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Evaluation And Comparison Of Smear Layer Removal Potency Of Two Different Irrigation Regimens In Apical Thirds Of Curved Mesial Root Canals Of Permanent Mandibular First Molar Using Stereomicroscope And Scanning Electron Microscope: An In Vitro Study.

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Abstract
Aim: *The Aim Of The Study Is To Evaluate And Compare The Smear Layer Removal Potency Of Two Different Irrigating Regimens In Apical Thirds Of Curved Mesial Root Canals Of The Permanent Mandibular First Molar.*
Materials And Methods: *Thirty First Molars Of The Human Mandible With Moderate Curvature That Were Extracted Due To Periodontal Issues Were Gathered. The Teeth Have Been Irrigated Using A Designated Irrigation Solution And Equipment Following The Preparation Of The Canals. Teeth Were Randomly Divided Into Three Experimental Groups Based On The Different Endodontic Irrigants Employed Namely Group 1:5.25% Sodium Hypochlorite,17% Edta And Saline (N=10), Group 2: Side Vented Polypropylene Irrigation Tip Group With Hebp [Twin Kleen] And 5% Sodium Hypochlorite(N=10) And Group 3:Apical Negative Pressure Group With Hebp [Twin Kleen],Side Vented Polypropylene And 5% Sodium Hypochlorite(N=10). To Measure The Amount Of Dye Penetration Brought On By The Removal Of The Smear Layer, Teeth Were Submerged In A Solution Containing 1% Methylene Blue Dye For 24 Hours. The Teeth Were Then Split Longitudinally Into Two Halves To Be Viewed Under Stereomicroscope And Sem.*
Results: *Group 3 Had Better Removal Of The Smear Layer Along With The Smear Plug Followed By Group 2and Group 1 With A Statistically Significant Difference Present.*
Conclusion: *Apical Negative Pressure Technique And Side Vented Needle Irrigation Technique With Continuous Chelating Agent Resulted In The Better Removal Of The Smear Layer Than With The Conventional Irrigation Technique.*
Keywords: *Apical Negative Pressure Irrigation, Hebp, Moderately Curved Canals, Scanning Electron Microscope, Smear Layer,Side Vented Needle Irrigation, Stereomicroscope.*

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I. INTRODUCTION

The elimination of germs from the root canal system, prevention of reinfection, and promotion of periapical healing are essential for the effectiveness of endodontic therapy. Under continual irrigation, inflammatory and necrotic tissue, microbes/biofilms, and other debris are removed from the root canal area by shaping it using hand and rotational devices. Instrumentation seeks to make irrigation, disinfection, and filling more efficient.[1] Irrigation is used during root canal debridement, which enables cleaning that is more thorough than what would be possible with just root canal instrumentation. The smear layer and smear plug play a critical role in the success of pulp therapy. It reduces irrigants' or obturating materials' ability to penetrate the canals by 25% to 49%. The debris blocks the orifices of the dentinal tubules, forming smear plugs that reduce dentin permeability by 86%, and together they prevent the ingress of intracanal disinfecting agents and sealers into blocked dentinal tubules. The smear layer and smear plug contain both organic (debris from pulpal and bacterial tissues) and inorganic (dentinal chips and debris) components. To create a hermetic seal, it is essential to remove this layer.[2]

Irrigants are frequently delivered using syringe irrigation with the tip near the working length (WL) [3,4,5]. The irrigation solutions can pass through the root canal walls thanks to the side-vented needles, which block irrigation apically. The flow pattern, solution speed, and apical wall pressure—all significant factors affecting irrigation effectiveness and safety—are all significantly influenced by needle tip design.[6]

In order to reduce the possibility of irrigant extrusion through the apical foramen, negative pressure irrigation was offered as a replacement way to distribute the irrigants inside the root canal [7,8]. A syringe and needle are used to inject irrigant into the pulp chamber, and a fine suction tip placed close to the working length (WL) generates the necessary negative pressure to push the irrigant into the canal. [7,9]

The apical negative pressure (ANP) system more effectively delivers the irrigant to the working length by positioning a tiny aspirating needle at the working length. The requirement for specialised instruments, which must be imported at a significant cost, is a drawback of the ANP system. To avoid incurring such a significant cost, a straightforward ANP kit was created in order to test its effectiveness in removing the smear layer from the apical third of the root canal surface.

