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Evaluation of efficacy of different intermittent irrigants in prevention of orange brown precipitate formation - An in vitro study

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Abstract

Aim: Aim of the study was to evaluate the efficacy of different intermediate endodontic irrigants to prevent the formation of orange-brown precipitate between sodium hypochlorite and chlorhexidine

Material and methods: Access opening for forty freshly extracted mandibular premolars was done, canals were instrumented and samples were randomly divided into four groups based on irrigation solutions used. In Group 1 canals were irrigated with 3% NaOCL followed by saline and 2% CHX. In Group 2,3,4 canals were irrigated with

70% Isopropyl alcohol,40% isopropyl alcohol and 10% citric acid as intermediate irrigants between NaOCL and CHX. All the samples were sectioned longitudinally and the canal sufaces was observed under stereomicroscope for evaluation. The results were tabulated as per scoring criteria and statistically analyzed using Mann-Whitney U test.

Results: The precipitate is measured by using image analysis system. Group 1 and 3 showed more precipitate and group 2 and 4 showed very minimum amount of precipitate.

Conclusion: Citric acid used as intermittent irrigant had the least amount of PCA formation in the canal system. until the threshold requires to cause biological damage in human is determined, the combination of NaOCL and CHX should be avoided.

Keywords: Sodium hypochlorite, chlorhexidine, interaction of irrigants, precipitate, intermittent irrigation.

Introduction

The success of endodontic treatment is both the appropriate chemical irrigating agents and tightly sealed root canal system. The use of effective irrigants supports the removal of intracanal micro-organisms and obtaining effective disinfection and it increases probability of successful of endodontic treatment.[1,2]

Sodium hypochlorite is a basic rinsing agent used in endodontic treatment due to its broad spectrum of activity and pulp tissue dissolving potential. The problem in endodontic cases of secondary infections, in which E fecalis is the main representative of bacterial flora. These bacteria have significant resistant to sodium hypochlorite.[2] However, there is a safety concern if NaOCl is extruded out of the root canal apex into the periapical tissues, resulting in destructive tissue damage. NaOCl also corrodes and weakens endodontic instruments and has a disagreeable odor. Thus, 2% chlorhexidine gluconate (CHX) have been considered as alternate irrigant.[4]CHX is a broad-spectrum antimicrobial agent that acts by absorbing on to the microbial cell wall and disrupts the membranes of microbes. It has been suggested for use as an root canal irrigant and intracanal medicament because of its lower toxicity when compared with NaOCl. CHX has comparable antibacterial efficacy to NaOCl and has the advantage of having substantivity. However, CHX lacks the tissue dissolution capabilities of NaOCl.[3,4]

Therefore alternate use of NaOCL and CHX is advocated to take advantage of the beneficial qualities of both solutions and to enhance their antimicrobial property.[7]

The association leads to the formation of form dense orange brown flocculate parachloro aniline resulting chemical smear layer which covers the dentinal tubules. The presence of 4-chloroaniline (PCA) in an amount directly related to the concentration of NaOCl used and how effective heating CHX can produce this by-product as well, which is difficult to remove from the root canal and could cause darkening of the dental structures and hamper the radicular sealing during obturation. It has been shown that aniline derivatives have different levels of toxicity. [5,6,9,10,20]

An intermediate intracanal flush with distilled water, isopropyl alcohol, and citric acid has been suggested to eliminate the residual NaOCl before using CHX to prevent formation of precipitate.70% Isopropyl alcohol drying has been suggested by many researchers to offer clean, well-dried root canals and to help penetration of the sealing cement into the dentinal tubules.[6]For both ZOE and Ca(OH)2–based sealers, the presence of moisture was essential for their setting reactions, usually accelerating the setting process. Flushing of root canals with 70% isopropyl alcohol was reported to achieve excessive drying of root canal dentin. This action, in turn, did not offer enough wetness to the desired setting reaction of

both sealers, and probably has an adverse effect on their wetting and adhesiveness to root canal dentin.[6] So in this study tried 40% isopropyl alcohol to avoid excessive drying of root canal.

