IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 22, Issue 6 Ser.9 (June. 2023), PP 59-65
www.iosrjournals.org

Comparison Of Debris Removal By Chloroquick High And 0.2% Chitosan Nanoparticle Containing Irrigants Activated By Passive Ultrasonic Irrigation –A Confocal Laser Scanning Microscopic Analysis

Dr. Macherla Kranthi Kumar[1*,] Dr Dunnala Sowjanya[2], Dr. Sudhendra Deshpande[3], Dr Sreeha Kaluvakolanu[4,] Dr. K Lakshmi Bhavya[5], Dr Kasireddy Jyothsna[6,] Dr Jahnavi Javvadi[7]

*(Postgraduate KIMS, Amalapuram[*1], Senior Lecturer, KIMS Amalapuram[2], Professor and Head, KIMS, Amalapuram[3,] Reader, KIMS Amalapuram[4,] Senior Lecturer, KIMS Amalapuram[5,] Senior Lecturer, KIMS Amalapuram[6,] Postgraduate KIMS Amalapuram[7])*

Abstract:
Background: The root canal system has a complex anatomy with areas that cannot be touched mechanically by endodontic instruments, such as isthmuses, fins and recesses in oval-shaped canals.[1] Irrigants have a central role in endodontic treatment. There is no data exist in the literature comparing the ChloroQuick High and 0.2 % Chitosan nanoparticles activated by passive ultrasonic irrigation technique. Hence the aim of this study is to compare debris removal by ChloroQuick High and 0.2% Chitosan nanoparticle irrigating solutions activated by passive ultrasonic irrigation.
Objectives:
1. To compare the efficiency of ChloroQuick High and 0.2% Chitosan nanoparticle in debris removal as final irrigants.
2. To compare the efficiency of passive ultrasonic irrigation and manual dynamic agitation in debris removal.
Materials and Methods: Thirty extracted human mandibular premolar teeth were decoronated at the level of cementoenamel junction to standardize the length of 15mm. After access opening, the working length was determined. Biomechanical preparation was done with ProTaper gold rotary files SX-F3 size. Irrigation was performed with 5.25% NaOCI. According to final irrigation protocols, samples were divided into Group I (n=10) ChloroQuick high, Group II (n=10) 0.2% Chitosan nanoparticle, Group III (n=10) Saline (control group). Based on the agitation of irrigating solutions each group is subdivided into two subgroups (n=5) Sub Group A- Passive ultrasonic irrigation (PUI) and Sub Group B – Manual Dynamic Agitation (MDA). Debris removal is examined at Coronal, Middle, Apical third of roots using confocal laser scanning microscopy (CLSM). Data was analysed using one way analysis of variance, and Friedman's tests.
Results: PUI Showed better debris removal than MDA at all levels. The depth of irrigant penetration is highest for Chloroquick High followed by Chitosan nanoparticle & least for Saline. Debris removal was more at coronal than at middle & apical. All the results are statistically significant.
Conclusion: Passive ultrasonic activation of ChloroQuick High showed better smear layer removal at all levels. Hence, it can be used as an alternative for routine irrigation protocol.
Keywords: 0.2 % Chitosan nanoparticle, ChloroQuick High, Confocal laser scanning microscope, Passive ultrasonic irrigation.

Date of Submission: 09-06-2023 Date of Acceptance: 19-06-2023

I. Introduction

The root canal system has a complex anatomy with areas that cannot be touched mechanically by endodontic instruments, such as isthmuses, fins and recesses in oval-shaped canals.[1] Irrigants has a central role in endodontic treatment. Among them, the novel irrigants include, ChloroQuick high (innovations endo, India) contains 18% HEBP and 5.25% NaOCl, need to be premixed with surfactant tween 80 for a complete root canal irrigation solution.[2] Chitosan is a biopolymer derived by the partial deacetylation of chitin obtained from crustacean shells.[3] Silva et al.[4] recommended using 0.2% chitosan for 3 min to remove the smear layer without causing dentinal erosion. The accumulated hard tissue debris (AHTD) within root canal irregularities may be

inaccessible to conventional syringe-and-needle non-activated irrigation (NAI).[5] Additional supplementary methods, such as passive ultrasonic irrigation (PUI) and other activation methods have been proposed to drive irrigants solutions into these root canal complexities.[6] There is no data exist in the literature comparing the ChloroQuick High and 0.2 % Chitosan nanoparticles activated by passive ultrasonic irrigation technique. Hence the aim of this study is to compare debris removal by ChloroQuick High and 0.2% Chitosan nanoparticle irrigating solutions activated by passive ultrasonic irrigation.