The goal of the current study was to assess how irrigation, root curvature, and continuous chelating agent affected the elimination of the smear layer.

II. MATERIALS AND METHODOLOGY

A detailed protocol explaining the purpose and procedures of the study were submitted and ethical clearance was obtained from the institution. [VDCW/IEC/237/2021]

Thirty mandibular first molar teeth extracted due to periodontal conditions with moderate curvature were taken and the presence of curvature was confirmed with the radiograph (Schneiders technique). The exclusion criteria involved: Teeth with carious involvement in crown/root surfaces, teeth having external and internal root resorption, teeth with open apices,root canal treated and restored teeth.[10]

The selected teeth were then thoroughly cleaned to remove debris and calculus and stored in distilledwater.

IRRIGATION REGIMEN:

Following incubation, the samples were randomly segregatedinto three groups, each consisting of 10 samples.

o Group I - 5.25% sodium hypochlorite,17% Ethylenediaminetetraacetic acid (EDTA), and Saline

o Group II – Double Side Vented Polypropylene needle irrigation group with 1-Hydroxyethylidene-1,1- bisphosphonate (HEBP) [Twin kleen] and 5% sodium hypochlorite

o Group III – Double Side Vented Polypropylene needle with Apical negative pressure group with HEBP [Twin kleen] and 5% sodium hypochlorite (figure:1)

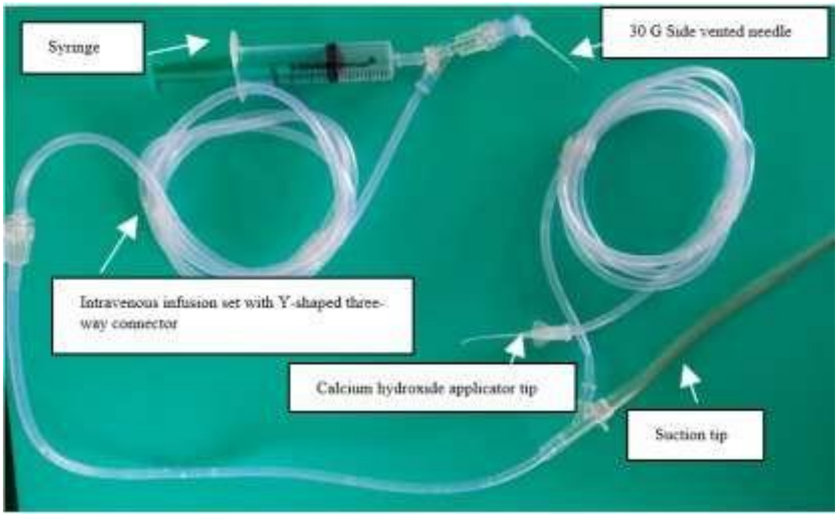


Figure 1: The two IV sets were connected using a Y-shaped three-way connector. One end will be used to deliver irrigant while the other aspirates. The last end connects to a high-powered suction to operate Further, they were decoronated using a diamond disc (1 mm) to a root canal length of 14 mm to obtain standardization. The position of the apical foramen and the canal patency was established with the No 10 K file (Mani, Japan). For working length estimation, the file was inserted in the canal and the working length was 1 mm short of the length of the file when the tip was visible beyond the apex. Before preparing the root canal, the apical end were sealed using wax to prevent the escape of the irrigants periapically. Biomechanical preparation was done using Protaper rotary files (DentsplyMaillefer, Ballaigues, Switzerland) until the working length increased the canal size as per sequence until the F2 file. After using each file and before preceding the next file, the root canals were irrigated with the assigned group irrigant in the pre-decided sequence and time. Transparent nail polish lacquer was used to coat the teeth. Staining was done with 1% methylene blue dye by immersing the teeth in the solution for 24 hr to detect the amount of dye penetration caused by the removal of the smear

