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Comparative Evaluation Of Retrievability Of Calcium Hydroxide From Apical Third Of Root Canal With Commercially Available Root Canal Irrigants: An In-Vitro Study

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Abstract
Context: Calcium hydroxide is the most widely used intracanal medicament to aid chemomechanical preparation to thoroughly clean the root canal system. However, it is important to completely remove the medicament for predictable success of the root canal treatment.
Aim: This study was aimed to compare the effectiveness of irriganting solutions and irrigation methods to remove calcium hydroxide from the root canal system.
Material and Methodology: Eighty extracted single rooted teeth were selected and instrumented using ProTaper Gold rotary files, followed by application of calcium hydroxide. After incubating for a week, teeth were randomly allocated into 8 groups (n=10) based on irrigating solution (17% EDTA, 1% Phytic Acid, 3% NaOCl, 70% Ethanol) and method of irrigation used (PUI and NAI). The sample teeth were then sectioned horizontally to assess the residual calcium hydroxide on the canal walls under Confocal Laser Scanning Microscope.
Statistical analysis: The difference in the mean value of the two parameters was assessed by using analysis of variance (ANOVA). Tukey's test was used for post hoc multiple comparisons between different groups. The level of significance was fixed at $p<0.05$.
Results: None of the irrigants were able to completely remove the calcium hydroxide from the root canal. However, irrigation with 70% Ethanol presented significantly cleaner root canal walls in NAI.
Conclusion: 70% Ethanol presented with least amount of residual calcium hydroxide in comparison with phytic acid and commercially available irrigants i.e., EDTA and NaOCl in both NAI and PUI.
Keywords: *Intracanal Medicament, Calcium Hydroxide, Ethanol, Phytic Acid*

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I. Introduction

Periapical pathosis is commonly caused by bacterial invasion of diseased root canals, resulting from pulpal inflammation triggered by bacterial infection.[1] Periapical and pulpal inflammation is an immune system's self-defense strategy in reaction to prolonged bacterial stimulation, therefore, endodontic therapy concentrate on removing bacteria and their byproducts from root canal system.[2] Although chemo-mechanical root canal preparation helps to reduce the bacterial counts, it has been suggested that additional medication be used between appointments to thoroughly clean the root canal system and improve therapeutic outcomes.[3] Owing to its well-established antibacterial activity against the majority of endodontic pathogens and presentation of highly alkaline environment in which most organisms cannot thrive, calcium hydroxide is commonly used intracanal medicament .[4]

In order to attain optimal filling material adaption, the dentin wall must be cleared of debris, smear layer, and intracanal medicament.[5] The efficacy of root canal sealers to seal a canal may be adversely affected if the intracanal dressing is not entirely removed prior to obturation.[6] Moreover, the physical characteristics of sealers, their ability to penetrate dentinal tubules, the strength of their binding with root dentin, and even the filling of lateral canals are also impacted. Due to the higher likelihood of filling material dislodgment and fluid percolation from lateral canals, coronal and apical pathways, these problems may indicate a route for bacterial infiltration.[7]-[10] Additionally, apical leakage is thought to be the most frequent reason for endodontic failure and is impacted by a variety of factors, including the existence or absence of a smear layer, the chemical and physical characteristics of the materials used to fill root canals, and different filling techniques. An improperly constructed apical seal increases the likelihood of fluid seepage which may result in continuation of periradicular inflammation. To establish a good apical seal and the effectiveness of endodontic therapy, it is therefore essential to remove any intracanal medication from the apical third.