Objectives:
1) To compare the efficiency of ChloroQuick High and 0.2% Chitosan nanoparticle in debris removal as final irrigants.
2) To compare the efficiency of passive ultrasonic irrigation and manual dynamic agitation in debris removal.

II. Material And Methods

Study site: Department of conservative dentistry and Endodontics KIMS, Amalapuram, Andhra Pradesh, India

Study duration: Three months: January 2023 to March 2023

Sample size: 30 samples were taken.

Ethical considerations:
Institutional ethical committee approval was obtained before conducting the study.

Methodology:
Preparation of teeth:
Thirty recently extracted human mandibular premolar teeth were collected for this study.

Radiographs were taken for evaluation and to exclude teeth with caries, fractures, calcification, multiple canals, resorptions. Teeth which were caries free, having a single canal and a straight mature root, were included in the study. All the samples were stored in distilled water until further used in the study. All the samples were cleaned and free off debris and calculus with piezo ultrasonic scaler unit (Satelec Acteon, Merignac, France). The root was separated from the crown to obtain a root length of 15 mm, with Bucco-lingual oriented cuts, with the aim of establishing stable reference point with help of diamond disk by cuspal reduction. A size 10 K file (Mani Inc., Tochigi, Japan) was introduced into the canal until it was visible at the apical foramen, and the working length was determined by reducing 1 mm from this length. Working length radiographs were taken.

Root canal preparation:

The Glide path was established with 15k file and then root canal instrumentation were done with Protaper Gold (PTG; Dentsply Maillefer, Ballaigues, Switzerland) with basic sequence SX, S1, S2, F1, F2, F3, operated in continuous rotation motion at 300 rpm and a torque of 5.2 Ncm by an electric motor (CanalPro CL2 Endo motor; Coltene, Whaledent Pvt Ltd). Irrigation with 2.5 mL of 5.25% NaOCI (Prime dental products Pvt. Ltd, Mumbai) was performed between each file, using a 27G needle (Orikam Healthcare, India) inserted 1 mm shorter than the working length. Canals were dried with paper points (Dentsply-Maillefer, Ballaigues, Switzerland). Apex was covered with composite resin (Tetric N Ceram, Ivoclar, Vivadent AG). Gutta-percha point (Dentsply-Maillefer, Ballaigues, Switzerland) was placed at the working length in order to avoid composite intrusion and removed after resin curing.

Groups:

Teeth were randomly divided into three groups (n = 10) according to final irrigation. Group I - ChloroQuick High (Innovations Endo, India), For Group II - 0.2 % Chitosan nanoparticle (Swatik Biotech PVT Ltd, Bengaluru), For Group III (control) saline solution. About 0.1 g of Rhodamine B dye (LOBA CHEMIE PVT. LTD., Mumbai, India) was mixed with 100 ml of each of irrigating solutions and used as the final irrigants.

For samples In Sub Group A (n=5) 3 ml of irrigating solution were delivered into canal & activated with Irri safe Size 20 # tips (Acteon, Merignac, France) & 3 W and 30 kHz piezoelectric ultrasound P5 Newtron XS unit (Satelec Acteon, Merignac, France). Irri safe Ultrasonic tip was placed 1 mm coronal to the working length and was used with 2 - 3 mm apical-coronal movements.[7] For Sub Group B (n=5) 3 ml of irrigating solution were delivered with 30 G Side vented needle (Orikam Healthcare, India) A fitted master cone F3 was grasped with tweezer and inserted 1mm short of the working length, push and pull strokes were performed manually at an approximate rate of 100 strokes per minute.[8] Roots were placed in Clear acrylic resin blocks and transversely sectioned to a thickness of 1 mm, using a slow-speed, water-cooled diamond saw (Leica SP1600 – Saw Microtome, Leica Microsystems Nussloch GmbH Heidelberger Str. 17-1969226 Nussloch Germany) at 2 mm

