Section A-Research paper
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Comparative evaluation of depth of penetration of sodium hypochlorite in radicular dentine with different irrigant activation techniques: An in-vitro confocal laser scanning microscopy study

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ABSTRACT

Introduction: The preparation and thorough cleaning of the root canal system are crucial components of endodontic therapy carried out using mechanical and chemical means.Some of the most popular endodontic irrigant solutions include sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine (CHX). Through the ages, irrigation and root canal system activation methods have been improved in order to increase the depth of penetration of sodium hypochlorite solutions into root canals. In order to assess and analyse the penetration depth of 5.25% NaOCl into dentinal tubules utilising various irrigant activation procedures, this study was conducted.

Aim: To evaluate and compare the depth of penetration of sodium hypochlorite in radicular dentinal tubules by manual dynamic agitation, XP Endo finisher files, sonic agitation, passive ultrasonic activation.

Materials and Methods: This in-vitro study was carried out on 68 extracted human mandibular premolars at the Department of Conservative Dentistry & Endodontics, Bharati Vidyapeeth (Deemed to be University), Dental College & Hospital, Sangli, Bapuji Dental college and hospital, Davanagere and Indian institute of science and Research, Pune. 68 teeth were decoronated, working length determination and BMP was done, after preparation teeth were randomly divided into 4 groups for final irrigation activation protocol.Group 1(Manual Dynamic Agitation), group 2(XP EndoFinisher), group 3(Sonic Agitation), group 4(Passive Ultrasonic Irrigation). After final irrigation protocol the teeth were sectioned at coronal, middle and apical 1/3rd and confocal imaging was done to determine the depth of penetration. The mean Depths of Penetration for each group were assessed using analysis of variance (ANOVA), and then paired wise comparisons were made using Tukey's Post hoc Test.

Results: The current in vitro study discovered that Group 2 (XP EndoFinisher system) had the deepest overall penetration of 5.25% NaOCl+R in radicular dentinal tubules, followed by Groups 3 (Sonic Agitation), 4 (Passive Ultrasonic Irrigation), and 1 (Manual Dynamic Agitation).

Conclusion: Within the limitations of the present study, it can be concluded that the XP EndoFinisher file system, followed by sonic agitation, provided the maximum overall depth of penetration of sodium hypochlorite. In the middle and coronal thirds, maximum

penetration was seen. No single technique reliably produced the best results across the entire length of the root canal.

INTRODUCTION

Thorough cleaning and preparation of the root canal system is an important part of endodontic treatment performed using mechanical and chemical methods. [1]

Various studies have reported that bacteria can penetrate dentinal tubules up to 300 µm. Enterococcus faecalis penetrates dentin to a depth of 500 µm and the infection front reaches 1000 µm. [2]

The depth of penetration of the irrigating solution and thus its effect on the dentin tubules is limited. [2] Removal of the smear layer improves the penetration of the irrigating solution into the dentinal tubules, thus improving the disinfection and sealing of the root canal system. [2]

Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX) are some of the most commonly used endodontic irrigant solutions. Modifications have been made throughout history to increase the depth of penetration of sodium hypochlorite solutions into root canals by using more effective irrigation and root canal system activation techniques. [1]

Activation systems for root canal irrigation can be divided into two main groups: manual agitation techniques and instrument agitation techniques. [1]

Hand agitation usually consists of using a syringe and a side-vented needle to flush with positive force. [1] Conventional irrigation during root canal treatment with a syringe and needle limits penetration beyond the main canal into the dentinal tubules (Ram 1977). [2]

A general device-based activation technique was developed to increase the effectiveness of irrigant solutions. These device activation technologies include sonic, ultrasonic, and newer systems such as plastic rotary files. [1]

Several single-file technologies for root canal measurements have recently been introduced, including the XP EndoFinisher system, the Fanta AF Max file system, and EasyClean.

The XP-Endo-Finisher is a rotatable nickel-titanium finisher file with a small core size ISO 25 and zero taper. Made of MaxWire (Martensite-Austenite Electropolished-FleX) for increased flexibility.

This allows the file to contact the root canal over its entire length, improving cleaning efficiency of the root canal wall with less impact on the dentin.

Studies throughout the literature have shown that sodium hypochlorite penetration can be increased with sonic and ultrasonic devices, but studies on the effects of activation using rotary files such as the XP EndoFinisher are lacking.

Therefore, the purpose of this study was to evaluate and compare the penetration depth of 5.25% NaOCl into dentin tubules using different irrigant activation techniques.

