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Research Article

COMPARATIVE EVALUATION OF TWO DIFFERENT INTERMEDIATE IRRIGATING SOLUTIONS IN PREVENTING ORANGE BROWN PRECIPITATE FORMED DURING ALTERNATE USE OF SODIUM HYPOCHLORITE AND CHLORHEXIDINE: *AN IN-VITRO STUDY*

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Aim: To evaluate the effectiveness of two intermediate endodontic irrigating solutions in eliminating the residual Sodium hypochlorite, thus preventing the formation of the Orange-brown precipitate when 2% Chlorhexidine is used as the final irrigant.
Materials and Methods: A total of 30 extracted human maxillary anterior teeth were selected, disinfected and decoronated to obtain a standardized length of 10 mm. The teeth were prepared with Protaper universal rotary files until F4.
Irrigation protocol: -2 ml of 2.5% NaOCl was used as an irrigant during instrumentation. The samples were randomly allocated to three groups of 10 samples each based on the use of 2.5 ml of intermediate irrigating solution for 60 seconds as specified below:
Group A (Control) No intermediate irrigating solution used
Group B - 6.25% Sodium metabisulfite.
Group C - 10% Citric acid
2 ml of 2% CHX was used as the final rinse. The roots were sectioned longitudinally and the canal surface were evaluated under Scanning Electron Microscope for the presence of Orange-brown precipitate.
Statistical Analysis Used: - One‑way ANOVA test and *post hoc* Tukey's test.
Results: Group 2 exhibited the least mean scores amongst all the experimental groups ($P < 0.05$), followed by Group 3 and Group 1. The difference between Group 2 and Group 3 was also statistically significant ($p < 0.05$).
Conclusion: 6.25% Sodium metabisulfite is more effective in eliminating the residual Sodium hypochlorite when compared to 10% Citric acid."

INTRODUCTION

Microbial infection is one of the major factors associated with endodontic failure. Although mechanical instruments can reduce microbial load, due to the complex internal anatomy of the root canal, it is difficult to achieve complete root canal disinfection.[1] During_biomechanical preparation of the root canal, an antimicrobial solution such as sodiumhypochlorite is used to completely remove necrotic pulp and microorganisms.[2] Chlorhexidine gluconate has been used in combination with NaOCl because of its low toxicity and antibacterial substantivity.[3,4] It has been found to have high efficacy against several strains of *E. fecalis*. Few studies advocate the alternate use of NaOCl and CHX to take advantage of the beneficial qualities of the twosolutions.[5]

After NaOCl interacts with CHX, an orange-brown precipitate is formed, called para-chloroaniline, which may be carcinogenic to humans and is known to have toxic and immunotoxic effects.[6,7]

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This deposit may interfere with the radicular seal during obturation.[7] It may greatly increase the possibility of unknown chemicals leaching into the periradiculararea and may also cause discoloration of the teeth.[6]

It is suggested to use an intermediate intracanal flush with 50% Citric acid, Isopropyl alcohol or distilled water before using CHX to eliminate residual NaOCl, thus preventing the formation of a precipitate. [6-9]

Sodium metabisulfiteis achemical reagent which is used as a disinfectant, antioxidant and preservative agent. [10-11]Its use in dentistry, howeveris limited to being a preservative in local anaesthetic solutions to prevent the oxidation of the vasoconstrictor- adrenaline.[12]

Citric acid is a demineralizing solution in a concentration of 10% to 50% and is used in root canal treatment to remove smear layers from prepared root canals. The biocompatibility of citric acid is 10% higher than 17% of EDTA, and is also effective in removing the smear layer. Citric acid interferes with the mechanism of action of NaOCl.[13]The use of 10%

citric acid has shown good results in smear layer removal when used as a final irrigation solution.[14]In vitro studies have shown that 10% citric acid has better biocompatibility than 17% EDTA-T and 17% EDTA.[15,16]

Therefore, the potential of both 10% Citric acid and6.25% Sodium metabisulfite in reducing the formation of orange‑brown precipitate when used as an intermediate intracanal irrigant between CHX and NaOCl appears to be self‑explanatory.

The null hypothesis of the present study stated that there would not be any difference between the effectiveness of10% Citric acid and 6.25% Sodium metabisulfite in the prevention of precipitate formation when used as an intermediary intracanal flush between the alternate use of NaOCl and CHX.

MATERIALS AND METHODS

40 human intact and mature permanent premolars with single canals which were extracted recently for periodontal reasons were selected for this study by direct clinical examination. After washing with distilled water and ultrasonic scaling, the specimens were immersed in a solution of 0.5% Chloramine T till use.

A standardized root length of 12 mm was achieved by decoronation of the teeth. Patency of the canal was evaluated using #10 K file and canals of teeth with_ canal obstructions were discarded. The actual length of each tooth was determined with a #10 K file, introduced into the canal until its tip emerged through the major apical foramen. The working length was established by deducting 1 mm from the actual length. The apices of the specimens were sealed with wax to avoid extrusion of irrigating solutions. The same operator prepared all the specimens using NiTi rotary files ('Protaper Universal, Dentsply, India') until size F3 as per the manufacturer's instructions. Glyde Prep Canal™ ('Dentsply India Pvt. Ltd., New Delhi, India') was to lubricate the canal during instrumentation and the canals were irrigated with 2.5% NaOCl between each file change using a 30G side vented needle. ('RC Twents Irrigation Needle, Prime dental Products Pvt. Ltd., Bhiwandi, India') introduced 2 mm short of the apex.

