	.00000	.00000		
P value (Intragroup)		0.0001	0.007	-	-		
Post hoc pairwise		Apical	Apical >coronal,middle				



Graph 1: Comparison of Group 1(Blue), Group 2(Red), Group 3(Green), Group 4(Purple) at apical, middle and coronal portions of root.

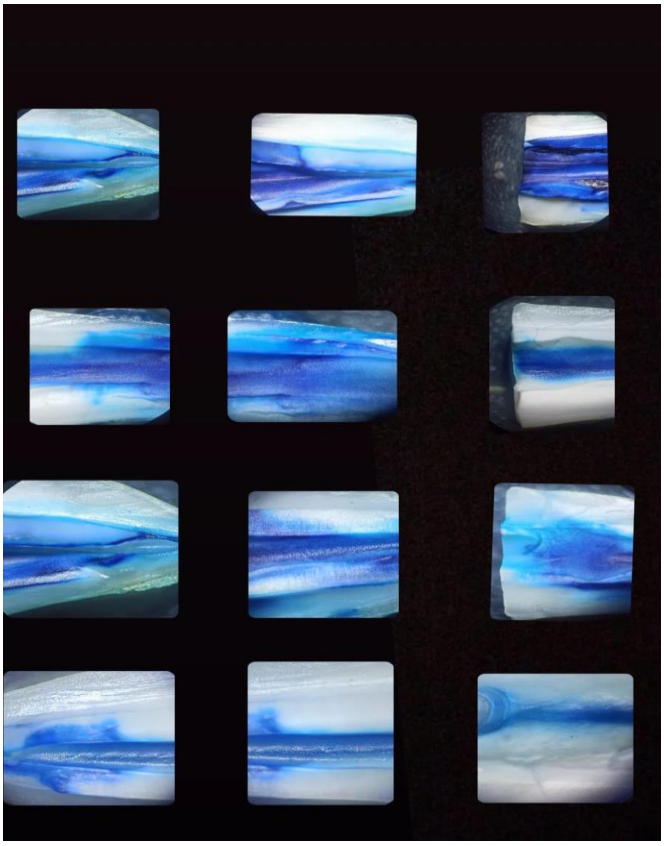


Fig 10: Stained sections under microscope

Discussion

Chelating agents are lubricating agent that remove smear layer, created during biomechanical preparation. The efficiency of chelating agent depends on application time, pH, concentration, and amount of the solution. If highly concentrated solutions are applied for long period, it causes roughness of the dentin surface. (19)

EDTA is the most widely used chelating agent. The activity of EDTA is due to the chelation of cations from the outer membrane of bacteria that produce a zone of bacterial growth inhibition, hence the antibacterial activity of EDTA is limited. [20] The properties of EDTA are self-limiting because of the neutral pH. Since 99% of EDTA exist as $EDTAH^3$, the exchange of Ca^{++} from dentin will occur by H^+ ion causing subsequent decrease in pH, hence increase in acidity; so, the effect of EDTA decreases with increase in acidity. [21]

Very recent studies have shown that neutral EDTA solution can reduce the Ca^{++}as well as non-collagenous proteins (NCP) and as concentration of NCP decreases in the apical third zone of root dentin. A decrease in NCP concentration in this area may result in a lesser degree of decalcification by EDTA. [22] As apical third of root canal is sclerosed, the smear layer may not be removed at neutral pH by EDTA. The larger molecules will bind to a smaller number of Ca^{++}into the narrow canals. Due to its higher molecular size than that of fumaric acid its demineralization effect may be limited. [23,24]

Unlike EDTA, the demineralization effect of fumaric acid depends on the concentration of hydrogen ions since it is an organic acid. The pH of 0.7% fumaric acid was obtained as 2.70, after calculation. Since dentin demineralization occurs at pH 4–5, fumaric acid can well demineralize and remove the smear layer. [23] As fumaric acid has two carboxylic groups always opposite to each other, it has shown significantly better smear layer removal in their 3-D orientation and can bind with more of Ca^{++}present in intraradicular dentin. It is non-toxic, biocompatible, anti-carcinogenic, anti-inflammatory, non-absorbable and growth modulatory in action. Metrogyl has proven to have antibacterial properties in many literatures, but its smear layer capacity was not evaluated. Therefore, fumaric acid was investigated as a root canal irrigant for smear layer removal, and metronidazole (Metrogyl) was chosen as an adjuvant to enhance its efficacy.

In 1957, the salts originally employed were acetate and hydrochlorite, both the salts suffer from relatively poor water solubility and were largely replaced by the digluconate, highly water soluble salt, which is a aqueous solutions of CHX and is more stable within the 5-8 pH range. [25] CHX is a very strong base and is

more stable in its salt form. CHX exhibits antimicrobial activity that is pH-dependent, with an optimal range of 5.5-7.0, corresponding to the pH of body surfaces and tissues [26] At the physiological pH, CHX readily dissociates, releasing the positively charged CH component. The cationic molecule of the drug, binds to extra-microbial complexes and negatively charged microbial cell walls, thereby altering the osmotic equilibrium of the cells providing its bactericidal effect. Low molecular weight substances will leak out at lower concentrations, especially potassium and phosphorous, resulting in a bacteriostatic effect. CHX has a bactericidal effect at higher concentrations due to precipitation and/or coagulation of the cytoplasm of bacterial cells, probably caused by protein cross-linking, resulting in cell death, [27,28] and leaving cell debris in the root canals, [29] which can be removed with a vigorous irrigation with distilled water.

The use of CHX gel as an intracanal medicament has been evaluated and shown to perform well (30). CHX gel as an endodontic irrigant was used in 2001, by Ferraz et al. [31] Due to its antimicrobial and substantivity properties, CHX can be used during all phases of root canal treatment including the disinfection of operatory field. In cases of open apex, root resorption, foramen enlargement and root perforation CHX has been recommended as an alternative to NaOCl, due to its biocompatibility, or in cases of allergy related to bleaching solutions. [32]

Conclusion

Within the limitation of our study, fumaric acid with metrogyl and fumaric acid with chlorhexidine is better in removing the smear layer from apical third with respect to EDTA and Fumaric acid, whereas in coronal and middle third no statistical difference was found between any groups.

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