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Comparative evaluation of prevention of an orange-brown precipitate formed in root canal space by the combination between 5% sodium hypochlorite and 2% chlorhexidine: an in vitro metallurgical microscopic analysis.

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Abstract

Context: The interaction between sodium hypoclorite when followed by chlorhexidine results in an orange-brown precipitate formation, known as para-chloroaniline which is possibly carcinogenic to humans and has toxic and immuno-toxic effects. An intermediate intracanal flush with 18% Etidronic acid and 50% Citric acid has been suggested to eliminate the residual sodium hypochlorite before using chlorhexidine to prevent the formation of a precipitate.

Aim: Aim of this study was to evaluate the thickness of an orange-brown precipitate formed in root canal space by the combination between 5% sodium hypochlorite and 2% chlorhexidine and its prevention by using 18% etidronic acid and 50% citric acid.

Study setting and design:- This was an experimental prospective in-vitro study conducted at dental learning institution.

Methods and materials: thirty single rooted human extracted teeth were used. Following cleaning and shaping, all teeth were decoronated and divided into 3 groups with 10 teeth in each group. In Group A (control group), canals were irrigated with 5% sodium hypochlorite and 2% chlorhexidine. In Group B and Group C (experimental groups), 18% etidronic acid and 50% citric acid was used as an intermediate flushes respectively between 5% sodium hypochlorite and 2% chlorhexidine.

Statistical analysis: The data was statistically analyzed using One way ANOVA test and Post Hoc Scheffe test.

Results:- In all the groups thickness of precipitate was present, Amongst which Group B (18% etidronic acid) exhibited the least mean thickness scores compared to Group A (5% NaOCl & 2% CHX) and Group C (50% citric acid).

Conclusion: Within the limitation of this study, it was observed that 18% etidronic acid had better efficacy in the prevention of orange brown precipitate when compared to 50% citric acid.

Keywords: chlorhexidine, citric acid, etidronic acid, metallurgical microscope, orange-brown precipitate, sodium hypochlorite.

Introduction

The prime etiology for vital soft tissue necrosis and inflammatory differences of apical region at the root vertex are microorganisms.[1] Enterococci which occur as single, or in pairs are facultative anaerobes that harbours the potential to populate with or without the presence of oxygen and possesses an increased resistance towards irrigating agents exhibiting high potential to reverse back in the canal after irrigation procedure.[2] Thus various irrigating solutions that are used, are recommended in definitive sequence in order to eradicate bacteria and achieve optimum and safe irrigation.

During mechanical and rotary instrumentation of the canal, irrigants that are used, play a notable task in the successful disinfection.[3-6] In higher range of concentrations, sodium hypochlorite (NaOCl) suspensions are less biocompatible causing periapical inflammation.[7] A layer of smear adheres to the dentinal wall during mechanical debridement in lower concentrations of NaOCl, where it is less potent against specific microorganisms.[8,9] It cannot be used as a final irrigant since it causes erosion of the dentinal wall and thus as a final irrigant, 2% chlorhexidine (CHX) should be used due to its low grade toxicity.[9-12]

When NaOCl merges with CHX, an orange-brown indissoluble precipitate is formed which is possibly carcinogenic to humans (Group 2B-International Agency for Research on Cancer 1993) and has toxic and immune-toxic effects.[7] The concern is that the removal of this precipitate completely using irrigating solutions is impossible since it might attach to the surface of root and slowly leak into periapical tissues but can be prevented if irrigating solutions are used in proper sequential manner using syringes with metal needles with adequate tip diameters.[13] Also the sealing of an obturating material of root canal with resin sealer might affect were hybrid layer is required.[14]

Etidronic acid is a chelating agent which is pharmacologically observed to reduce osteoclastic activity which prevents bone resorption[15] whereas citric acid is an organic acid used as intermediate irrigant In this study, a metallurgical microscope which guides at micron and submicroscopy level is used. The purpose of this study was to evaluate the orange brown precipitate and its thickness with prevention of an orange-brown precipitate

formed in root canal space by the combining 5% sodium hypochlorite and 2% chlorhexidine. An invitro metallurgical microscopic analysis.

Materials and methodology

Thirty single rooted human extracted mandibular premolar teeth were selected. The external surfaces of all thirty teeth were cleaned and stored in 0.9% saline. The working length was determined with #10k file (Dentsply tulsa, dental specialities) proposed into the canal until tip of the file was visible at the apical foramen and 0.5 mm was reduced. The apices of the single rooted extracted tooth specimens were sealed with wax. This prevented the leaking of the irrigants beyond the apex. The root canals of all the groups were instrumented with stainless steel #10k file to #60k file (Dentsply tulsa, dental specialities) using step back technique during instrumentation. The canals were irrigated using 1ml of 5% NaOCl (S.K enterprises, nashik), 2 mm from the working length using 27 gauge hypodermic needle. Decoronation of all the teeth at the cemento enamel junction was done.