One of the most commonly documented procedures for eliminating intracanal calcium hydroxide is the use of master apical file till the working length, along with profuse irrigation using sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA).[11]-[13] Furthermore, a number of studies showed passive ultrasonic method of irrigation (PUI) rather than non-activated irrigation (NAI) enhanced the efficacy of calcium hydroxide removal.[12],[14],[15]

While EDTA has been shown in numerous studies, relatively fewer studies have examined the effectiveness of another potent calcium chelator- Phytic acid. According to a recent study, rinsing root canals with alcoholic solutions at the end shown increased sealer penetration and dentin's radicular wettability.[16]

Therefore, the aim of the present study was to comparatively evaluate the cleanliness of root canal walls (perimeter) and depth of dentinal tubules in the apical third region of the root canal after attempting to remove the calcium hydroxide dressing with 17% EDTA, 1% Phytic acid, 3% NaOCl and 70% Ethanol using Confocal Laser Scanning Microscope (CLSM). Furthermore, effect of using different types of irrigation method i.e., Non Activated Irrigation (NAI) and Passive Ultrasonic Irrigation (PUI) was studied on these outcomes.

II. Material And Methodology

The current study was approved by the institutional ethical committee and in this study, chelators namely 17% EDTA (META–Biomed Co., Ltd, Korea) and 1% Phytic acid (Tokyo Chemical Industry Pvt. Ltd.) were compared with non-chelators namely 70% Ethanol (Absolute chemicals Pvt. Ltd.) and 3% NaOCl (Prime Dental Products Private Limited, Pune, India) to evaluate their effectiveness in removing calcium hydroxide medicament from the apical one third of the root canals of eighty single rooted extracted teeth.

Study design

1. Sample selection and preparation:

- The study included eighty single rooted teeth with single canals, teeth with root caries, cracks, open apices and resorption were excluded. To clean the calculus and soft tissue debris periodontal curette and an ultrasonic scaler was used.
- Specimens were decoronated with a Diamond Disc while heavily misted with water until uniform lengths of 15 mm were reached (Figure 1a).
- Each tooth had its access cavity adjusted using a round bur #BR 46 (Mani Inc., Japan) and from the observed length of the canal, 1mm was subtracted to establish the working length. Upon ascertaining the working length, biomechanical preparation was performed using ProTaper Gold Rotary files up to F3 #30.09% (Figure 1b).
- Between each file, irrigation with 3ml of 3% NaOCl was done and 2ml of 17% EDTA was used for final irrigation (Figure 1c).
- 1gm of calcium hydroxide powder, 2ml of propylene glycol, and 1% of rhodamine B dye were combined to create calcium hydroxide paste (Figure 1d) which was placed in each sample using lentulospiral 3mm short of working length (Figure 1e), radiograph was taken to check the complete filling of the canal (Figure 1f). The access cavities were temporarily closed with a cotton pellet and intermediate restorative material (IRM).

2. Sample storage:

- The samples were thereafter kept for seven days at 37°C and 100% humidity in an incubator. Thereafter, two coats of coloured nail varnish were placed on each sample covering the apical foramen as well, to prevent leakage of the irrigant.

3. Sample Preparation for irrigation:

- To simulate the vapor-lock effect, a closed system comprising cylindrical tubes filled with silicone impression material was created for each specimen (Figure 1g). The temporary restoration and the cotton pellet were then removed, opening the coronal access.

- The intracanal medication was first removed by irrigating using 10 ml of normal saline solution and instrumentation using master apical file, i.e. F3, upto working length.
- The specimens were then categorised into four groups depending on the type of irrigating solution used (n=20) and each group was further divided into two subgroups depending on the type of irrigation method as shown in Table 1.

Group I (N=20)	**Subgroup I A (N=10)**	17% EDTA/ Non Activated Irrigation (NAI)
	Subgroup I B (N=10)	17% EDTA / Passive Ultrasonic Irrigation (PUI)
Group II (N=20)	**Subgroup II A (N=10)**	1% Phytic Acid/ Non Activated Irrigation (NAI)
	Subgroup II B (N=10)	1% Phytic Acid/ Passive Ultrasonic Irrigation (PUI)
Group III (N=20)	**Subgroup III A (N=10)**	3% NaOCl/ Non Activated Irrigation (NAI)
	Subgroup III B (N=10)	3% NaOCl/ Passive Ultrasonic Irrigation (PUI)
Group IV (N=20)	**Subgroup IV A (N=10)**	70% Ethanol / Non Activated Irrigation (NAI)
	Subgroup IV B (N=10)	70% Ethanol / Passive Ultrasonic Irrigation (PUI)

