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Original Research Article

Comparative Assessment of the Effect of a Modified Irrigation Protocol on the Cleanliness of Moderately Curved Root Canals: An *In-vitro* Study

Gaurav Jain[1]*, Preeti Shukla[1], Pradyumna Misra[1], Shronika Shronika [1], Swadhinta Raj[1], Anupama Keerikkadu[1]

[1]Dept. of Conservative Dentistry and Endodontics, Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India

Abstract

Background: Adequate disinfection of root canal system is essential for the success of endodontic therapy and chemo-mechanical preparation of the root canal system during root canal treatment ensures the same. The varying anatomical complexities of root canal is one of the limiting factors for mechanical instrumentation of the canal alone, so judicious use of chemical irrigation assumes more responsibility in such cases. The present study assesses and compares the efficacy of modified irrigation protocols on the debridement and smear layer removal in moderately curved canals, and also its subsequent effect on chemical composition of root canal dentin.

Materials and Methods: 60 human single rooted teeth with moderately curved canals (10-30° curvature) were randomly divided into 3 groups (n=20). Irrigation protocol employed in Group I was 5ml of 3% NaOCl between each instrument, followed by 5ml of 17% EDTA for 30 seconds. Group II employed Reverse Irrigation (RI) protocol wherein 5ml of 17% EDTA was used between each instrument followed by 5ml of 3% NaOCl for 30 seconds. In Group III, Reverse Irrigation plus (RI+) was used which is similar to RI except that NaOCl used was agitated manually in the canals. Debridement of root canal dentin was assessed using Scanning Electron Microscope and the chemical composition of dentin was assessed by energy dispersive X-ray (EDX).

Results: The mean Hulsmann score at the middle third in all groups were comparable, wherein Group I at 1.8, in Group II at 2.9 while in Group III it was 1.5. At the apical third, the score in Group I was 2.5, Group II was 3.6 and Group II was at 1.5. The difference was significant ($p<0.05$). The atomic percentage of phosphorus, carbon, oxygen, sodium and magnesium in all groups was comparable.

Conclusion: Results showed that 17% EDTA during cleaning and shaping followed by 3% NaOCl rinse for 3 minutes and manual agitation for 30 seconds improved apical debridement in moderately curved canals without inducing dentin erosion.

Keywords: Curved root canals, EDTA, Irrigants, NaOCl, Smear layer.

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1. Introduction

Endodontic infections caused due to microorganisms lead to inflammation of pulpal and peri-radicular tissue and removal of this pathogenic microflora and infected tissue forms the core of endodontic therapy.[1] Adequate chemo-mechanical preparation of root canal space during the root canal treatment is an important contributing factor to the long-term success and subsequently in maintaining the endodontically treated tooth in normal form and function in the oral cavity.

Of the two aspects of chemo-mechanical preparation, mechanical preparation alone primarily focuses on removal of pulp tissue, infected dentin, and bacteria;[1] however, adjunctive use of chemical irrigants eliminates the tissue debris generated in the due process and also the remaining micro-organism, if any, present in root canal dentin.[2] The varying complex root canal morphology makes this step more challenging and affects efficiency of debridement as anatomy limits instrumentation leaving the nooks and crannies of the root canal system unprepared. Also, the risk of unwanted endodontic mishaps (transportation, ledge formation, perforation) increases multifold with variations like curved

*Corresponding author: Gaurav Jain
Email: gauravjs23@yahoo.com

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canals. It increases the risk of instrument separation too, restricting the use of instruments for mechanical debridement. Moreover, apical curvature in root canals further impedes efficient removal of smear layer formed. Hence, use of chemical irrigation becomes indispensable to efficient debridement and adequate disinfection during chemo-mechanical preparation of the root canal system especially in curved canal spaces.[3,4]