Therefore, the aim of study was to evaluate the efficacy of three different intermediate irrigants that are 70% isopropyl alcohol, 40% isopropyl alcohol, 10% citric acid to prevent the orange-brown precipitate formation and to attain maximum antimicrobial efficacy.

Materials and methodology

Forty single rooted mandibular premolars were collected for the study. External surfaces of specimens were cleaned of tissue remnants and stored in 0.9% saline. Access cavity opening was done for all teeth. Working length was determined with 10K file introduced into the canal until the tip of the file was visible at the apical foramen and 0.5 mm was deducted from it.

The root canals of all teeth were instrumented with stainless steel K files upto 40 size at the apex in a step-back preparation. Recapitulation was done before every next file change during instrumentation, canals were irrigated between each instrument change with 1 ml of 3% sodium hypochlorite, 2mm from the working length, using a 27-gauge hypodermic needle.

The root ends of the prepared teeth were inserted into the softened impression compound and allowed to set. This prevented extrusion of the irrigants out of the apex and allowed ease of handling during instrumentation.

All the prepared forty specimens were randomly divided into four groups of ten teeth each. In group 1, the specimens were irrigated with 2.5ml of 3% sodium hypochlorite followed by 2.5 ml of saline and a final flush with 2.5ml of 2% chlorhexidine. The other three groups received intermediate flushes of 70% isopropyl alcohol, 40% isopropyl alcohol and 10% citric acid in between sodium hypochlorite and chlorhexidine.

The coronal opening was sealed with softened impression compound. A thin longitudinal slot was made along the buccal and lingual aspect of the root using diamond discs, making sure to avoid perforation into the canal. Using a chisel and mallet, the roots were split longitudinally and one of the two parts of each split tooth was examined under stereomicroscope.

Root samples of each group were examined at the coronal, middle, and apical third levels. The thickness of the precipitate was measured from its outer surface to the inner dentinal wall at a uniplanar level.

Results

Stereomicroscopic examination of the specimens revealed clear canals with less evidence of precipitate deposition in group 4 ($p<0.05$) followed by Group 2 and 3 and Group 1 respectively. The Mann-Whitney U test analysis of variance revealed there is a significant difference between the three levels among four groups.

The mean thickness of the precipitate was maximum at the coronal and middle thirds and minimum at the apical levels of all groups. The thickness of the precipitate was more in Group 1 and 3 compared to Group 2.

Although Group 2 and Group 3 performed better than Group 1, the difference was not statistically significant. Thus Group 4 exhibited the least amount of orange- brown precipitate when compared with all the experimental groups ($p<0.05$)

Table 1: Comparison of four groups (1, 2, 3, 4) with thickness scores in coronal third by Kruskal Wallis ANOVA and Mann-Whitney U test

Groups	Mean	SD	Median	Mean rank
Group 1	0.88	0.27	0.92	35.50
Group 2	0.13	0.05	0.12	15.85
Group 3	0.36	0.10	0.39	24.85
Group 4	0.02	0.04	0.00	5.80
H-value	35.4380			
P-value	0.0001*			
Pair wise comparisons by Mann-Whitney U test				
Group 1 vs Group 2	p=0.0002*			
Group 1 vs Group 3	p=0.0002*			
Group 1 vs Group 4	p=0.0002*			
Group 2 vs Group 3	p=0.0009*			
Group 2 vs Group 4	p=0.0006*			
Group 3 vs Group 4	p=0.0002*			

$p<0.05$ indicates significant

A significant difference between four groups (1, 2, 3, 4) with thickness scores in coronal third ($H=35.4380$, $p<0.05$). It means that, the thickness is different in different groups. Further, the thickness is significantly higher group 1 and smaller in group 4 followed by group 2 and group 3.