Table 1: Sample Distribution

4. Irrigation Protocol:

- Non-activated irrigation was done in the samples of subgroup A using a side vented nickel titanium needle which was inserted at 2mm from the working length (Figure 1h).
- Passive ultrasonic irrigation was carried out in samples of subgroup B with a 21mm stainless steel ultrasonic file driven by Woodpecker's Uds-P Ultrasonic Scaler (Figure 1i). In both the methods of irrigation 6ml of solution was used.
- All the specimens were then irrigated with 5ml of normal saline solution as the final rinse, it was followed by horizontally sectioning all the specimens 3mm from the apex (Figure 1j) and the thickness of the sectioned sample was measured by a digital vernier caliper with an accuracy of 0.01mm. (Figure 1k)

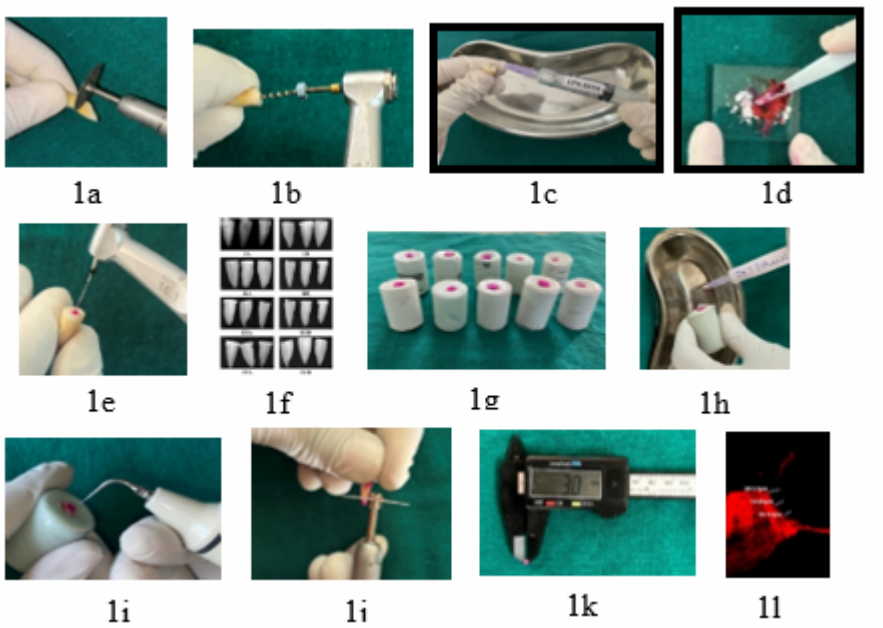


Figure 1

5. Confocal Laser Scanning Microscope

- These sectioned samples were then examined under Nikon Confocal Laser Scanning Microscope- Nikon A1R with helium neon laser as the light source and the excitation light source had a wavelength of 543nm and the fluorescent light was collected beyond 560nm for evidence of rhodamine B dye.
- The images obtained (Figure 2) were then analysed using imageJ software to calculate the percentage of clean perimeter of the root canal walls and depth of clean dentinal tubules.