Of the most commonly used irrigants, majority have actions that can be categorized under antimicrobial action, organic tissue dissolution activity and chelation action. Sodium hypochlorite (NaOCl) is a well-established endodontic irrigant used in various concentrations (0.5-5.25%) favored as primary irrigant by clinicians for use. While higher concentration (5.25%) achieve faster dissolution of organic tissue, it runs the risk of tissue toxicity when pushed inadvertently in the periapical region.[5] In some studies, even at lower concentrations (1-3%), it has proven to be an effective antimicrobial agent. Thereby, concentrations of sodium hypochlorite ranging between 2.5-3% are theorized to strike the right balance between efficiency and safety.[6] It is also observed that sodium hypochlorite has no effect on the inorganic dentin debris formed during mechanical preparation of the canal that leads to the formation of endodontic smear layer.[7] Hence the need arises for the combined use of irrigants with varying core action and techniques that augment the action of irrigants used, to enhance debridement of canals.[2] According to some studies, 0.5-2µm thick smear layer consisting of organic and inorganic debris, packed into canal walls, was shown to harbor bacteria and occlude 6% of the volume of mesial roots in mandibular molar as the mesial roots show moderate curvature.[4] Thus removal of smear layer is advocated in order to enhance canal disinfection. 17% EDTA is being routinely used by clinicians because of its chelation action. Researches have shown that larger volumes and longer duration of irrigation have more impact on canal debridement and this creates a space to look for the most impactful irrigation protocol.[8] Different irrigation protocols are routinely employed by clinicians and a modified irrigation protocol is a step away from the conventionally followed irrigation protocol. The present *in-vitro* study was conducted to assess the effect of modified irrigation protocol on debridement of moderately curved canals and subsequently, its effect on chemical composition of root canal dentin.

2. Materials and Methods

The present *in-vitro* study involved 60 extracted roots with moderately curved canals, having 10-20 degrees of curvature, as determined by Schneider's method (**Figure 1**) (radio graphing the tooth from buccolingual and proximal direction to determine the root canal curvature).[9] All teeth were decoronated to achieve standardized root length of 15mm till root apex. Canals were located using DG-16 (Dentsply Maillefer, Switzerland) and ISO size 10 K-Flex file (Dentsply Maillefer, Switzerland) was used to establish canal patency. The working length was confirmed and recorded by reducing 1mm from the file length after its emergence through apical foramen. An ISO size 15 followed by ISO size 20 hand K-files were than used to standardize the foramina. The teeth were then randomly divided into three groups (n=20) (**Table 1**) according to the irrigation protocol to be studied. The root canals were prepared using rotary nickel-titanium Hyflex® CM file system (ColteneWhaledent, USA) upto the working length, to size 30/0.06 taper, following different irrigation protocols as per group allocation.

Three groups were determined as follows: In Group I, irrigation protocol followed was 5 ml of CanalPro™ NaOCl 3% (ColteneWhaledent, USA) between each instrument, followed by 5 ml of CanalPro™ EDTA 17% (ColteneWhaledent, USA) for 30 seconds. In Group II, reverse irrigation (RI) was used i.e., 5 ml of 17% EDTA, followed by 5 ml of 3% NaOCl for 30 seconds. In Group III, reverse irrigation plus (RI+), similar to RI, was used with the only alteration being that NaOCl was allowed to interact with dentin for 3 minutes before manual agitation for 30 seconds. Final rinse in all the groups was done with distilled water (Nirlife, India). In all samples, root canal debridement was assessed using scanning electron microscope (SEM) in the middle and apical regions and the chemical composition of dentin such as atomic percentage of calcium, phosphorous, carbon, oxygen, sodium and magnesium after irrigation was assessed by energy dispersive X-ray (EDX). Data thus obtained were subjected to statistical analysis using SPSS Statistics 23 (IBM Corporation, Chicago). $p<0.05$ was considered significant. Institutional ethical clearance was obtained before starting the study from Institutional Human Ethics committee and Institutional Research & Development committee vide number #FG1CE21122020R.

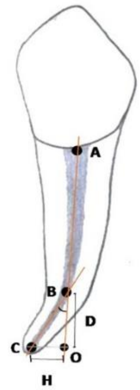

Figure 1: Schematic diagram showing determination of root canal curvature using Schneider's method (Schneider's angle- Angle formed between the lines CB and BO; Line AO is a straight line along the axis of root canal). Point 'A': Point at canal orifice. Point 'B': Point where canal starts to deviate. Point 'C': Point at apical foramen. Point 'D': Curvature distance- perpendicular line drawn from B to OC- BO. Point 'H': Curvature Height-OC.

Table 1: Distribution of teeth based on type of irrigation used

Groups	Group I	Group II	Group III
Method	Standard irrigation	Reverse irrigation	Reverse irrigation plus
Number	20	20	20

3. Results

The mean Hulsmann scores at middle third in Group I was 1.8, in Group II was 2.9 and in Group III was 1.5 (**Table 2**). At apical third, in Group I was 2.5, in Group II was 3.6 and in Group III was 1.5. The difference was significant ($p < 0.05$). The atomic percentage of calcium, phosphorous, carbon, oxygen, sodium and magnesium in all groups was comparable ($p > 0.05$) (**Table 3**).