Figure 1: Comparison of four groups (1, 2, 3, 4) with thickness scores in coronal third

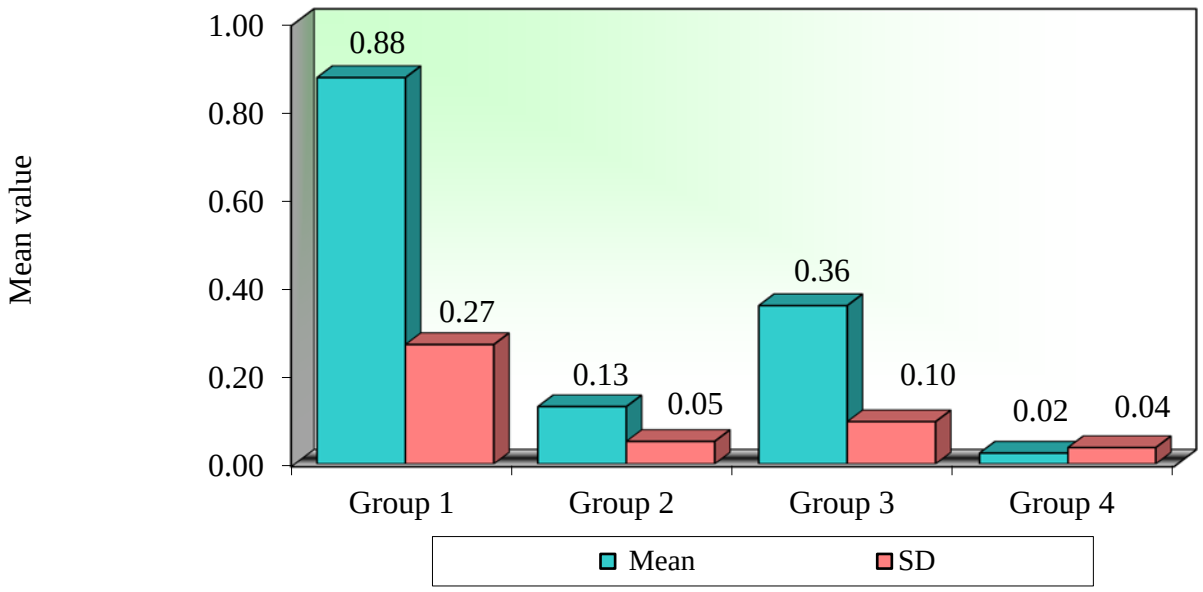


Table 2: Comparison of four groups (1, 2, 3, 4) with thickness scores in middle third by Kruskal Wallis ANOVA and Mann-Whitney U test

Groups	Mean	SD	Median	Mean rank
Group 1	0.46	0.14	0.41	35.00
Group 2	0.09	0.03	0.08	15.80
Group 3	0.26	0.08	0.26	25.70
Group 4	0.00	0.01	0.00	5.50
H-value	35.8970			
P-value	0.0001*			
Pair wise comparisons by Mann-Whitney U test				
Group 1 vs Group 2	p=0.0002*			
Group 1 vs Group 3	p=0.0007*			
Group 1 vs Group 4	p=0.0002*			
Group 2 vs Group 3	p=0.0003*			
Group 2 vs Group 4	p=0.0002*			
Group 3 vs Group 4	p=0.0002*			

p<0.05 indicates significant

Figure 2: Comparison of four groups (1, 2, 3, 4) with thickness scores in middle third