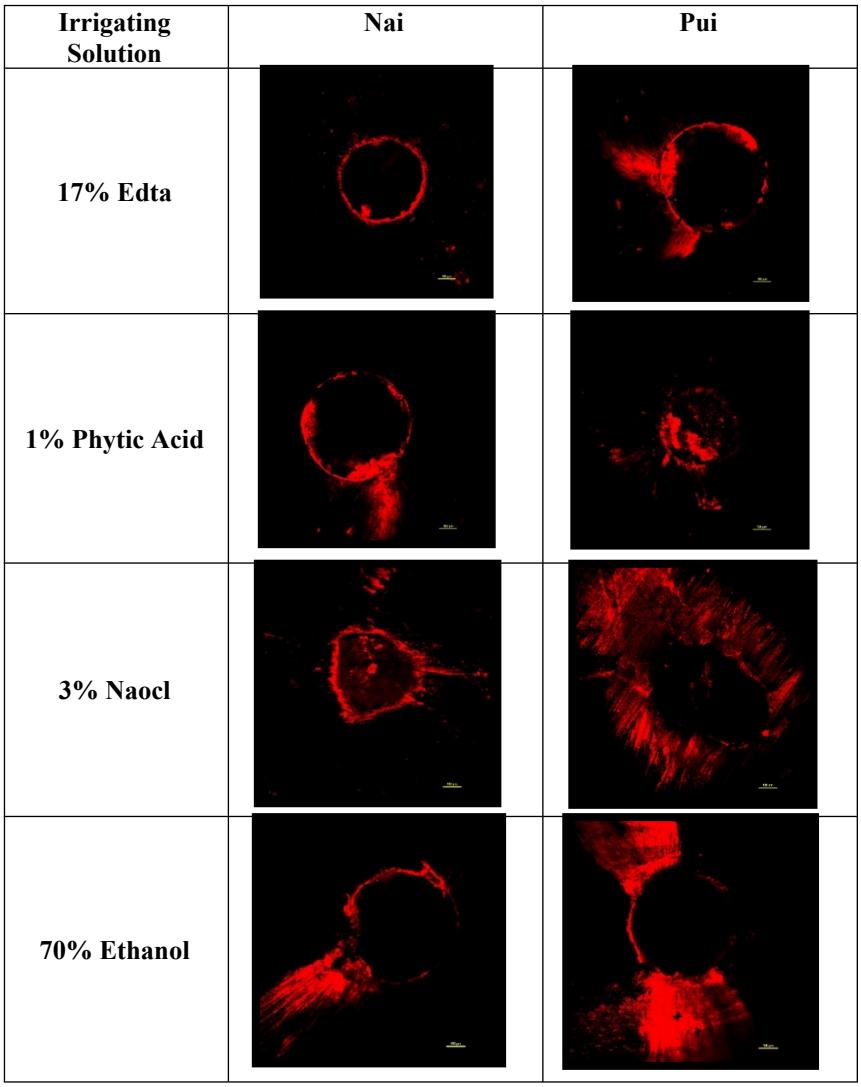


Figure 2

%age of clean root canal walls= clean perimeter/ total perimeter x 100 (As proposed by Moon et al.)[17]

Wherein, Total perimeter = The total perimeter of the root canal walls
Clean perimeter = The perimeter along the root canal walls where there was no sign of leftover medicament.

- Depth of clear dentinal tubules was measured from the root canal wall to the closest point where calcium hydroxide could be observed (Figure 1l). The data were averaged to produce a single value for each section.

The values obtained were subjected to suitable statistical analysis.

Statistical analysis:

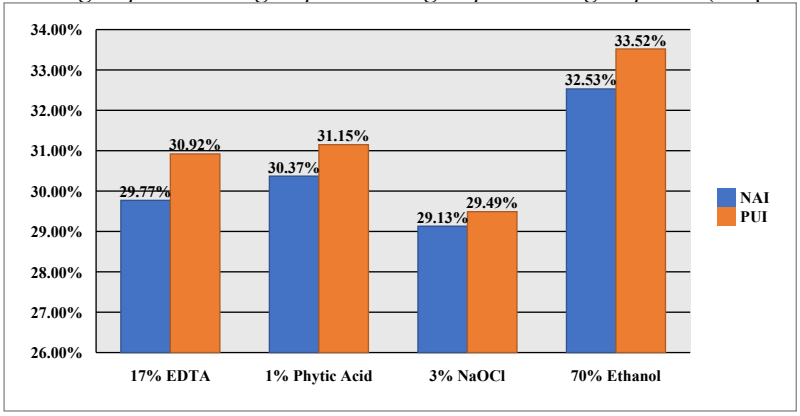
Statistical analysis was done by using SPSS 27.0 software. Prior to analysis, normality testing of the data was done using Shapiro-Wilk test which showed that the data were normally distributed ($P<0.05$). Thereafter, the difference in the mean value of the two parameters was assessed by using analysis of variance (ANOVA). Post hoc multiple comparisons between different groups was done using Tuckey's test. The level of significance was fixed at $p<0.05$.