Table 2: Assessment of mean Hulsmann scores in the middle and apical regions

Region	Group I	Group II	Group III	p value
Middle	1.8	2.9	1.5	0.04
Apical	2.5	3.6	1.5	0.05

Table 3: Comparison of chemical composition of dentin in all groups

Chemical	Group I (%)	Group II (%)	Group III (%)	p value
Calcium	27	28	29	0.94
Phosphorous	11	12	14	0.95
Carbon	22	24	18	0.08
Oxygen	34	30	35	0.81
Sodium	3	3	3	1
Magnesium	2	2	2	1

4. Discussion

The role of microorganisms as the causative agent and in perpetuation of pulpal and periapical diseases has been well established in animal models and human studies.[10-12] Array of measures have been studied to reduce/eliminate microorganisms from infected root canal systems, including the use of various instrumentation techniques, irrigation regimens and intra-canal medicaments. There is no evidence in the literature to show that mechanical instrumentation alone results in a bacteria-free root canal system,[1] and considering the complex anatomy of the root canal pulp space, this is anticipated. So, irrigation is considered a necessity to completely and effectively debride the root canals and eliminate all microorganisms to assure an aseptic environment. The effectiveness of any irrigation protocol depends on its ability to reach all corners of the prepared root canal space and navigating all kinds of anatomical variations like canal curvature.[13] The volume and wetting properties of the irrigants, as well as techniques that enhance its action like use of ultrasonic,[2,13] are some of the important factors that can affect the efficiency of an irrigation regimen.

Chemical treatment of the root canal can be arbitrarily divided into irrigants, rinses, and inter-appointment medicaments. Sodium hypochlorite (NaOCl) was first recognised as an antibacterial agent in 1843 when hand washing with hypochlorite solution between patients produced unusually low rates of infection transmission between patients. It was first recorded as an endodontic irrigant in 1920,[14] and is now in routine worldwide use. However, a number of studies showed the inefficacy of NaOCl in removing inorganic debris.[7] It leaves the prepared canal walls covered with a smear layer.

Smear layer, which is a film of debris attached to dentin surface, is composed of excised dentin chips, remnants of vital or necrotic pulp tissue, microorganisms and their by-products, and chemical remnants.[15,16] Literature shows that the smear layer has detrimental influence on adhesion in root canal because it acts as a physical barrier between the filling material and canal walls, undermining sealer penetration and formation of intratubular tags but more importantly, harbours microorganisms, making their elimination an indispensable step in disinfection.[17] Curved root canals, with limited accessibility, make removal of smear layer more difficult.[3,4] Conventional irrigation methods have demonstrated effective debridement in the coronal third, but their efficacy decreases apically.

Moreover, passive irrigation is done to avoid inadvertently pushing the debris and the irrigant beyond apex,[18] but it limits the fluid exchange ability, especially beyond the curvature in curved canals. For efficient debridement, several chemical agents in use today have different properties that are used for cleaning root canals but none of theavailable endodontic irrigants appease all ideal physicochemical properties to act simultaneously on the organic and inorganic components of smear layer and debride the hideaways too.

Efficient, routinely employed, protocol for removal of smear layer is the use of sodium hypochlorite (NaOCl) in concentrations ranging from 0.5% to 5.25% and 17% ethylenediaminetetraacetic acid (EDTA) as initial and final irrigating solutions, respectively.[19] B. Ciucchi *et al.* found in their study that final rinsing with EDTA did not produce the expected smear-free surfaces in the apical region of the canal.[19] This decline of efficiency along the apical part could be attributed to the curvature of the canal which hindered the optimal flow of the irrigant in the region below the curvature. Hence the present study was conducted with the aim to assess the effect of a modified irrigation protocol and their subsequent effect on the cleanliness of moderately curved canals.

An adequate coronal pre-enlargement accurately defines what initial apical instrument should be used, and it results in a more precise anatomical diameter at working length.[20-22] Adequate root canal access increases the efficacy of irrigants and control of instruments.[23] Boutsioukis *et al.*,[24] reported that the 9% tapered file size 30 apical preparation of Protaper F3 allows the deep placement of the irrigation needle and a higher shear stress to develop along canal walls during irrigation as seen in the present study where Hyflex® CM file size 30/0.06 taper was used as final shaping and finishing file. Some studies promote adjunctive methods to improve the action of irrigant used.[2] The present *in-vitro* study investigated different irrigation methodologies, where in one of the groups, a size 30/0.06 taper gutta-percha point was used to manually agitate the solutions. This allowed the air locked in the apical region, beyond the curvature, to escape, ensured homogeneity of the solution and its dispersion.