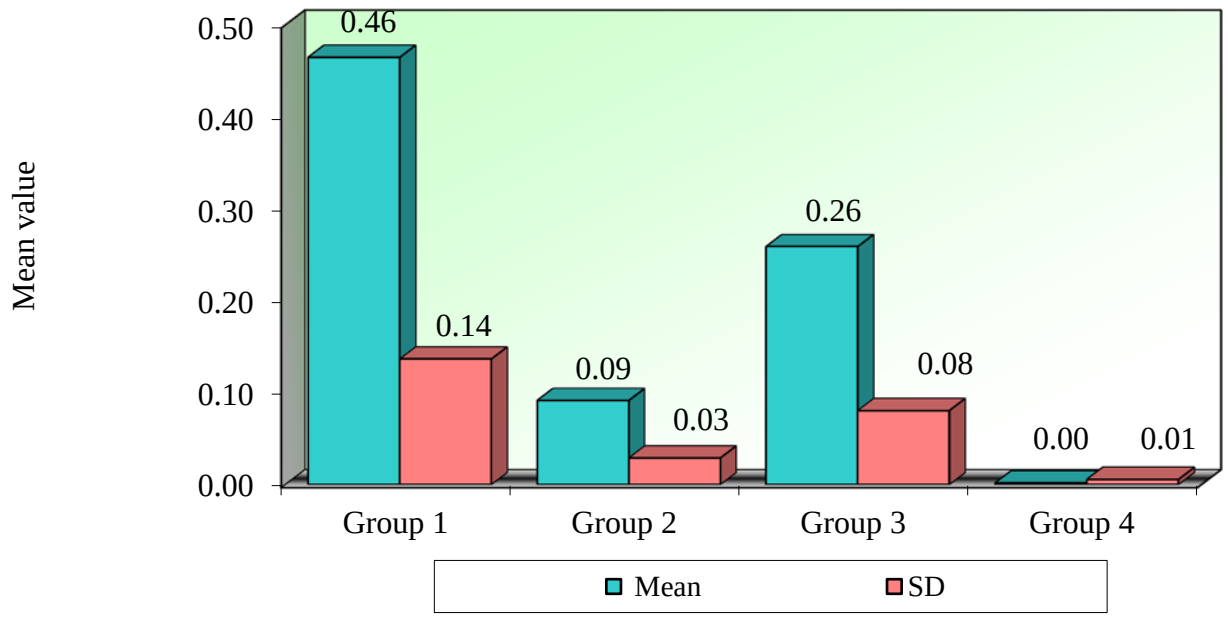
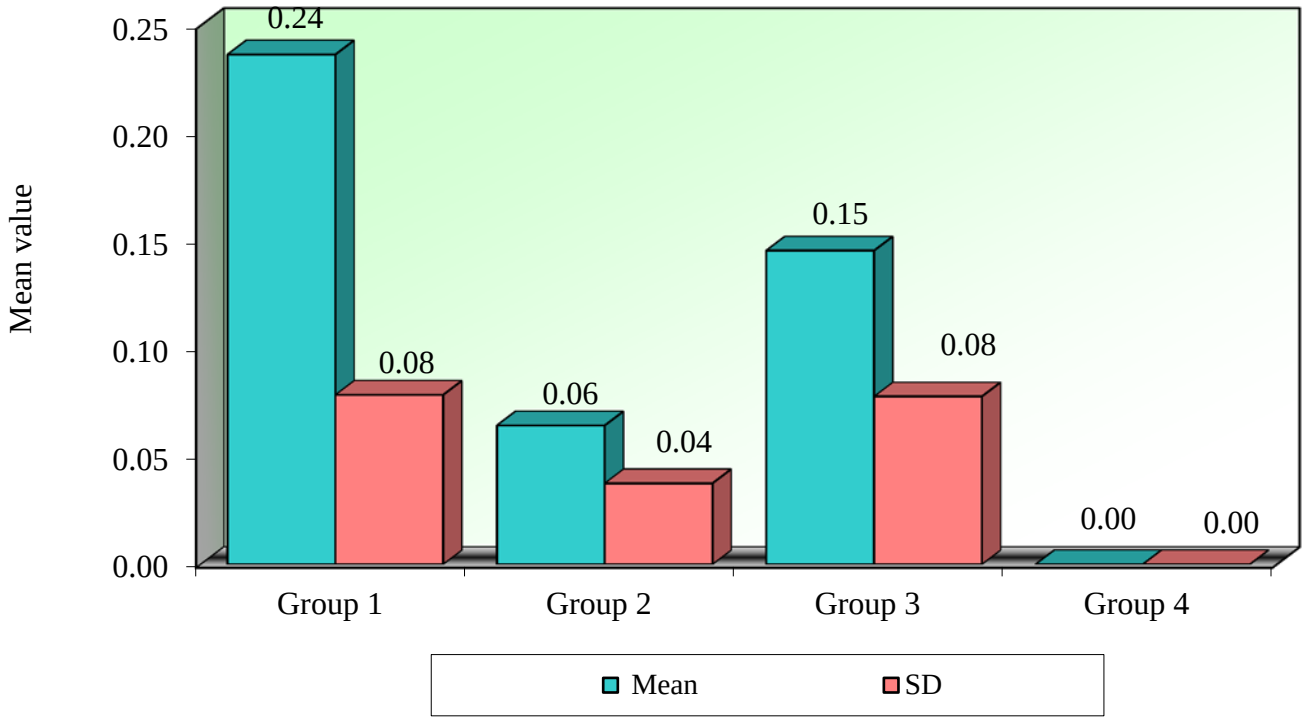


