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Original Research Article

An In vitro Metallurgical Microscopic Analysis to Evaluate the Prevention of an Orange Brown Precipitate Formed in Root Canal Space by the Combination Between 5.25% Sodium Hypochlorite and 2% Chlorhexidine

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ABSTRACT

Aim: The aim of this study was to evaluate the precipitate and its thickness formed by interaction between 5.25% sodium hypochlorite and 2% chlorhexidine and its prevention by using 18% etidronic acid and 99% absolute alcohol.
Methodology: 30 single rooted human extracted teeth were used. Following cleaning and shaping, all teeth were decoronated and divided into one 3 groups with 10 teeth in each group. All canals of teeth were irrigated with 5.25% sodium hypochlorite and 2% chlorhexidine. As control Group, in group A saline was used as intermediate flush. In experimental groups, group B and group C, 18% etidronic acid and 99% absolute alcohol was used as an intermediate flushes respectively.
Statistical Analysis:- one way variance of analysis and Tukey's test was used.
Results: Group A and B samples showed significant concentration of precipitate respectively. Group C showed less evidence of precipitate.
Conclusion: Within the limitation of this study, it was observed that 99% absolute alcohol had better efficacy in the prevention of orange brown precipitate when compared to 18%etidronic acid.

Key Words: Root canal space, sodium hypochlorite, chlorhexidine.

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. [2,3] 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. [4-9] In higher range of concentrations, sodium hypochlorite suspensions are less biocompatible causing periapical inflammation. [10] A layer of smear adheres to the dentinal wall during mechanical debridement in lower concentrations of sodium hypochlorite, where it is less potent against specific microorganisms. [11-13] It cannot be used as a final irrigant since it causes erosion of the dentinal wall and thus as a final irrigant, 2% chlorhexidine should be used due to its low grade toxicity. [14-17]

When sodium hypochlorite merges with chlorhexidine, an orange brown indissoluble precipitate is formed. [10] 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. [18] Also the sealing of an obturating material of root canal with resin sealer might affect were hybrid layer is required. [19]

Etidronic acid is a chelating agent which is pharmacologically observed to reduce osteoclastic activity which prevents bone resorption [20] whereas absolute alcohol act as a volatile agent that completely provides a dry and clean dentinal surface and enhances better penetration of sealer. [21,20]

In this study an upheaval of optical microscopy, with a metallurgical microscope which guides at micron and submicroscopy level is used. The intention of the this study was to evaluate the orange brown precipitate and its thickness with prevention of a orange brown precipitate formed in root canal space by the combining 5.25% sodium hypochlorite and 2% chlorhexidine. An invitro metallurgical microscopic analysis.

MATERIALS AND METHODS

30 single rooted human extracted teeth were selected. The external surfaces of all 30 specimens were cleaned and stored in 0.9% saline. Gates Glidden drills #2 and #3 (DENTSPLY TULSA, DENTAL SPECIALITIES) were used for coronal flaring .The working length was determined with 10#k 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 ends of the single rooted extracted tooth specimens were mounted in plaster. This prevented the leaking of the irrigants beyond the apex and enhanced ease of handling during the instrumentation. The root canals of all the experimental groups were instrumented with stainless steel 10# k file to #60k file (DENTSPLY TULSA, DENTAL SPECIALITIES) using step back technique during instrumentation. The canals were irrigated using 1ml of 5.25% NaOCl (REACHEM LABORATORY CHEMICALS PVT. LTD, CHENNAI), 2 mm from the working length using 27 gauge hypodermic needle. Decoronation of all the teeth at the cemento enamel junction was done.

INHIBITION OF ETIDRONIC ACID AND ABSOLUTE ALCHOHOL

30 specimens were then randomly divided into one control and two experimental groups, 10 teeth in each group. In group A(control group), canals were irrigated using 17% EDTA (*DENTSPLY/*MAILLEFER, BALLAIGUES, SWITZERLAND) and 5ml of 5.25% NaOCl (REACHEM LABORATORY CHEMICALS PVT. LTD,CHENNAI) followed by 5ml of saline and 5ml of 2% chlorhexidine (CALYPSO, SEPTODONT, INDIA). In group B and C (Experimental groups), 5 ml of 17% etidronic acid (TOKYO CHEMICAL INDUSTRY CO. LTD, JAPAN) and 5ml of 99.9% absolute alcohol (HONYON INTERNATIONAL,INC.,CHINA) were used as intermediate flushes between 5.25% sodium hypochlorite (REACHEM LABORATORY CHEMICALS PVT. LTD, CHENNAI) and 2% chlorhexidine (CALYPSO, SEPTODONT, INDIA) respectively. The canals were dried immediately with sterile paper points to ensure complete dryness