Thirty teeth were then randomly divided into 1 control and 2 experimental groups, 10 teeth in each group. In group A(control group), canals were irrigated with 5ml of 5% NaOCl followed by 5ml of saline and 5ml of 2% CHX (V-Consept, Vishal dentocare pvt. Ltd, Gujarat). In group B and C (Experimental groups), 5 ml of 18% etidronic acid (Zodenta, Neelkanth dental and surgical factory, Jodhpur) and 5ml of 50% citric acid (Modern industries, Nashik) were used as an intermediate flushes between 5% NaOCl and 2% CHX respectively. The canals were dried immediately with sterile paper points to ensure complete dryness.

Two longitudinal grooves were made along the buccal and lingual surfaces of the roots with diamond disc. The roots were then sectioned using mallet and chisel into halves. Evaluation of the coronal, middle and apical third of each canal was done using metallurgical microscope, since the surface of all the sectioned roots were smooth, etching of the surfaces before placing under metallurgical examination was not required. The sections were examined under metallurgic microscope at 50X magnification using “Ziess” computer software program as shown in “figure 1.”

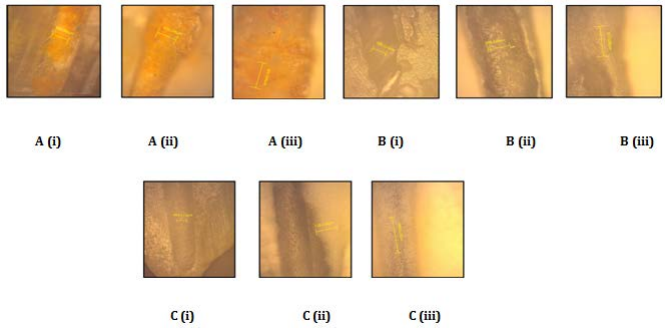

Fig. 1: Magnification at 50X for group A,B and C at (i)apical, (ii)middle and (iii)coronal third.

Results

The evidence of the precipitate deposited was maximum at the coronal third in GROUP A (956.490 µm) compared to the coronal and middle thirds of GROUP B and GROUP C. The mean thickness was more at the middle and coronal third of all groups and least at the apical third of GROUP B (384.204 µm). The obtained readings were analyzed using one way ANOVA test(table 1) and Post hoc scheffe test(table 2). The post hoc scheffe test disclosed a statistically significant difference between GROUP A, GROUP B and GROUP C in coronal, middle and apical third (p.value < 0.001).

Table 1: One way ANOVA test

Coronal third	N	Mean	SD	F	df	P value
Group A	10	956.490	5.2779	745.8	2	< 0.001; Highly significant
Group B	10	874.158	5.7409			
Group C	10	905.744	2.9299			
Total	30	912.131	34.8047			

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Group B	10	874.158	5.7409			
Group C	10	905.744	2.9299			
Middle third	N	Mean	SD	F	df	P value
Group A	10	849.787	3.0196	2761.5	2	< 0.001; Highly significant
Group B	10	644.221	8.8608			
Group C	10	718.013	5.4934			
Total	30	737.340	86.6920			
Apical third	N	Mean	SD	F	df	P value
Group A	10	545.084	4.7144	1566.7	2	< 0.001; Highly significant
Group B	10	384.204	9.7430			
Group C	10	451.881	2.7906			
Total	30	460.390	67.3698			

Table 2: Post Hoc Scheffe test for pair wise comparison

(I) Groups	(J) Groups	Mean Difference for coronal third(I-J)	SE	P value
Group A	Group B	82.332*	2.150952	< 0.001
	Group C	50.746*	2.150952	< 0.001
Group B	Group C	-31.586*	2.150952	< 0.001
(I) Groups	**(J) Groups**	**Mean Difference for middle third(I-J)**	**SE**	**P value**
Group A	Group B	205.566*	2.802489	< 0.001
	Group C	131.774*	2.802489	< 0.001
Group B	Group C	-73.792*	2.802489	< 0.001
(I) Groups	**(J) Groups**	**Mean Difference for apical third (I-J)**	**SE**	**P value**
Group A	Group B	160.880*	2.886064	< 0.001
	Group C	93.203*	2.886064	< 0.001
Group B	Group C	-67.677*	2.886064	< 0.001