(apical), 5 mm (middle) and 8 mm (coronal) from the apex of the root.[7] The samples were then bonded onto glass slides and ground with wet silicon carbide papers. The slides were examined with a confocal laser scanning microscope (LSM 880, Zeiss Microscopes, Germany) at x10 and x20 under Ar/HeNe laser excitation with a wave length of 543 nm. The point of deepest penetration was measured from the canal wall to the point of maximum dye penetration in µm. For each section, the circumference of the root canal was outlined and the portion of the root canal perimeter showing tubular penetration was outlined and measured. Images were analyzed by Ziess LSM 880 image examiner software (ZEN Blue version) and imageJ software.

Statistical analysis: Data were analyzed using SPSS 23.3 software. One-way ANOVA test used to compare data between groups. P value below 0.05 is considered significant.

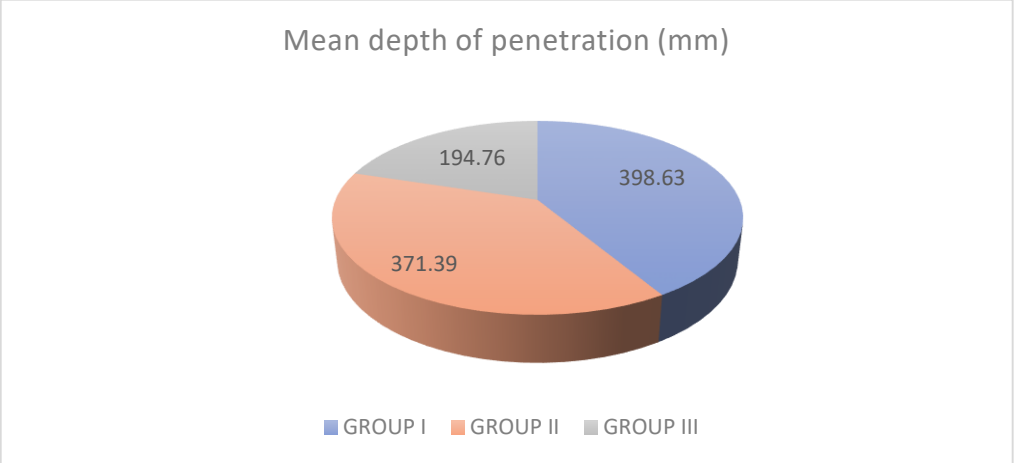
III. Results

Depth of penetration: With passive ultrasonic activation of irrigants, at all positions, highest depth of penetration was noted with ChloroQuick High (398.63) followed by 0.2% Chitosan nanoparticle (371.39) and normal saline (194.76). These differences were statistically significant, as evident from P value (0.000).

Table 1: Depth of penetration among 3 groups of samples- with passive ultrasonic activation with P value- ANOVA analysis

ANOVA ANALYSIS	SS (sum of squares)	Df (degree of freedom)	MS (mean squares)	F	p
Between groups	245,010.505	2	122,505.252	213.169	0.000
Within groups	15,516.540	27	574.687		
Total:	260,527.045	29			