layer.The teeth were then divided longitudinally into two half using a diamond disc for stereomicroscopy and scanning electron microscopy. The stained samples were examined at a 4x magnification under a stereomicroscope to identify the area of dye penetration in the apical third. All of the specimens' individual dye penetration areas were measured using ImageJ software. Utilizing SEM examination, the surface alterations on the root dentin were identified. There was gold sputtering. After that, they were examined with a SEM at a magnification of 5000. Each sample's apical third was used to take photomicrographs, which were then analysed for alterations to the dentinal surface.[10]According to Gutmann et al.'s method[11], the residual smear layer was evaluated as follows:

- Score 1: a little or no smear layer covering up to 25% of the specimen; tubules visible and patent
- Score 2: a little to moderate or patchy amounts of smear layer covering between 25% and 50% of the specimen; many tubules visible and patent
- Score 3: moderate amounts of scattered or aggregated smear layer covering between 50% and 75% of the specimen; minimal to no tubule visibility or patency
- Score 4: heavy smear layer covering over 75% of the specimen; no tubule orifices visible or patent.

STATISTICAL ANALYSIS

The statistical analysis was done by using SPSS software Version 25.0. Utilising the Kruskal Wallis test, the three research groups were compared under stereomicroscopy. The Chi-square test was used to compare the three research groups under the SEM. Less than 0.05 was maintained as the p-value for statistical significance.

III. RESULT

The following conclusions were reached based on the study's observations:
The 30 teeth were separated into three groups of 10, each grouping. All groups were evaluated for the degree of dye penetration (measured in microns) in the apical third.

Stereomicroscope findings of dye penetration

Table 1 Descriptive statistics of the study groups under stereo microscope

Types of microscope	Groups	N	Mean	Std. Deviation
Stereo	Group I	10	.0790	.02025
	Group II	10	.3800	.17512
	Group III	10	1.0290	.35510

The mean and standard deviation of the group I, II and III under stereomicroscope were 0.08±0.02, 0.38±0.18 and 1.03±0.36 respectively.

Table 2 Comparison between the means of three study groups under stereo microscope

Type of microscope	Groups	Mean	Std. Deviation	p-value
Stereo	Group I	.0790	.02025	**0.000***
	Group II	.3800	.17512	
	Group III	1.0290	.35510	

*Significant

When comparing the means of the three study groups under stereomicroscope, the mean was higher in group III (1.03±0.36) followed by group II and III respectively and the result was also statistically significant with the p-value of 0.000.
Group III (mean 1.0290) (fig 4) showed the highest amount of dye penetration indicating maximum smear layer removal, second being Group II (mean 0.3800)(fig 3), and least dye penetration was seen in Group I (mean 0.0790)(fig 2). (P = 0.001) The outcomes were statistically significant.
When compared to Group I (Fig. 2), Groups III (Fig. 4) and II (Fig. 3) showed a significant difference in the elimination of the smear layer in the apical third.

Stereomicroscope Image for Conventional Irrigation (Fig 2)

Stereomicroscope Image for double side vented irrigation needle with HEBP (Fig 3)

Stereomicroscope Image for double Side Vented Polypropylene needle with Apical negative pressure group with HEBP (Fig 4)

Dentin patency as seen under the scanning electron microscope

The effect of the irrigation regimes on the smear layer and dentinal tubules as well as changes in the dentinal surface were evaluated using SEM.

Table 3 Comparison of the frequencies between the study groups under SEM							
	Study groups		SEM			Total	p-value
			1	2	3		
	Group I	Count	1	4	5	10	**0.000***
		% of Total	3.3%	13.3%	16.7%	33.3%	
		Count	8	2	0	10	
		% of Total	26.7%	6.7%	.0%	33.3%	
	Group III	Count	10	0	0	10	
		% of Total	33.3%	.0%	.0%	33.3%	
Total		Count	19	6	5	30	
		% of Total	63.3%	20.0%	16.7%	100.0%	

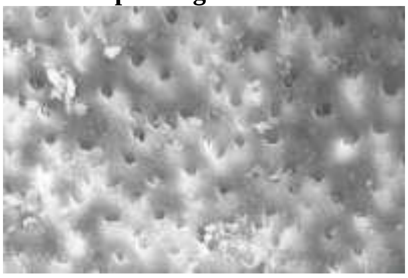
When comparing the frequencies between the study groups under SEM, score 1 was more in group III at about 33.33% followed by score 2, and score 3 was more in group II and the result was statistically significant with a p-value of 0.000.