* The mean difference is significant at the 0.05 level

Discussion

NaOCl is one of the effective and commonly used irrigant, having tissue dissolving properties with antimicrobial efficacy, although it has the potential to damage the periapical tissue if its extruded from the apex.[16]

CHX is a cationic bisguanide which is known for its broad spectrum antimicrobial substantivity and low grade toxicity with no tissue dissolving properties.[17] Thus, CHX cannot be used alone as an irrigant and is supplemented to NaOCl.[18] The biochemical mechanism of NaOCl is justified by its saponification and amino acid reactions.[19] When NaOCl is used as an irrigant followed by CHX (where CHX is a dicationic acid (pH 5.5-6) that donates protons to NaOCl, which is alkaline in nature (pH 7-9) and can accept protons from CHX an acid-base reaction takes place. This leads to evolution of an insoluble substance referred to as 'precipitate'.[20,21]

The insoluble precipitate which is formed when NaOCl is merged with CHX is an orange-brownish precipitate which arises significance as it causes discoloration of dentinal structures and also affects the patency of the dentinal tubules by obliterating them.[22]

In this study, it was observed that when 5% NaOCl was interacted with 2% CHX, a thick brownish precipitate was formed in the canals. There was no much change seen in the remaining of debris caused by mechanical instrumentation although, the remnant of this precipitate gives potential concerns with leaking of this precipitate into the surrounding tissues. It has been demonstrated by various authors in several studies[23-25,10] that the formed precipitate contained parachloroaniline (PCA) which causes toxicity in human beings leading in cyanosis and methemoglobinomia. On the contrary, in a recent study by Ekim Onur Orban et al.[26] it was reported that the interaction between NaOCl and chlorhexidine leads to the formation of brown precipitate, however it does not contain PCA (by performing non destructive methods such as 1H-NMR, HPLC, GC and TLC) and that mass spectrometry may not be a proper method to reveal the presence of PCA from the reaction of NaOCl and CHX.

In the current study however, the analysis of the precipitate formed and its thickness was done by examining the root canals at coronal, middle and apical third using an optical metallurgic microscope and the

efficacy in the removal of the precipitate by 18% etidronic acid and 50% citric acid used as intermediate irrigants between 5% NaOCl and 2% CHX was inspected. According to several studies,[27,28] etidronic acid is a weak chelating agent that encounters less dentin surface, than other commonly used chelators as EDTA which avails 300 seconds to completely disrupt and remove the smear layer. The mechanism of etidronic acid pharmacologically can be explained by the bone resorption/formation which remains in equilibrium toward the formation side and hence makes bone stronger and on the long run, it prevents bone calcification, thus known for its remedy in Paget's disease and osteoporosis.[29]

In the present study, the group with 18% etidronic acid (group B) had showed better efficacy in preventing the formation of the brown precipitate in comparison with group A. Etidronic acid is used as a possible alternative to citric acid or EDTA, in that it showed minimal short tissue interaction with sodium hypochlorite and appeared to have adequate calcium chelating capacity.

Etidronic acid is nontoxic and has been systemically applied to treat bone diseases. Less aggressive calcium complexing agents such as 7-10% etidronic acid could be administered during the whole course of root canal preparation. Etidronic acid could be mixed chair side with NaOCl without fearing any loss of NaOCl activity.

The measurements of the thickness of this brown precipitate occuring at coronal, middle and apical aspect of root canals were accurately noted using an innovation of optical microscopy, that is optical metallurgical microscope with bright field illumination having extra wide eyepiece of 10x with 4x,10x ,20x and 40x magnification with or without a 50x and 100x, 1000x infinite plan with achromatic objectives, and color filters (part no.IM 3000) and has revealed subjective differences in the morphology of the root surface showing maximum thickness at the coronal level compared to middle and apical third. This may be observed, since various anatomic constraints of teeth provide a hindrance for irrigation at the apical aspect due to which there was more concentration of precipitate at the coronal and middle third.

Thus, 18% etidronic acid and 50% citric acid can be advised to be used as intermediate irrigants between NaOCl and CHX in order to prevent the formation of PCA however it is seen that 18% etidronic acid has a better efficacy in the removal of PCA as compared to 50% citric acid.

Conclusion

Within the limitations of this study, 18% etidronic acid and 50% citric acid showed comparable prevention of the orange brown precipitate to each other at the coronal, middle and apical third. However the best prevention of orange brown precipitate is provided by 18% etidronic acid. Still further research with more number of samples along with different techniques would be helpful.

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