STATISTICAL ANALYSIS

Evaluation of the coronal, middle and apical third of each canal using metallurgical microscope

All the teeth were then sectioned longitudinally, followed by a transverse section since the precipitate thickness was

measured from its outer surface to inner dentinal wall at uniplanar level. 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 then examined under metallurgic microscope at 50x magnification and with a light microscope of 40x magnification using "Ziess" computer software program. The root samples were measured at the coronal, middle and apical third levels

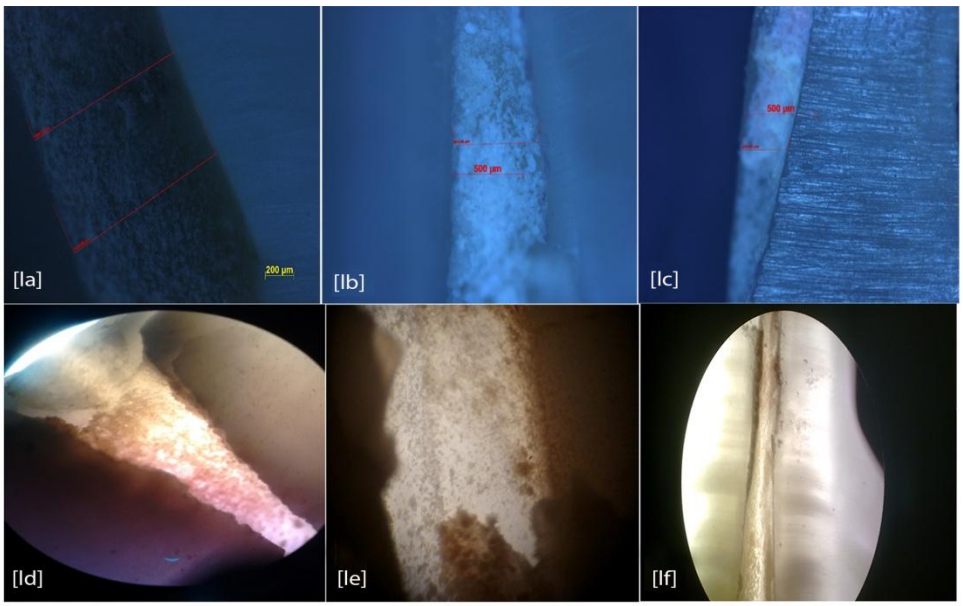

Figure 1: Metallurgical microscopic examination at 100x (la) Thickness of precipitate at the coronal third is more compared to the (lb) middle third (lc) and apical third and also at 50x (ld) Thickness of precipitate at the coronal third is more compared to the (le) middle third (lf) and apical third.

Table 1: Mean thickness of precipitate at the coronal,middle and apical third for all groups and p values (ANOVA test).

Class		N	Mean	Std. Deviation	F	p
Coronal	Absolute alcohol	10	818.064	125.408		
	Etidronate	10	864.290	127.504		
	Precipitate	10	997.401	80.966	6.748	.004
Middle	Absolute alcohol	10	744.774	113.888		
	Etidronate	10	802.419	92.859		
	Precipitate	10	874.311	92.859	4.182	.026
Apical	Absolute alcohol	10	399.075	118.534		
	Etidronate	10	499.286	72.180		
	Precipitate	10	555.323	118.248	5.655	.009

Table 2: Multiple comparison of mean difference between coronal,middle and apical thirds for all groups with p values(tukey's test)

Group	(i) class	(j) class	Mean difference (i-j)	P
Absolute alcohol	Coronal	Middle	73.290	.369
		Apical	418.989	.000
	Middle	Apical	345.699	.000
Etidronate	Coronal	Middle	61.871	.364
		Apical	365.004	.000
	Middle	Apical	303.133	.000
Precipitate	Coronal	Middle	123.090	.025
		Apical	442.078	.000
	Middle	Apical	318.988	.000