Group I – Showed a moderate amount of scattered aggregated smear layer covering dentinal tubules indicating

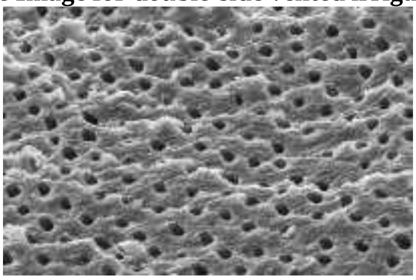
minimum tubule visibility and patency. (Fig 5)
Group II – Showed the minimum amount of smear layer covering the dentinal tubules. A maximum number of tubules were visible and patent when compared to the other two groups.(Fig 6)

Group III – Showed little or patchy amount of smear layer covering the dentinal tubules. Many of the dentinal tubules were visible and patent. (Fig 7)

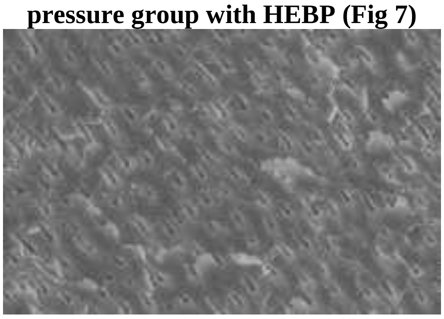
Scanning Electron Microscope Image for Conventional Irrigation (Fig 5)



Scanning Electron Microscope Image for double-side vented irrigation needle with HEBP (Fig 6)



Scanning Electron Microscope Image for double Side Vented Polypropylene needle and Apical negative pressure group with HEBP (Fig 7)



IV. DISCUSSION

This study compared the ability of the double-sided vented polypropylene irrigation tip and traditional irrigation, both of which are often used, to remove the smear layer from the apical third of the root canal in order to evaluate the effectiveness of the built ANP kit.Because they are often tiny, curved, and frequently have isthmuses between them, the mesial canals of the permanent root mandibular first molar canals were chosen. [12]. Khaord P. et al. examined the efficacy of various irrigation techniques on the removal of smear layers in the apical thirds of the mesial root canals of permanent mandibular first molars. They came to the conclusion that final irrigant activation with sonic and MDA was more effective than with CI in removing smear layers, and the present study's apical negative pressure irrigation technique supports this conclusion. [2]

Due to the complexity of its anatomy, the apical area has been referred to as "the critical area." Because it is frequently located apical to a canal curve, it is the portion that is furthest from the access opening [12]. It has been suggested to use fine irrigation tips to deliver irrigants to the apical region of canals [13]. When treating curved canals, it can be difficult to get the irrigation needle to the area around the apical constriction, thus it's important to worry about whether the irrigants are getting there so that effective debridement can take place. So, for clinical success, putting a focus on full irrigation of the apical area may be useful. [14], and to my knowledge, this is the first study to report that side-vented polypropylene tips which are more flexible and can adapt to the canal curvature and its side vent provide effective smear layer removal.