Graph 1: Depth of penetration of all groups- with passive ultrasonic activation

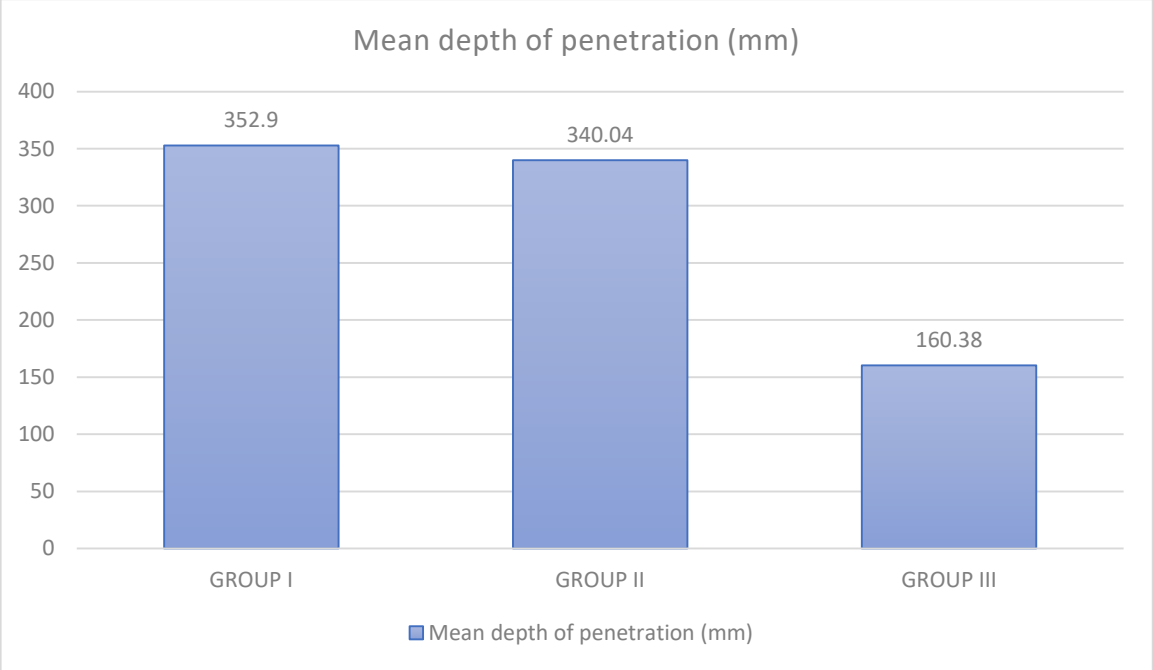


With manual dynamic agitation of irrigants, at all positions, highest depth of penetration was noted with ChloroQuick High (352.9) followed by 0.2% Chitosan nanoparticle (340.04) and normal saline (160.38). These differences were statistically significant, as per P value (0.000).

Table 2: Depth of penetration among 3 groups of samples- with manual dynamic agitation of irrigants with P value- ANOVA analysis

ANOVA ANALYSIS	SS	df	MS	F	p
Between groups	231,690.152	2	115,845.076	209.660	0.000
Within groups	14,918.490	27	552.537		
Total:	246,608.642	29			

Graph 2- Depth of penetration among 3 groups of samples- with manual dynamic agitation of irrigants



Similar observations were made with regard to the dye penetration along the canal wall perimeter with both passive ultrasonic and manual dynamic irrigation (Table 2 & Graph 2)

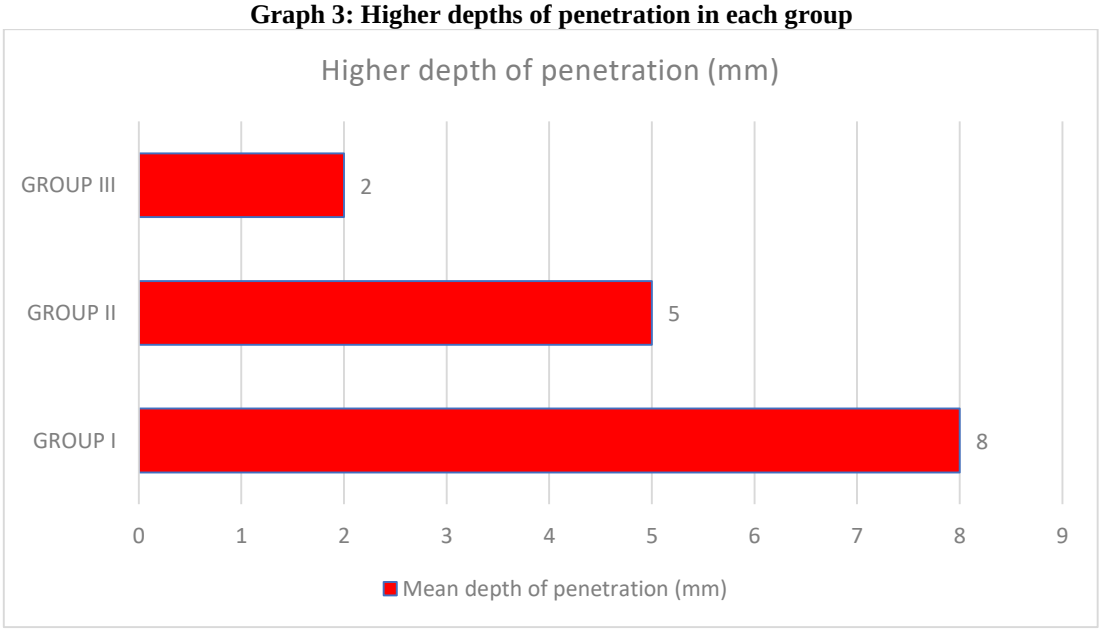
Higher depths of penetration:

Within each group, higher depths of penetration and greater dye penetration along the canal wall perimeter were observed at 8 mm followed by 5 mm and 2 mm with both irrigation techniques.

Table 3: Higher depths of penetration in each group

ANOVA ANALYSIS	SS	df	MS	F	p
Between groups	180.000	2	90.000	115.701	0.000
Within groups	21.002	27	0.778		
Total:	201.002	29			

Graph 3: Higher depths of penetration in each group



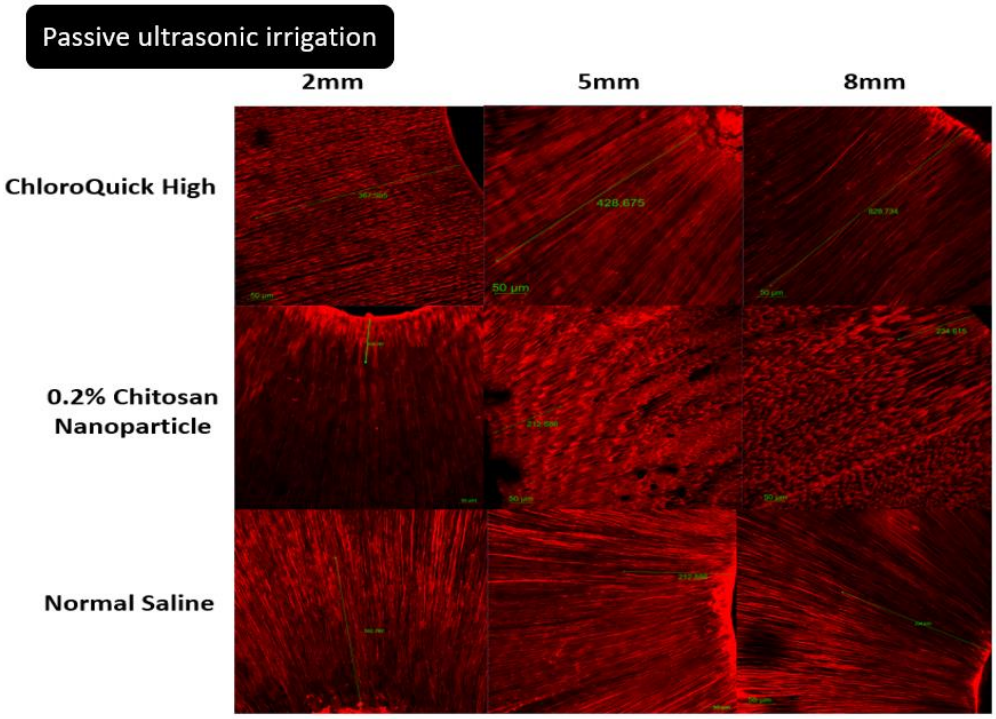


Image 1: Passive ultrasonic irrigation

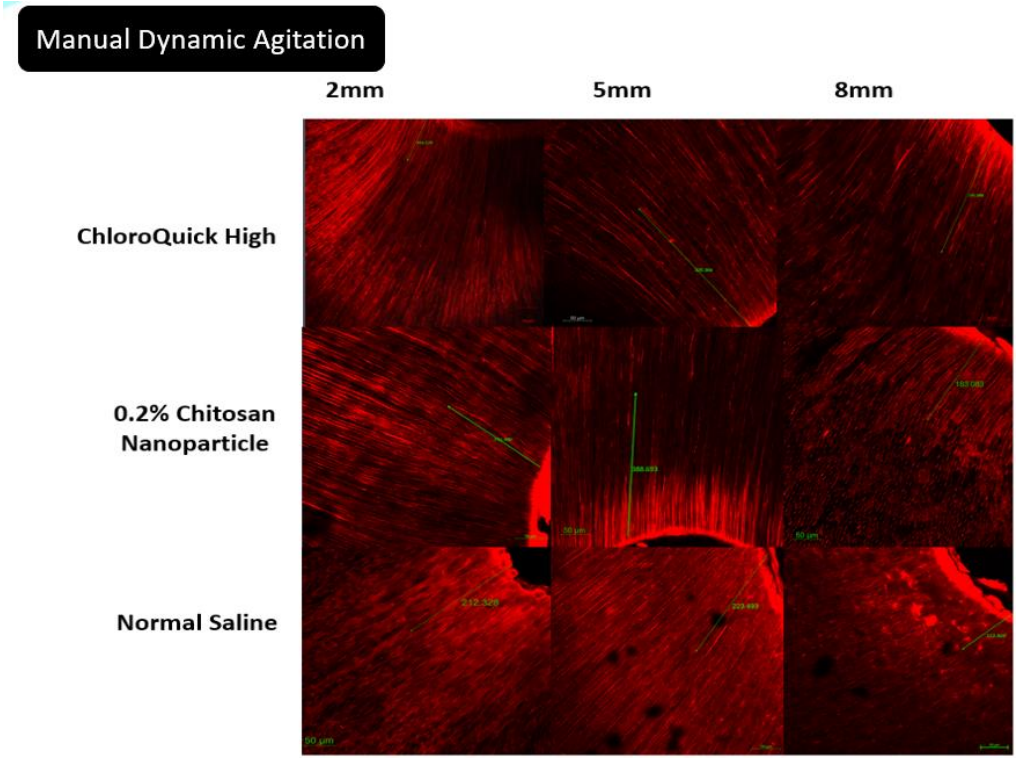


Image 2: Manual dynamic agitation

IV. Discussion

The goal of root canal instrumentation is to achieve aseptic root canal system based on the combined use of mechanical instrumentation and two or several irrigating solutions and to obtain a continuous taper. Louis Grossman stated: "mechanical instrumentation should be followed by irrigation of canal in order to wash out fragments of pulp tissue and dentinal shavings.

A review evaluated the literature on PUI and has described its efficient antibacterial effect, better removal of smear layer, better cleaning efficacy in curved root canals and canal isthmuses.

Etridonic acid (HEBP) which is a soft chelating agent that appears to have a nominal effect on dentine walls and still acts on the smear layer. Lottanti *et al*. showed that HEBP could be used in combination with NaOCl without affecting its proteolytic or antimicrobial properties.