MATERIALS & METHODS

Ethical approval: The institutional ethics committee of Bharati Vidyapeeth (Deemed to be University) medical college and hospital, Sangli (BV(DU)MC & H/Sangli/ IEC/ Dissertation 2020-21/ D-52) has approved the present study.

Source of data: This in-vitro study was carried out on 68 extracted human permanent mandibular premolars at the Department of Conservative Dentistry & Endodontics, Bharati Vidyapeeth (Deemed to be University), Dental College & Hospital, Sangli, Bapuji Dental college and hospital, Davanagere and Indian institute of science and Research (IISER), Pune.

Inclusion criteria: Extracted single rooted mandibular premolars with mature apex.

Exclusion criteria: Extracted mandibular premolars with, cracked tooth, shape or size anomalies, internal or external resorption of root and teeth that are previously treated with Root Canal Treatment.

SAMPLE PREPARATION

Using an ultrasonic scaler, the extracted teeth were cleaned of all debris and periodontal fiber remains before being stored in 0.1% Thymol solution for preservation and disinfection until use. Decoronation of teeth was performed to standardize a root length of 15 millimeters. After inspecting the tip of a 10 k-file in the apical foramen, 0.5 mm was subtracted from the length of the file, and that file length was deemed the working length. Biomechanical preparation was performed with Protaper gold files until F4 with recapitulation and canal patency maintained with number 10 k-files. Following each tool change during biomechanical preparation, canals were irrigated with 3 ml of 5.25% sodium hypochlorite and 3 ml of normal saline. (BMP).At the end of cleaning and shaping procedure, the canals were irrigated with 3 ml of 5.25% of sodium hypochlorite, followed by irrigation with 3 ml of normal saline.Following irrigation with normal saline, the canals were dried with paper points. (Dentsply, Sirona, USA). The canals were irrigated with 3 ml of 17% EDTA for 1 minute to clear the smear layer, followed by 3 ml of normal saline.Following BMP, two coats of nail polish were applied around the root surface, and modelling wax was used to seal off the apex to avoid irrigant leakage. The canals were dried with paper points of size F4. (Dentsply, Sirona, USA). Following biomechanical preparation, the teeth were randomly assigned to one of four test groups for the final irrigation activation procedure. The volumes of irrigant solutions were standardized at 3ml for all 4 groups. For final irrigation protocol, 0.1 gm of Rhodamine B was diluted in 500 ml of 5.25% sodium hypochlorite.

Group 1 (Manual Dynamic Agitation)

Manual dynamic agitation with well-fitting F3 gutta-percha cone. Activation- 100 strokes/minute. Total activation time was 1 minute.

Group 2 (XP-endo finisher file System)

Activation with XP-endo finisher file (FKG, La Chaux-de-fonds, Switzerland) at 800 rotations per minute and 1 Ncm torque. The XP-endo Finisher file was inserted into the root canals filled with the (NaOCl+R) and activated for 1 min using slow and gentle 7–8 mm lengthwise movements to contact the full length of the canal.Activation: 3 cycles of 20 seconds. Total activation time was 1 minute.

Group 3 (Sonic Agitation)

Sonic agitation with EndoActivator at 10,000 cycles per minute. Activation: 3 cycles of 20 seconds. Total activation time was 1 minute.

Group 4 (Passive Ultrasonic Irrigation)

Passive ultrasonic activation with tip size no.20. Parameters were kept according to manufacturer's instructions. Activation cycle: 3 cycles of 20 seconds. Total activation time was 1 minute.

After final irrigation, the teeth were sectioned horizontally with a Hard tissue microtome at 3 mm, 6 mm, and 12 mm from the apex to produce 200-micron thick sections.

The teeth were sectioned at Bapuji Dental College and Hospital in Davanagere, Karnataka, and then inspected under a confocal laser scanning microscope (Leica, Mannheim, Germany) at the Indian Institute of Science and Research in Pune to determine depths of penetration.

Using a measuring tool included in the software, the point of deepest penetration of sodium hypochlorite was determined from the canal wall to the point of deepest penetration of dye. (FIG 1, 2, 3 &4)

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was used to evaluate mean Depths of Penetration across multiple groups, followed by paired wise comparison using Tukey's Post hoc Test.

For data display, simple/multiple bar charts were used.

In the preceding analyses, a p-value of less than or equal to 0.05 (p0.05) was considered statistically significant.