The major goals of root canal therapy are complete root canal debridement, eradication of microorganisms and their metabolic products, as well as removal of organic and inorganic materials from the root canal space.[15]

The smear layer should be removed for the following reasons:

1. Its volume and thickness are unexpected, and a significant amount of it is made up of water.
2. Necrotic tissue, bacteria, and their byproducts.
3. Reduce the degree to which disinfectants can penetrate.
4. Create a barrier between the filler materials and the canal wall to prevent the creation of an adequate seal.
5. A loosely adhered structure that could be a route for bacterial contamination and leaking between the dentinal walls and the root canal filling. Therefore, removing it would make canal filling easier..[16]

After chemo-mechanical preparation, Machado R et al. compared the removal of the smear layer using 17% ethylenediaminetetraacetic acid (EDTA) by conventional application (CA), passive ultrasonic irrigation (PUI), Easy Clean (EC), and XP-Endo Finisher (XPF), and came to the conclusion that no irrigation method was able to completely remove the smear layer, particularly in the apical third. Any form of activation underperformed compared to the conventional application of the chelating solution. In the current investigation, smear layer removal was more effective with side vented needle irrigation than it was with the apical negative pressure approach, which also removed the smear plug.

The primary organic components of dentin, collagen and pulpal remains, are effectively dissolved by the antibacterial agent NaOCl. The organic portion of the smear layer is damaged by hypochlorite, which enables its total removal by subsequent irrigation with EDTA or citric acid (CA). Hypochlorite alone cannot completely remove the smear layer.[18]

Effectively dissolving inorganic material, including hydroxyapatite, include EDTA and citric acid.Disodium EDTA, pH 7, at a concentration of 17% is the most frequently used, but other research have shown that solutions with lower concentrations (for example, 5%, 10%, and even 1%) equally eliminate the smear layer after NaOCl irrigation.[18]

Etidronate, also known as hydroxyethylidene bisphosphonate (HEBP) (1-hydroxyethylidene-1, 1-bisphosphonate), is a decalcifying drug with negligible short-term interactions with sodium hypochlorite. It has lately been proposed as a potential substitution for EDTA or citric acid.[19] Both 9% HEBP and 18% HEBP generated considerably slower demineralization kinetics than 17% EDTA. [20]. In a study by Ashraf H. et al.[21] to compare the effectiveness of 17% ethylenediaminetetraacetic acid (EDTA), 18% etidronate, and Er: YAG laser in removing the Smear layer, it was found that polypropylene side vented needles and the apical negative pressure technique were more effective than EDTA and etidronate.

When treating curved canals, it can be difficult to reach the area around the apical constriction with the irrigation needle; therefore, it should be a worry as to whether the irrigants are being given to this area so that effective debridement can take place. So, for clinical success, putting a focus on full irrigation of the apical area may be helpful.[22]The irrigation solutions can pass through the root canal walls thanks to the side-vented needles, which stop irrigation fluid from flowing apically.Because of the increased pressure at the apical foramen, improved irrigant replacement, and increased canal wall shear stress, open-ended needles may increase the likelihood of periapical irrigant and debris extrusion.[23]Ghivari and Kubasad[24] 2011 evaluated the effectiveness of different irrigating needles. Results showed that a side-vented needle enables better removal of debris in the middle and apical third of the root canal compared with the single-beveled needle.

The ANP system cleans up the apical root canal and stops irrigation fluid from seeping into the surrounding tissue. According to Siu and Baumgartner [25], canals irrigated with the ANP system had their working length effectively reduced by 1 mm. Additionally, Mancini et al. [26] observed that root canals irrigated using the ANP system had the cleanest root canal walls 1 mm from the apex when comparing the ANP, sonic, and PUI systems. The results of those two experiments are consistent with those of this one, indicating that the key to successfully eliminating the smear layer from the apical third of the root canal is the penetration of the irrigant into that portion of the canal. De Gregorio et al. [27] evaluated how well various irrigation methods allowed irrigants to flow to the working length. Their findings demonstrate that the ANP system, with all specimens (100%) watered at the working length, was noticeably more successful. Only 65%, 40%, and 0% of the working length of the root canals could be reached by the PUI, acoustic, and CNI systems, respectively.

Limitation: The absence of a defined rating scale for the thickness of the smear layer is a limitation of this study. As a result, unlike in the study where the Guttman Rating scale was employed, it is not possible to quantitatively evaluate the smear part.

V. CONCLUSION

The straightforward ANP kit created for this study is more effective than CI at administering irrigants to remove the smear layer from the apical third of the root canal surface. This is followed by the use of a side vented needle and a continuous chelating agent.

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