The smear layer removal efficiency in the study was quantified using Hulsmann scoring criteria which scored the dentinal surfaces based on percentage of patent dentinal tubules. Completely patent dentinal tubules, more than 50% patent dentinal tubules, less than 50% patent dentinal tubules and nearly all of the dentinal tubules occluded with smear layer were the scored 1,2,3 and 4 respectively. In the present *in-vitro* study, it was found that the mean Hulsmann scores at middle third in Group I was 1.8 (**Figure 2**), in Group II was 2.9 (**Figure 3**) and in Group III was 1.5 (**Figure 4**). At apical third, in Group I was 2.5 (**Figure 5**), in Group II was 3.6 (**Figure 6**) and in Group III was 1.5 (**Figure 7**).

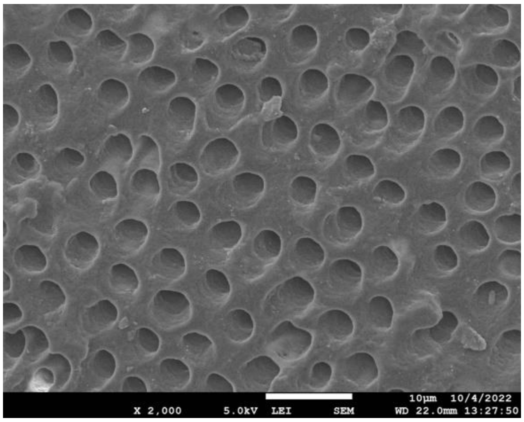

Figure 2: SEM image of middle-third root canal dentin in Group I

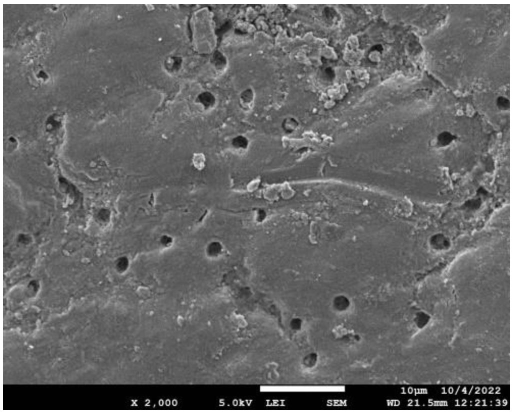

Figure 3: SEM image of middle-third root canal dentin in Group II

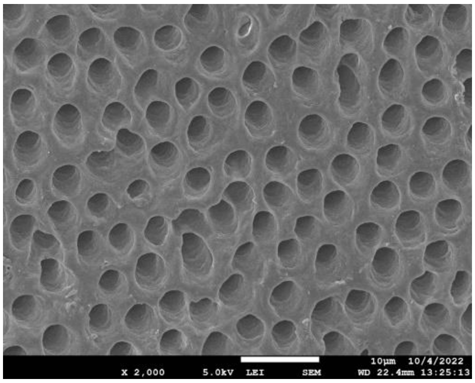

Figure 4: SEM image of middle-third root canal dentin in Group III

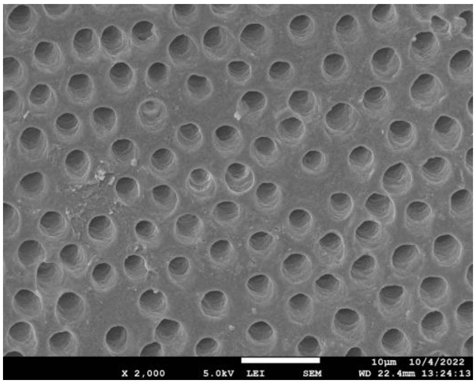

Figure 5: SEM image of apical-third root canal dentin in Group I

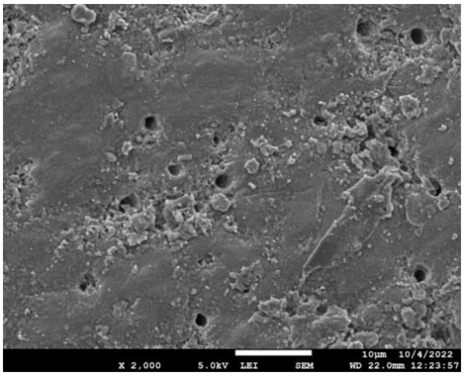

Figure 6: SEM image of apical-third root canal dentin in Group II

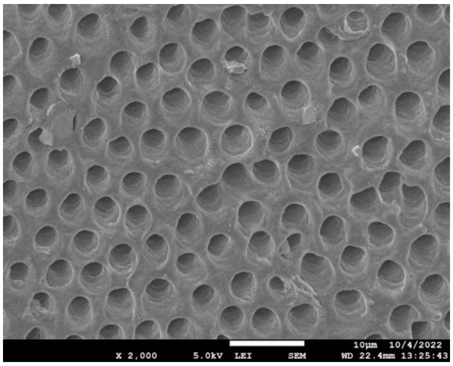

Figure 7: SEM image of apical-third root canal dentin in Group III

The results of the present study were in accordance to the previous studies by Voegeli G *et al.*, that assessed the hypothesis that modifying the sequence of sodium hypochlorite (NaOCl)/ethylene diamine tetra-acetic acid (EDTA) irrigation during root canal shaping would improve apical cleanliness in moderately curved canals in 45 root canals which were irrigated with NaOCl followed by EDTA in one group and the sequence of irrigants used were modified in other test groups.[3] The procedure aimed to assess any significant association of debridement with certain irrigation protocol in moderately curved canals. It was observed that in the apical region, cleanliness was better in the group which was first irrigated with EDTA, followed by NaOCl and manual agitation for 30 seconds than the group that commonly followed routine irrigation protocol. Odd ratios found in the present *in-vitro* study substantiated the same.

The improved debridement of root canal dentin in group I compared to Group II can be associated with the use of EDTA as the final rinse which improves the smear layer removal. The enhanced smear layer removal in Group III, in contrast to Group I, even though NaOCl is used as the final rinse, can be ascribed to the use of manual agitation in Group III. Studies have shown that different methods of activation of irrigants augment their smear layer removal efficacy.[4]