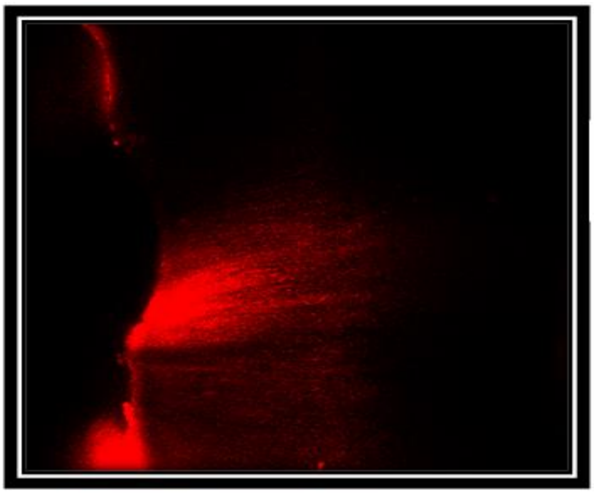
SPSS (Statistical Package for Social Sciences) software version 20 was used for all statistical studies.

RESULTS

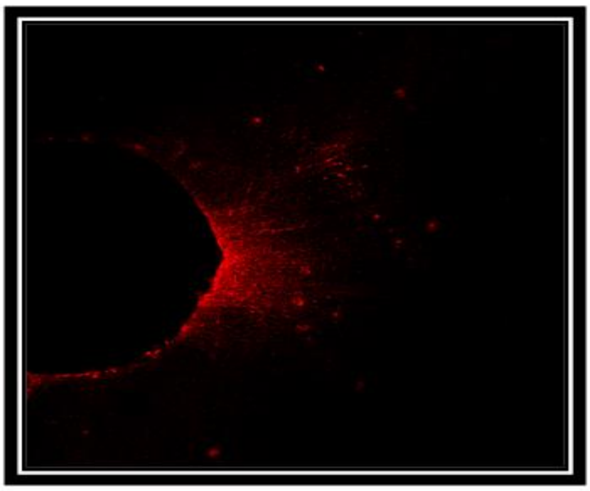
The current in vitro research found that (XP EndoFinisher system) Group 2 had the deepest overall penetration (Fig 2). With group 2 (XP EndoFinisher file system), the greatest depth of penetration in the coronal 1/3rd was 995.27 µm. With a mean of 1147.90 µm, group 3 (Sonic Agitation using EndoActivator) achieved the greatest depth in the middle third (Fig 3).

Also, with a mean of 788.07 µm, group 4 (Passive ultrasonic irrigation using IrriSafe) had the greatest entry in the apical 1/3rd (Fig 4). However, when comparing the experimental groups, group 3 (Sonic Agitation (EndoActivator)) demonstrated generally satisfactory depth of penetration, followed by groups 4 and 1. (Table 1)

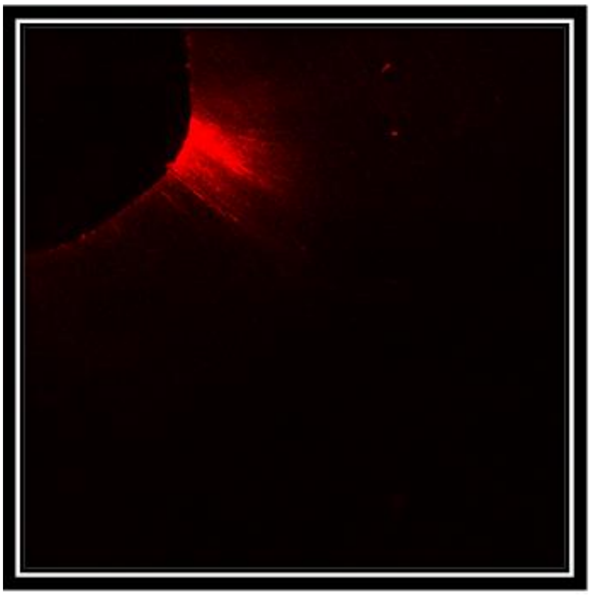
Fig 1: - Confocal laser Scanning Microscopic images for penetration depth of 5.25% Sodium Hypochlorite when activated with Manual Dynamic Agitation (Group 1)