Table 3: Comparison of four groups (1, 2, 3, 4) with thickness scores in apical third by Kruskal Wallis ANOVA and Mann-Whitney U test

Groups	Mean	SD	Median	Mean rank
Group 1	0.24	0.08	0.21	34.20
Group 2	0.06	0.04	0.06	16.65
Group 3	0.15	0.08	0.12	25.65
Group 4	0.00	0.00	0.00	5.50
H-value	33.7800			
P-value	0.0001*			
Pair wise comparisons by Mann-Whitney U test				
Group 1 vs Group 2	p=0.0002*			
Group 1 vs Group 3	p=0.0046*			
Group 1 vs Group 4	p=0.0002*			
Group 2 vs Group 3	p=0.0025*			
Group 2 vs Group 4	p=0.0002*			
Group 3 vs Group 4	p=0.0002*			

p<0.05 indicates significant

Figure 3: Comparison of four groups (1, 2, 3, 4) with thickness scores in apical third



Discussion

The irrigation of root canals is paramount during biomechanical preparation due to its noteworthy role in the eradication of microorganism, tissue dissolution, removal of debris as well as smear layer, especially in areas untouched during biomechanical preparation which amount to almost 40%-60% of total surface area.[7]

To mimic the scenario of precipitate formation *in vitro*, the mandibular premolars were selected for this study since they provided wider canal diameter. It also facilitated splitting of the roots to observe the radicular surface of the canal.[14,16]

In contemporary endodontic practice 3% NaOCl was used as the irrigating solution although, there have been concerns related to the reduced efficacy of tissue dissolving capability.[7]

The disadvantage of NaOCL is its lack of ability to remove smear layer and it does not work on dentin inorganic substance. Therefore, NaOCL should be used alternately with chelating compounds like EDTA, citric acid which allows for the liquidation of the smear layer from the walls of the prepared canal. [12,13,14,15]

CHX exhibits broad-spectrum antimicrobial action and substantivity, but it has no tissue dissolution ability. To combine the desirable effect of irrigants, they have been used in combination and in sequence.[3,6,12] Hence, studies on the possible effects of the interaction between them have gained importance.

Kuruvilla et al suggested that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination was better than that of either component.[5,8]

Therefore, in the present study the association of NaOCl and CHX was used, aiming at combining the best properties of those 2 irrigant solutions and improving the antimicrobial action.