[9] ChloroQuick (innovationsendo, India) is a combination of NaOCl and HEBP. ChloroQuick high contains 18% HEBP and 5.25% NaOCl, whereas ChloroQuick low contains 9% HEBP and 3% NaOCl. The highlight of such combination of NaOCl and HEBP is that the NaOCl does not surrender its biological, antibacterial, and tissue dissolving properties, whereas the reduction and elimination of the inorganic element are done with the help of HEBP. In a study conducted by Hegde V, Thakkar P, concluded that ChloroQuick High shows better removal of smear layer.[2]

Chitosan, a natural polysaccharide, is prominently used in dentistry because it is biocompatible, biodegradable, bioadhesive, and non-toxic, with broad-spectrum antimicrobial properties and chelating activity.[4] According to the literature, final irrigation of the root canal with chitosan has the advantage of removing the smear layer in addition to inhibiting bacterial recolonization. Polliana Vilaça Silva ANTUNES et al., concluded that final irrigation of the root canal with 15% EDTA or 0.2% chitosan achieved comparable effects in terms of reducing dentin microhardness, penetrating endodontic sealer through the dentinal tubules, and bond strength.[10] In the present study, ChloroQuick High showed better results due to combination of 5.25% NaOCL+ 18% HEBP in ChloroQuick High might have contributed to increased depth of penetration compared to Chitosan .[2]