Coronal 1/3$^{rd.}$

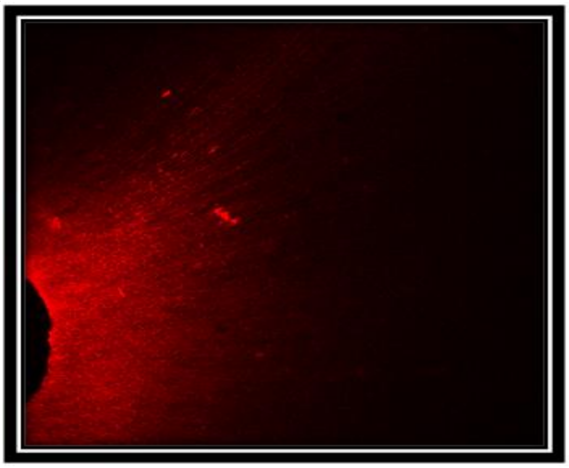


Middle 1/3$^{rd.}$

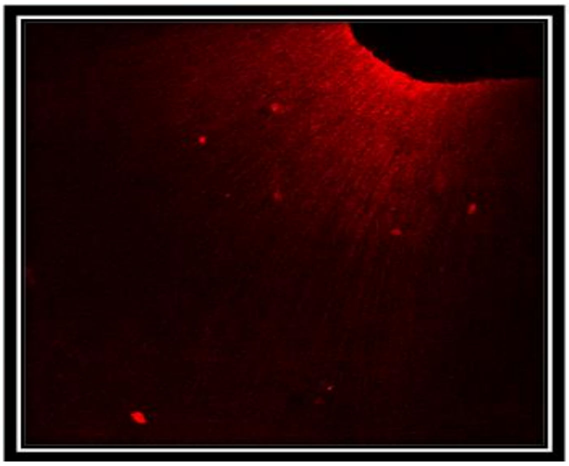


Apical $1/3^{rd.}$

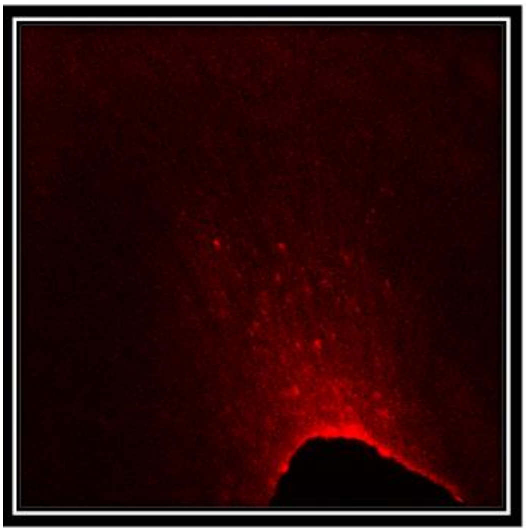
Fig 2: - Confocal laser Scanning Microscopic images for penetration depth of 5.25% Sodium Hypochlorite when activated with XP Endo finisher file system (Group 2)