III. Result

No group was able to completely remove the calcium hydroxide dressing. The percentage of clean perimeter among root canal irrigants used with non-activated method of irrigation was found as follows: *Subgroup IVA> Subgroup IIA> Subgroup IA> Subgroup IIIA* (Graph 1)

Furthermore, subgroup IVA had significantly higher percentage of clean perimeter in comparison with subgroup IA (p=<.001), subgroup IIA (p=.007), subgroup IIIA (p=<.001). (Table 2)

The percentage of clean perimeter among root canal irrigants used with passive ultrasonic irrigation was found as follows: *Subgroup IVB> Subgroup IIB> Subgroup IB> Subgroup IIIB* (Graph 1)



Graph 1: Comparison of Clean Perimeter between Irrigation Methods

Subgroup IVB the difference in the clean perimeter percentage in comparison with Subgroup IIIB is statistically significantly (p=.002) but non-significant with Subgroup IB and IIB. (Table 2)

Group		Mean Difference	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
IA	IIA	-.59623	.775	-2.2753	1.0828
	IIIA	.64278	.733	-1.0363	2.3218
	IVA	-2.76309*	<.001*	-4.4421	-1.0840
IB	IIB	-.22583	.996	-2.9871	2.5355
	IIIB	1.42744	.512	-1.3339	4.1888
	IVB	-2.59874	.071	-5.3600	.1626
IIA	IA	.59623	.775	-1.0828	2.2753
	IIIA	1.23901	.212	-.4400	2.9181
	IVA	-2.16685*	.007*	-3.8459	-.4878
IIB	IB	.22583	.996	-2.5355	2.9871
	IIIB	1.65327	.385	-1.1080	4.4146
	IVB	-2.37291	.114	-5.1342	.3884
IIIA	IA	-.64278	.733	-2.3218	1.0363
	IIA	-1.23901	.212	-2.9181	.4400
	IVA	-3.40587*	<.001*	-5.0849	-1.7268
IIIB	IB	-1.42744	.512	-4.1888	1.3339
	IIB	-1.65327	.385	-4.4146	1.1080
	IVB	-4.02618*	.002*	-6.7875	-1.2649
IVA	IA	2.76309*	<.001*	1.0840	4.4421
	IIA	2.16685*	.007*	.4878	3.8459
	IIIA	3.40587*	<.001*	1.7268	5.0849
IVB	IB	2.59874	.071	-.1626	5.3600
	IIB	2.37291	.114	-.3884	5.1342
	IIIB	4.02618*	.002*	1.2649	6.7875

Table 2: Multiple comparison (Post Hoc test) - Mean clean perimeter percentage

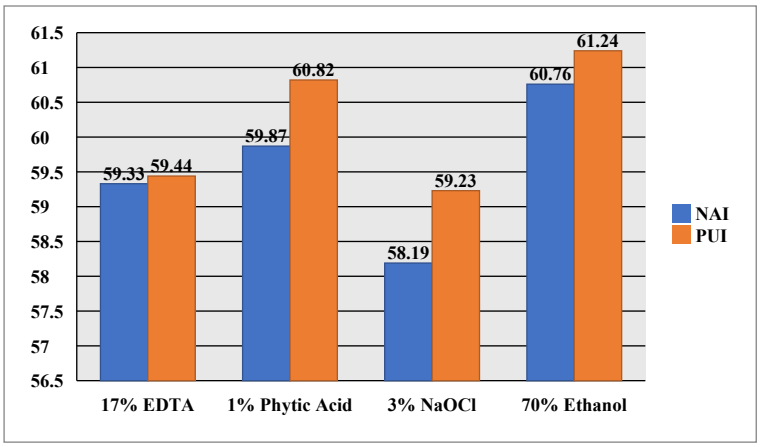
The sequence of depth of clean dentinal tubules was as follows: *Group IV> Group II> Group I> Group III* in both activated and non-activated method of irrigation. Nevertheless, no statistically significant difference was seen between the groups. (Table 3, Graph 2)