Passive Ultrasonic Irrigation (PUI) oscillates at an ultrasonic frequency of 25 - 30 kHz. Due to this oscillation, it has the potential to create acoustic streaming, Cavitation and aids in the deeper penetration of irrigants laterally. A review evaluated the literature on PUI and has described its efficient antibacterial effect, better removal of smear layer, better cleaning efficacy in curved root canals and canal isthmuses.[11] Better smear layer removal and penetration depth in experimental groups of PUI is might be due to method of activation of irrigating agent than manual dynamic agitation. Confocal laser scanning microscopy (CLSM) is an excellent tool for evaluation of dentinal tubule penetration on account of its ability to produce standardised, reproducible 3-D imaging, and the detailed information it provides at lower magnifications (10x) by means of fluorescent rhodamine-marked sealants.[7]

The present study showed that, in all groups maximum depth of irrigant penetration is seen in coronal thirds than in the middle and apical thirds (Fig. 1&2). These results are in agreement with previous studies.[11,12,13] This might be because the coronal third of the root canal had the smear layer removed more effectively than the apical third. It must also be considered that larger number and diameter of dentinal tubules exist in coronal areas, irrigating solutions in experimental groups may have induced significantly greater penetration depth of irrigating solutions.[14]

V. Conclusion

Within the limitations of this In vitro study, it may be concluded that PUI group showed better penetration of ChloroQuick High irrigant into dentinal tubules than manual dynamic agitation group, at all three levels. Acoustic microstreaming plays an important role in the efficacy of PUI. The use of ChloroQuick High with PUI application can be considered as an alternative for final irrigation in the clinical practice, as it can penetrate into the dentinal tubules.

References

[1]. Chuppani Dastgerdi A, Navabi M, Rakhshan V. Isthmuses, accessory canals, and the direction of root curvature in permanent mandibular first molars: an in vivo computed tomography study. Restor Dent Endod. 2019;45: e7.

[2]. Hegde V, Thakkar P. Effect of continuous soft chelating irrigation protocol on removal of smear layer. Endodontology 2019; 31:63-7.

[3]. Kesim B, Burak AK, Üstün Y, Delikan E, Güngör A. Effect of chitosan on sealer penetration into the dentinal tubules. Niger J Clin Pract 2018; 21:1284-90.

[4]. Silva PV, Guedes DF, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: A new solution for removal of smear layer after root canal instrumentation. Int Endod J 2013; 46:332-8.

[5]. Versiani MA, Alves FR, Andrade-Junior CV, Marceliano-Alves MF, Provenzano JC, Rôças IN, et al. Micro-CT evaluation of the efficacy of hard-tissue removal from the root canal and isthmus area by positive and negative pressure irrigation systems. Int Endod J. 2016; 49:1079-87.

[6]. Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J. 2007; 40:415-26.

[7]. Llena C, Forner L, Cambralla R, Lozano A. Effect of three different irrigation solutions applied by passive ultrasonic irrigation. Restor Dent Endod. 2015; 40:143-8.

[8]. Machtou PP. Manual dynamic activation technique. Clin Dent Rev. 2018; 2:1-5

[9]. Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. Int Endod J 2009; 42:335-43.

[10]. Antunes PVS, Flamini LES, Chaves JFM, Silva RG, Cruz Filho AMD. Comparative effects of final canal irrigation with chitosan and EDTA. J Appl Oral Sci. 2019; 28:1-7

[11]. Moon YM, Shon WJ, Baek SH, Bae KS, Kum KY, Lee W. Effect of final irrigation regimen on sealer penetration in curved root canals. J Endod 2010; 36:732-6.

[12]. Kara Tuncer A, Tuncer S. Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. J Endod 2012; 38:860-3.

[13]. Yoo YJ, Lee W, Kim HC, Shon WJ, Baek SH. Multivariate analysis of the cleaning efficacy of different final irrigation techniques in the canal and isthmus of mandibular posterior teeth. Restor Dent Endod 2013; 38:154-9.
[14]. Al-Jadaa A, Paqué F, Attin T, Zehnder M. Necrotic pulp dissolution by passive ultrasonic irrigation in simulated accessory canals: impact of canal location and angulation. Int Endod J 2009; 42:59-65.