Coronal $1/3^{rd.}$

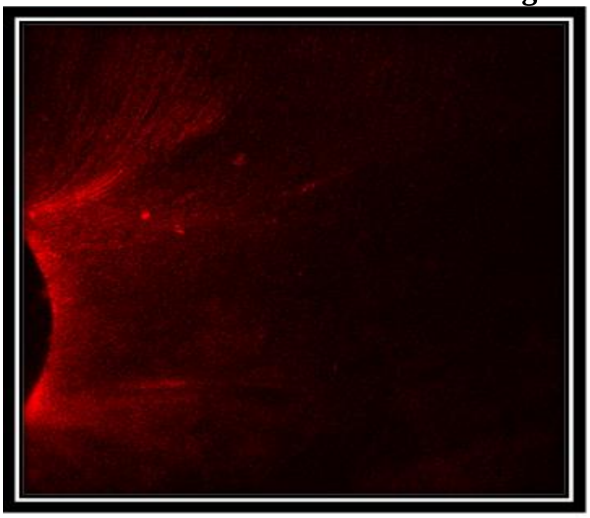


Middle $1/3^{rd.}$

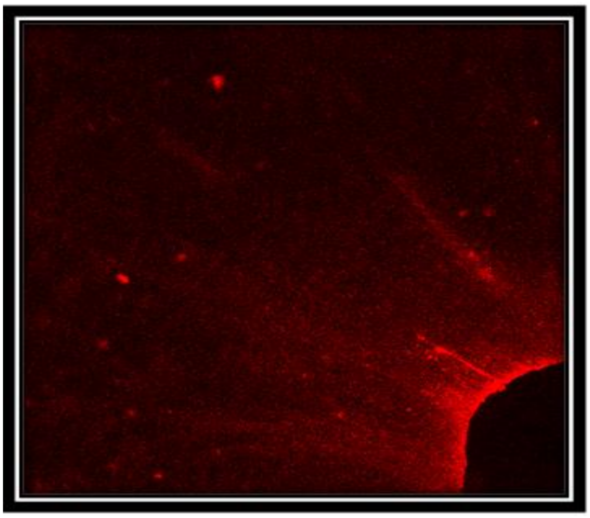


Apical 1/3$^{rd.}$

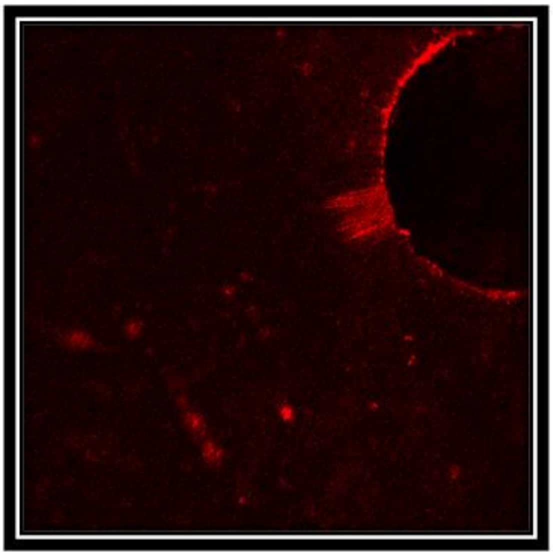
Fig 3: - Confocal laser Scanning Microscopic images for penetration depth of 5.25% Sodium Hypochlorite when activated with Sonic Agitation [Endo Activator] (Group 3)



Coronal 1/3$^{rd.}$

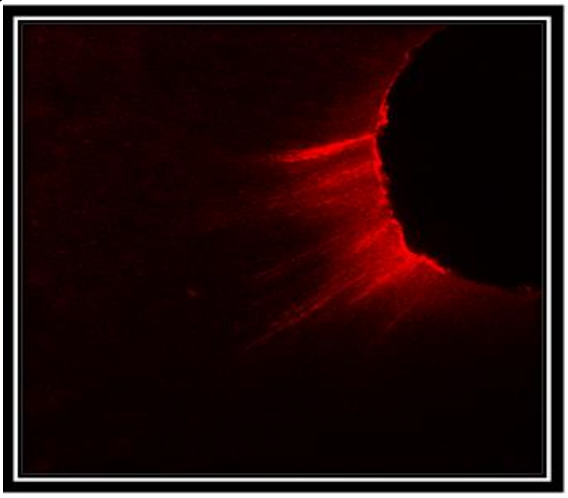


Middle 1/3$^{rd.}$

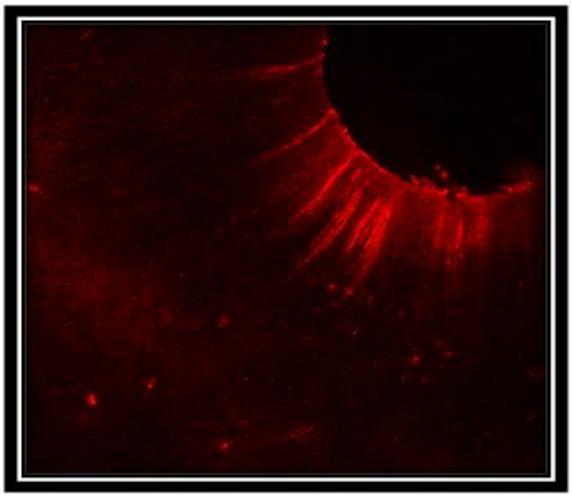


Apical $1/3^{rd.}$

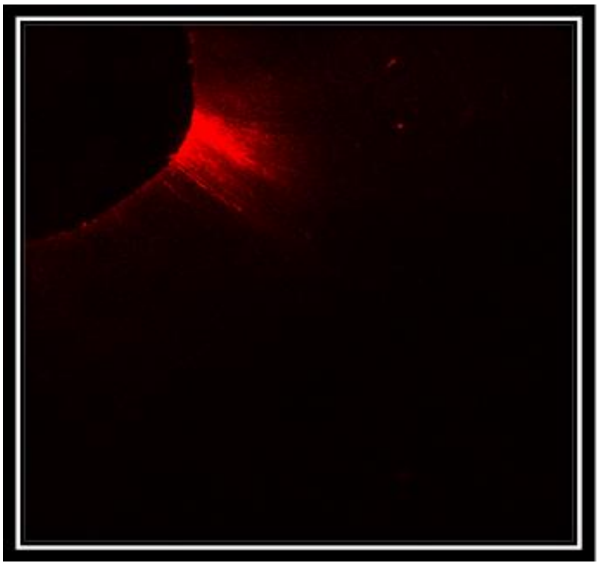
Fig 4:- Confocal laser Scanning Microscopic images for penetration depth of 5.25% Sodium Hypochlorite when activated with Passive Ultrasonic Irrigation [IRRI Safe] (Group 4)



Coronal $1/3^{rd.}$



Middle $1/3^{rd.}$



Apical 1/3rd.