Group		Mean Difference	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
IA	IIA	-.54000	.978	-4.1849	3.1049
	IIIA	1.13800	.835	-2.5069	4.7829
	IVA	-1.43200	.717	-5.0769	2.2129
IB	IIB	-1.38200	.867	-6.2116	3.4476
	IIIB	.21400	.999	-4.6156	5.0436

	IVB	-1.79500	.750	-6.6246	3.0346
IIA	IA	.54000	.978	-3.1049	4.1849
	IIIA	1.67800	.606	-1.9669	5.3229
	IVA	-.89200	.912	-4.5369	2.7529
IIB	IB	1.38200	.867	-3.4476	6.2116
	IIIB	1.59600	.810	-3.2336	6.4256
	IVB	-.41300	.996	-5.2426	4.4166
IIIA	IA	-1.13800	.835	-4.7829	2.5069
	IIA	-1.67800	.606	-5.3229	1.9669
	IVA	-2.57000	.246	-6.2149	1.0749
IIIB	IB	-.21400	.999	-5.0436	4.6156
	IIB	-1.59600	.810	-6.4256	3.2336
	IVB	-2.00900	.680	-6.8386	2.8206
IVA	IA	1.43200	.717	-2.2129	5.0769
	IIA	.89200	.912	-2.7529	4.5369
	IIIA	2.57000	.246	-1.0749	6.2149
IVB	IB	1.79500	.750	-3.0346	6.6246
	IIB	.41300	.996	-4.4166	5.2426
	IIIB	2.00900	.680	-2.8206	6.8386

Table 3: Multiple comparison (Post Hoc test) – Depth of clean dentinal tubules

Passive ultrasonic irrigation was found to have slightly better results than non-activated method of irrigation but no significant difference was found.



Graph 2: Comparison of Depth of Clean depth of dentinal tubules between Irrigation Methods

IV. Discussion

The only goal of utilising intracanal medicament is to restrict and/or avoid the growth of microorganisms between appointments and to create an environment that promotes healing.[18] Calcium hydroxide is the most commonly used and is also considered the 'gold standard' intracanal medicament in endodontics.[19] A study, concluded that it causes denaturation of pro-inflammatory mediators for example, interleukin1α (IL-1α), tumor necrosis factor α (TNFα) and calcitonin gene-related peptide (CGRP), which is a potential mechanism by which it contributes to periapical repair.[20]

However, removing the calcium hydroxide is equally vital as removing the smear layer and debris from the root canal before obturation as it will promote better adaptation of the filling material to the canal walls.[6] White RR et al. concluded that the ingress of root canal sealers or filling materials into the dentinal tubules increase the surface area contact of the sealers to the prepared walls of the canal which in turn could potentially increase the root canal system's sealing. [21]

When it comes to removing $Ca(OH)_2$, mechanical instrumentation with a master apical file (MAF), in conjunction with copious irrigation with NaOCl and EDTA is the most often utilised approach.[22]

Several previous studies have revealed, $Ca(OH)_2$ residues on the root canal walls have been found in nearly all specimens, regardless of the file system and removal procedure employed. These remnants are primarily packed in the apical one third of the root.[23]-[27] Therefore, in the present study, apical third of root canal system was studied.

In the present study, NaOCl removed least amount of calcium hydroxide in both the methods of irrigation (PUI & NAI), similar results were seen in studies conducted by Da Silva JM et al. (2011),[28] Eymirli A et al. (2017),[29] Poornima P et al. (2022).[30] However, a study conducted by Tasdemir T et al. (2011)[31] found that passive ultrasonic irrigation with NaOCl is better than a combination of NaOCl and EDTA. Moreover, it

was found that irrigating with EDTA gave better results as compared to sodium hypochlorite which can be attributed to its chelating property, similar outcome was seen in a study by Rodig et al. (2010).[32] Calt and Serper (1999)[33] demonstrated that, in contrast to NaOCl alone, treatment with EDTA and NaOCl resulted in full elimination of calcium hydroxide from the root canal. Other authors employing the identical irrigating methods including EDTA and NaOCl did not attain similar results, and reported small to extensive residues of $Ca(OH)_2$ Lambrianidis et al. (1999),[34] Kenee et al. (2006)[22].