RESULTS

An optical metallurgic microscopic examination at 50X and 40X was used to determine the thickness of the precipitate at coronal, middle and apical third of the canal. The mean values of specimens revealed orange brown precipitate formed by the interaction between sodium hypochlorite and chlorhexidine. The precipitate was seen settled on all surfaces of the canals in GROUP A whereas the distribution of the precipitate was more sparse in GROUP B. GROUP C had no confirmation of precipitate deposition (Figure1). The evidence of the precipitate deposited was maximum at the coronal third in GROUP A compared to the coronal and middle thirds of GROUP B and GROUP C. The mean thickness was more at the

middle(p=0.02) and coronal third(p=0.004) of all groups and least at the apical third.(Table 1)

The obtained readings were analyzed using one way variance of analysis and Tukey's test.(Table 1 and 2). Analysis of variance disclosed a significant and major difference was seen between GROUP A and GROUP C (p=0.000) at the apical third. However no much remarkable difference was seen between GROUP A and GROUP B at coronal and middle third(p=0.369).

DISCUSSION

Sodium hypochlorite 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. [22] Chlorhexidine is a cationic bisguanide which is known for its broad spectrum antimicrobial substantivity and low grade toxicity with no tissue dissolving properties. [23] Thus, chlorhexidine cannot be used alone as an irrigant and is supplemented to NaOCl. [24] The biochemical mechanism of sodium hypochlorite is justified by its saponification and amino acid reactions. [25] When sodium hypochlorite is used as an irrigant followed by chlorhexidine(where chlorhexidine is a dicationic acid (pH 5.5-6) that donates protons to sodium hypochlorite, which is alkaline in nature (pH 7-9) and can accept protons from chlorhexidine) an acid-base reaction takes place. This leads to evolution of an insoluble substance referred to as 'precipitate'. [26,27]

The insoluble precipitate which is formed when sodium hypochlorite is merged with chlorhexidine 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. [28] In this study, it was observed that when 5.25% sodium hypochlorite was interacted with 2% chlorhexidine, 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 [29-31,10] 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.* [32] it was reported that the interaction between sodium hypochlorite and chlorhexidine leads to the formation of brown precipitate, however it does not contain parachloroaniline (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 Parachloroaniline (PCA) from the reaction of sodium hypochlorite and chlorhexidine.

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 99% absolute alcohol used as intermediate irrigants between 5.25% sodium hypochlorite and 2% chlorhexidine was inspected. According to several studies, [33,34] 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. [34]

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. Absolute alcohol which was used as an intermediate irrigant in group C had shown significant efficiency (p=0.000) in the removal of precipitate as compared to group B in the apical third was seen. Absolute alcohol lowers the dentinal surface tension and being volatile in nature it evaporates completely and provides a dry and clean dentinal surface enhancing better penetration of sealers allowing the irrigating solutions to flow into the unimpeded entire length of the root canal and into various intricacies that exist in root canal system. [35] Moorer *et al.* [36] reported that the mechanism of action of alcohols as a disinfectant are coagulation of proteins, dehydration of cells and disruption of membrane. Since the diffusion of water into cell membrane is more easier, 100% absolute alcohol completely denatures the external cell membrane proteins. [37] On metallurgical examination, Group C had very less amount of precipitate at the apical third compared to group B. (fig.1) A similar study by Shashikala Krishnamurthy and Sunu Sudhakaran *et al.* [10] has also cited that the formation of the precipitate can be prevented by using absolute alcohol as intermediate flush.

The measurements of the thickness of this brown precipitate auguring 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 (fig.1). 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, 99% absolute alcohol and 18% etidronic acid can be advised to be used as intermediate irrigants between sodium hypochlorite and chlorhexidine in order to prevent the formation of brown precipitate however it is seen that absolute alcohol has a better efficacy in the removal of brown precipitate as compared to etidronic acid.

CONCLUSION

Within the limitations of this study, 18% etidronic acid and 99% absolute alcohol 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 99% absolute alcohol. Still further research with more number of samples along with different techniques would be helpful.

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Conflicts Of Interest

There are no conflicts of interest.

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