Fig/Table 5: Descriptive statistics of depth of penetration of sodium hypochlorite in radicular dentinal tubules by manual dynamic agitation, XP Endo finisher files, sonic agitation, passive ultrasonic activation respectively (in µm)

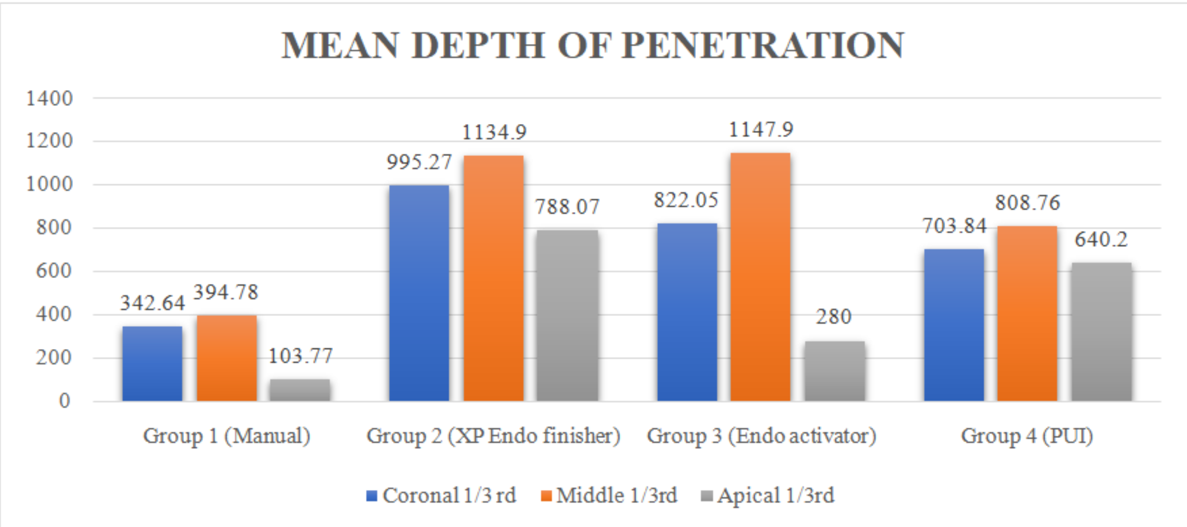
Coronal 1/3rd	Mean	SD	SE	Minimum	Maximum
Group 1 (Manual)	342.64	33.93	8.22	287.23	398.55
Group 2 (XP Endo finisher)	995.27	122.55	29.72	836.35	1335.26
Group 3 (Endo Activator)	822.05	79.81	19.35	675.61	973.68
Group 4 (PUI)	703.84	42.43	10.29	603.2	783.6
Middle 1/3rd	**Mean**	**SD**	**SE**	**Minimum**	**Maximum**
Group 1 (Manual)	394.78	52.26	12.67	267.0	488.5
Group 2 (XP Endo finisher)	1134.90	57.22	13.87	1024.89	1198.3
Group 3 (Endo Activator)	1147.90	95.57	23.17	984.34	1307.69
Group 4 (PUI)	808.76	11.62	27.07	516.18	957.21
Apical 1/3rd	**Mean**	**SD**	**SE**	**Minimum**	**Maximum**
Group 1 (Manual)	103.77	21.57	5.23	75.8	150.3
Group 2 (XP Endo finisher)	788.07	78.1	18.94	712.4	1043.29
Group 3 (Endo Activator)	280.0	42.88	10.4	206.24	345.3
Group 4 (PUI)	640.2	46.56	11.29	531.69	712.4

Fig/Table 6: Overall Comparative statistics of depth of penetration of sodium hypochlorite in radicular dentinal tubules by manual dynamic agitation, XP Endo finisher files, sonic agitation, passive ultrasonic activation respectively using One-way Anova F test