In spite of the fact that, EDTA aids in the elimination of the smear layer and chelates calcium from calcium hydroxide intracanal medicament, it has been proven to lower the modulus of elasticity and flexural strength of root dentin, which results in an increased risk of root fracture.[35] Hence, phytic acid which is a substitute chelator solution with comparatively milder deleterious effects was used in the present study and its biocompatibility and smear layer removal efficacy has been recently demonstrated.[36] Behl M et al. (2023)[37] concluded in his study, that, in the apical third, chitosan and phytic acid performed better than EDTA and glycolic acid, but the difference was not statistically significant. A similar result was obtained in the present study wherein Group II: phytic acid fared better compared to Group I: EDTA but the difference was not statistically significant. Phytic acid's superior outcomes can be due to its hexaphosphate chemistry, which partially ionises at physiological pH, where anionic charges are neutralised by cations, making it an excellent chelating agent of multivalent calcium cations.[38]

Ethanol is also an alternative agent to overcome the detrimental effects of EDTA on dentin. Ethanol is a known surface tension depressant and tissue desiccant, and its antibacterial qualities make it suitable for canal debridement (CDC Guideline for Disinfection and Sterilisation in Healthcare Facilities 2008). Various types of alcohol have been used to conveniently remove sealer from mixing surfaces and pulp chambers. Alcohol as a last rinse before sealer placement improved penetration of the sealer and wettability of root canal dentin, although its use is still anecdotal.[39] 70% ethanol was utilised in this investigation because it evaporates slowly, ensuring an acceptable contact period with the walls of the root canal.[40] according to the current study, Group IV: 70% Ethanol brought out the best results as compared to the other irrigants used in the study. Mean percentage of clean perimeter found with Group IV was found to be statistically significant in comparison with all the other groups in NAI group whereas in PUI method of irrigation mean percentage of clean perimeter was statistically significant with Group IIIB only.

Previous research investigating the effect of $Ca(OH)_2$ dressing on the ingress of root canal sealers in the dentinal tubules, reported that $Ca(OH)_2$ may obstruct the dentinal tubules[41] and/or the lateral canals[42] and prevent or reduce the penetrability of root canal sealers, whereas a study conducted by Cruz AT et al. reported that $Ca(OH)_2$ dressing did not impede with the apical penetration of the tested sealers (AH Plus and MTA Fillapex).[43] However, Araghi S et al. (2020) reported that presence of residual $Ca(OH)_2$ negatively affected the sealing potential of totalfill BC sealer and showed increased microleakage.[44]

However, tubular penetration of root canal sealers is important to entomb the bacteria present there to prevent bacterial repopulation.[43] Therefore, it is important to remove the calcium hydroxide dressing from the dentinal tubules to ensure flow of sealers into the dentinal tubules. That is why depth of clean dentinal tubules was evaluated in the present study.

In the present study Group IA (70% Ethanol) presented a higher depth of cleanliness in comparison to the other groups in both the methods of irrigation. Ethanol decreases dentinal roughness and increases the surface free energy that may be the possible reason for better penetration of the irrigant solution and thus better removal of calcium hydroxide. A similar result was found in a study conducted by De Lima Dias-Junior LC.[16]

According to the findings of the current investigation, using 70% ethanol to remove $Ca(OH)_2$ from the apical third may be a more conservative and predictable strategy with fewer risks to root canal dentin, as well as may potentially improve sealer penetration.

V. Conclusion

With limitations of the study, in-vitro study design and low volume of irrigating solution, 70% ethanol gave the best results in both NAI and PUI method of irrigation. However, more investigations and clinical trials with 70% ethanol are needed to prove its efficacy in removing calcium hydroxide residues using modern imaging technologies and to validate its safety for periapical tissues.

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