Basraniet al found a brown color precipitate (PCA) when chlorhexidine was mixed with sodium hypochlorite in the test tube. This precipitate is an insoluble neutral salt formed by the acid base reaction between NaOCl and CHX.[5]

The parachloro aniline flocculate formed adheres to the dentinal walls, acts as a smear layer and interfering in marginal sealing and favoring leakage and has immunotoxic effects (Group2B-International Agency for Research on Cancer 1993).[7,20]

The present study was designed to assess the inhibition of precipitate formation specifically on the radicular dentinal surface. The prepared specimens were evaluated using Stereomicroscope at magnification setting of ×40 to provided a well magnified two-dimensional view for quantifying the surface precipitate formed.[7,11]

The Stereomicroscopic results of Group I and Group 3 showed the root canal lumen coated extensively with the precipitate. The other groups with the intermediate irrigation 70% isopropyl alcohol and 10% citric acid revealed substantial reduction in the area covered by the precipitate.

Thickness of the precipitate was found maximum at the coronal third and least at the apical third. This can be attributed to the decreased amount of irrigants reaching the apex because of inherent anatomic constraints and limitations of the irrigation modality. An invitro study conducted by Krishnamurthy and Sudhakaran observed the similar results with maximum precipitate thickness at coronal third and least at apical third. [5,7]

Study done by Padmaja Singh examined the surface of the root canal as well as the amount of the chlorine in the root canal. The amount of chlorine present recorded in wt% and subjected to analysis. The chlorine content is more throughout the teeth when chlorhexidine irrigated with sodium hypochlorite without any intermediate irrigant,

than using 70% isopropyl alcohol as intermittent irrigant. This indicates that the precipitate that occluded the dentinal tubule, which contains chlorine in significant amount. The use of Isopropyl alcohol as an intermediate flush between NaOCl and CHX prevented the formation of the precipitate.[5,6,11]

Alcohol is a volatile, highly electronegative and can penetrate deeply to remove the residual NaOCl present in the canals. However, the volatility of alcohol-based irrigants restricts their shelf life.[5,11]

Due to dewatering properties of alcohols, many clinicians use 70% isopropyl alcohol to dry canals before the filling. Flushing of root canals with 70% isopropyl alcohol was reported to achieve excessive drying, and probably has an adverse effect on wetting and adhesiveness of sealers to root canal dentin.[2]

SEM findings showed a less depth of penetration of ZOE-based sealers to radicular dentinal tubules, compared with either Ca(OH)2 and resin-based sealers, which could be related to materials thixotropic characteristics. In addition to that biocompatibility of alcohol still stands as an issue.[6]

To avoid disadvantages of 70% isopropyl alcohol, this study used 40% isopropyl alcohol as a low concentration biocompatible agent evaluated whether it can prevent the precipitate better than 70% isopropyl alcohol. However, 40% Isopropyl alcohol cannot prevent the precipitate as effectively as 70% isopropyl alcohol.[19]

In Group 4, 10% citric acid used as intermediate irrigant showed no evidence of precipitate formation on the canal walls. Previous studies conducted by Matthias Zehnder and Prado et al were able to provide possible explanation to the clear canals obtained in this group. An exothermic reaction takes place when citric acid reacts with sodium hypochlorite leading to formation of bubbles by release of chlorine gas (Cl2) rendering sodium hypochlorite ineffective.[11,13,16]

Conclusion

Within the limitations of this in vitro study the following conclusions were drawn

The interaction between the NAOCL and CHX resulted in the formation of an orange-brown precipitate, that is difficult to remove and can compromise the seal of the obturated root canal.

The formation of precipitate can be prevented by the use of 10% citric acid and 70%isopropyl alcohol.

The use of saline and 40% isopropyl alcohol minimizes the amount of precipitate formed by the interaction but could not totally eliminates its presence.

Even though 40%isopropylalcohol seems to be ineffective in removal of precipitate, further more studies are to be conducted to evaluate the efficacy of 40% isopropyl alcohol and to reduce the disadvantages of 70% isopropyl alcohol

However, further research is warranted to determine the mechanism of diffusion of PCA into periradicular tissues and the threshold required to cause biological damage.

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