	Coronal 1/3rd	Middle 1/3rd	Apical 1/3rd
Group 1 (Manual)	342.64 (33.93)	394.78 (52.26)	103.77 (21.57)
Group 2 (XP Endo finisher)	995.27 (122.55)	1134.9 (57.22)	788.07 (78.1)
Group 3 (Endo Activator)	822.05 (79.81)	1147.90 (95.57)	280.0 (42.88)
Group 4 (PUI)	703.84 (42.43)	808.76 (11.62)	640.2 (46.56)
Anova 'F' test	F = 213.04	F = 309.57	F =641.39
P value, Significance	p<0.001**	p<0.001 **	p<0.001**

**p<0.001 – highly statistically significant difference

Fig/Table 6: Graphical representation of comparative statistics of mean of depth of penetration of sodium hypochlorite in radicular dentinal tubules by manual dynamic agitation, XP Endo finisher files, sonic agitation, passive ultrasonic activation



DISCUSSUION

Because of their suitability for the study design, single-rooted teeth with a single canal were chosen for the present study. Because premolars are commonly extracted for orthodontic purposes, mandibular 1st premolars were chosen.

All the teeth were decoronated to standardize a root length of 15mm to reduce the variability between the samples, even though this is considered as a technical limitation as there is no reservoir for the pooling of the NaOCl.

A 30-gauge needle with a tip diameter of 0.30 mm was used in this study, along with a preparation up to size F4 (Protaper Gold) whose tip corresponds to 0.40. This enabled for improved reaching and free movement of the needle tip without binding at 1-2mm from the apex, resulting in better and deeper irrigant action, particularly in the apical third. (Zhu et al. 2013, Urban et al. 2017). [5]

The study included closing the apices of the teeth with modeling wax and coating the external root surface with double layers of nail varnish. Clinically, the tooth roots are encased in the bony socket and periapical tissues, resulting in air entrapment in the root canal and periodontal ligament, preventing irrigation penetration in the apical portion of the canal. [5]

As a result, the sealed root apices allow for the irrigant solution to serve as a reservoir for the irrigation activation process without periapically leaking.

Rhodamine B is a substance that dissolves in water. It is frequently used as a tracer dye in water to identify flow and transport route. Rhodamine B was thought to enter the dentin because it is water soluble and has a low molecular weight. Rhodamine B dye was used to stain the samples due to its better solubility and fluorescence. [23]

In the current study, teeth were sectioned using a hard tissue microtome before microscopic analysis. Because hard tissue microtomes are heavier and more solid, they can cut the tooth without causing vibration. This results in uniformly thick portions with no microcrack inductions. These portions are critical for microscopic analysis. In the current study, 200-micron thick sections were created to enable laser from microscopic analysis to penetrate the sections effectively.[25]

Confocal Laser Scanning Microscopy (CLSM) was used for the microscopic analysis in this study because it gives detailed information about the presence and distribution of irrigant inside the dentinal tubules along the canal walls. It acquires images from multiple sections, which are then reconstructed to produce the final picture. [4]

In the current study, the mean depths of penetration for Manual Dynamic Agitation (Group 1) in the coronal 1/3rd, middle 1/3rd, and apical 1/3rd were 342.64 µm, 394.78 µm, and 103.77 µm, respectively.

The mean levels of penetration for the XP Endo finisher system (Group 2) in the coronal, middle, and apical thirds were 995.27 µm, 1134.9 µm, and 788.07 µm, respectively. Sonic Agitation (Group 3) had mean levels of penetration of 822.05 µm, 1147.90 µm, and 280.0 µm in the coronal, middle, and apical thirds, respectively.

Likewise, the mean depths of penetration for Passive Ultrasonic Irrigation (Group 4) in coronal $1/3^{rd}$, middle $1/3^{rd}$ and apical $1/3^{rd}$ was 703.84 µm, 808.76 µm and 640.2 µm respectively.

According to the findings of this study, the XP EndoFinisher (Group 2) had the greatest depth of penetration in the coronal and apical thirds, while the EndoActivator (Group 3) had the greatest depth of penetration in the middle third.

The maximum penetration of sodium hypochlorite in radicular dentine in the XP EndoFinisher (Group 2) could be explained by its special manufacturing process from a proprietary alloy with a shape-memory based design, allowing this single file with a size 25 diameter and zero taper to change from the Martensitic-phase to the Austenitic-phase within the root canal during rotation mode, allowing the file to adapt properly to the canal shape and expand inside the [8]

The unique spoon shape of the XP Endo finisher, with a length of 10 mm from the tip and a depth of 1.5 mm, allows the file to expand to 6 mm in diameter, or 100-fold when compared to an equivalent-sized file, allowing it to easily clean canal irregularities and effectively penetrate the dentin.[5]

Similar to this study, Zand et al. (2020) investigated the efficacy of XP in smear layer removal with various solutions, as removing the smear layer opens the dentinal tubules for sodium hypochlorite penetration. In accordance with our findings, they determined that XP promotes smear layer removal when combined with NaOCl and EDTA. [5]

Our results were in agreement with Ghorbanzadeh et al. 2016, Rajakumaran and Ganesh. (2019) who stated that XP endo finisher system is more effective than passive ultrasonic irrigant activation in increasing the depth of irrigant penetration inside the dentinal tubules at the middle and the coronal thirds.[1]

When compared to group 4, group 3 (Sonic agitation by EndoActivator) produced better outcomes. (PUI using IrriSafe).