Furthermore, literature shows that when in contact with irrigants, dentin changes its physical, chemical and structural properties.[25-30] NaOCl was shown to change the flexural strength and modulus of elasticity of dentin.[27,28] EDTA has shown to alter the microstructure of dentin. EDTA demineralizes the inorganic components of dentin by the chelation of calcium ions present in the hydroxyapatite, the main inorganic compound of dentin.[27,29] This might translate into endodontically treated teeth that are more susceptible to crown or root fracture. However, contradictory to this, the tested protocols showed no such result. Although higher amount of EDTA was used in Group II and Group III as compared to Group I, the EDX analyses showed that the irrigation protocols did not have any detrimental effect on the inorganic composition of root dentin. The atomic percentage of calcium, phosphorous, carbon, oxygen, sodium and magnesium in all groups was comparable. This might be attributed to the fact that final rinsing with NaOCl in Group II and Group III, removes EDTA remaining in the canal, oxidizes it, eliminating the risk of prolonged chelation.[31]

5. Conclusion

The irrigation protocol with NaOCl followed by EDTA is the gold standard for efficient disinfection of the root canal space and while it allows clinicians to clean canal surfaces in most cases, studies suggest that cleaning the apical third of curved canals is cardinal to achieve canal disinfection. The experimental protocol of irrigation employed in the present study suggested that 17% EDTA during shaping followed by 3% NaOCl rinse for 3 minutes and manual agitation for 30 seconds, improved apical debridement in moderately curved canals without inducing erosion of dentin, when compared to the routinely implemented irrigation protocols.

6. Ethical Approval

FG1CE21122020R via Ref. SDC/IRDC/2021/F/01

7. Author Contribution

1. Gaurav Jain: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review editing.
2. Preeti Shukla: Data curation, Investigation, Resources, Software, Validation.
3. Pradyumna Misra: Data curation, Formal analysis, Resources, Supervision.
4. Shronika Shronika: Data curation, Formal analysis, Software.
5. Swadhinta Raj: Data curation, Methodology, Software.
6. Anupama Keerikkadu: Data curation, Methodology.

8. Source of Funding

None.

9. Conflict of Interest

None.

10. Acknowledgement

None

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