This was consistent with the findings of the study conducted by Gu Y, Perinpanayagam H, Kum DJ, Yoo YJ, Jeong JS, Lim SM, Chang SW, Baek SH, Zhu Q, and Kum KY. (2020).[9]

They concluded that sonic agitation was more efficient than ultrasonic agitation in promoting irrigant and sealer penetration of dentinal tubules. [9]

Sonic activation can generate strong hydrodynamic intracanal ripples. These waves form bubbles that oscillate within the specified solution. These bubbles will grow and collapse as an implosion, emitting shockwaves that dissipate at a rate of 25,000 - 30,000 times per second.[4]

Yan Shen et al. (2022) described the bioacoustics effect of sonic irrigation in which shockwaves produced by sonic agitation transport disinfecting agents deep into biofilms by breaking their protective mechanism, resulting in bacterial death. This process effectively cleans debris from the root canals' middle and apical thirds. [4]

In this study, Group 4 (Passive Ultrasonic Irrigation Using IrriSafe) performed worse than Groups 2 and 3, but better than Group 1.

The ultrasonic device's underperformance could be attributed to inadvertent contact between the ultrasonic file and canal walls, which could have reduced its efficacy.

The findings contradicted the findings of Vadhana S et al. (2015), who claimed that the PUI group demonstrated superior penetration of 2% CHX irrigant into dentinal tubules. [3]

Manual dynamic agitation (Group 1) failed to perform as well as the other groups in this study. The reason for this is that the energy produced by the push-pull action of manual activation (3.3 Hz) is much lower than sonic energy (1 - 6 kHz) and ultrasonic energy (25-30kHz). [3,4]

The current study found that the maximum depth of penetration of sodium hypochlorite is in the coronal and middle thirds of all groups.

According to Morgental et al., these findings could be attributed to the fact that the diameter of the dentinal tubules in the middle and coronal thirds is larger and more patent with less ramification than in the apical third, resulting in deeper irrigant penetration. (2013). [5]

These results agreed with Rajakumaran and Ganesh et al. (2019), who stated that dentinal tubules are larger and more densely packed in the coronal and middle thirds of the root canal, while they are narrower in the apical third, resulting in better irrigant penetration in the coronal and middle thirds. [24]

Despite using different methodologies, Paque et al. (2006), Giardino et al. (2017), and Galler et al. (2017) all found similar findings. (2019). [5,17]

This regional variation can be ascribed to apical dentine characteristics such as increased peritubular sclerosis that progresses in a coronal direction beginning at 30 years of age, as well as decreased tubular density. (Mjor & Nordahl 1996, Mjor et al. 2001, Paque et al. 2006). [24]

Furthermore, dentinal sclerosis, which forms naturally along the mesio-distal direction of teeth, has been shown to be more pronounced apically than in any other segment of the root canal. (Russell et al. 2013, Giardino et al. 2017, Generali et al. 2018).[2]

Data from Spoorthy et al. (2013) and Ismail et al. (2016) support this hypothesis, as they discovered deeper irrigant and sealer infiltration when several agitation methods were used in a single canal.[24]
Overall, the current research found that group 2 had the greatest depth of penetration of 5.25% sodium hypochlorite into radicular dentine. (XP EndoFinisher).
Furthermore, the greatest depth of penetration was observed in the coronal and middle thirds.

CONCLUSION

Within the constraints of the current study, it can be determined that the overall depth of penetration of sodium hypochlorite was greatest with the XP EndoFinisher file system, followed by sonic agitation by EndoActivator. Maximum penetration was observed in the middle and coronal thirds.
No single technique reliably produced the best results across the entire length of the root canal. The agitating mechanisms of these techniques are most likely to blame for the regional variations in penetrative efficacy. This would imply that each technique is best suited for a specific area of the canal, and that a combination of agitation techniques may be needed to achieve maximum irrigant penetration along the entire length of the root canal.

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