After the completion of mechanical instrumentation, all the samples were flushed thoroughly with 2.5 ml of 2.5% NaOCl using a 30G side vented needle. The samples were randomly allocated to three groups of10 samples each based on the use of 2.5 ml of intermediate irrigating solution for 60 seconds as specified below:

Group A - No intermediary intracanal irrigant used (Control group);

Group B ‑6.25% Sodium metabisulfite;

Group C ‑10% Citric acid

Following which, all the samples were irrigated with a final wash of 2.5 ml of 2% CHX gluconate solution. Paper points of corresponding apical preparation size were used to dry the root canals. Along the buccal and lingual surfaces of the roots two longitudinal grooves were made with water‑cooled diamond disc and then sectioned using chisel and mallet.

The exposed surfaces of the root canal were examined for the orange‑brown precipitate using a 'Scanning Electron Microscope'. The images were then transferred to a computer and evaluated using image editing software ('Adobe Photoshop CS5; Adobe Systems, San Jose, CA, USA') by a blinded independent examiner.

'The quantum of orange‑brown precipitate was scored as per criteria suggested by Arslan *et al*. as follows:

Score 0: -The root canal surface was completely free of orange‑brown precipitate;

Score 1: -Orange‑brown precipitate present in < half of the root canal surface;

Score 2: -Orange‑brown precipitate covered more than half of the root canal surface;

Score 3: -The root canal surface completely covered with orange‑brown precipitate.'

The obtained data were subjected to one‑way ANOVA test and *post hoc* Tukey test for statistical analysis.

RESULTS

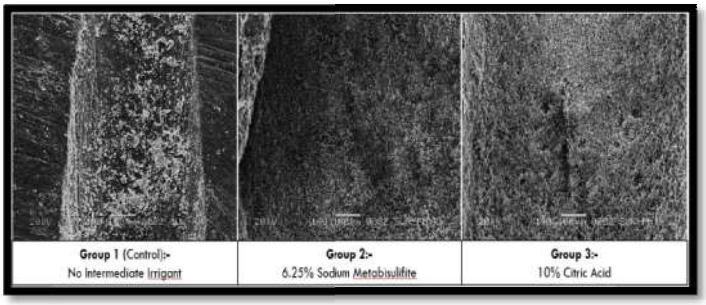

Figure 1 Representative SEM images from each group. (a)Control group (b) 6.25% Sodium Metabisulfite group (c)10% Citric acid group.

One‑way ANOVA test showed that Group 2 displayed least mean scores amongst all the experimental groups ($P < 0.05$), followed by Group 3 and Group 1 respectively (Table 2). The *post hoc* Tukey's test showed a statistically significant difference between Group 2 as compared to all the other experimental groups, whereas Group 1 exhibited the maximum amount of orange‑brown precipitate (Table 4). The difference between Group 2 and Group 3 was also statistically significant ($p < 0.05$). Thus, Group 2 displayed the least amount of orange‑brown precipitate among all the groups. Therfore, 6.25% Sodium metabisulfite is more effective in eliminating_the residual sodium hypochlorite when _compared to 10% Citric acid.

***'DATA* Summary:**

Table 1 ANOVA values for the presence of Orange‑brown precipitate

	N	∑X	MEAN	∑X²	Variance	Standard deviation	Standard error	95% Confidence Interval	
								Lower Bound	Upper Bound
Control	10	26	2.6	70	0.266667	0.516398	0.163299	2.28	2.92
6.25% Sodium Metabisulfite	10	2	0.2	2	0.177778	0.421637	0.133333	-0.061	0.461
10% Citric acid	10	8	0.8	8	0.177778	0.421637	0.133333	0.539	1.061
Total	30	36	1.2	80	1.268966	1.126484	0.205667		

Standard weighted-means analysis

Table 2 Summary of ANOVA values

ANOVA Summary (Independent Samples k=3)					
Source	**SS**	**Df**	**MS**	**F**	**P**
Treatment (between groups)	31.2	2	15.6	75.21	**<.0001**
Error	5.6	27	0.207407		
Total	36.8	29			

Tukey HSD Test

Table 3 *Post hoc* scores for the presence of Orange‐brown precipitate'

HSD [.05] = 0.51; HSD [.01] = 0.65		**M1 = Mean of Group 1** **M2 = Mean of Group 2** **M3 = Mean of Group 3**
M1 vs M2	**P<.01**	
M1 vs M3	**P<.01**	**HSD** = The absolute [unsigned] difference between any two sample means required for significance at the designated level. HSD [.05] for the .05 level; HSD [.01] for the .01 level.
M2 vs M3	**P<.05**	

DISCUSSION

Endodontic microflora is polymicrobial in nature. Biomechanical preparationcan reduce the bacterial number in the root canals but does not completely eliminate it.[1] Hence, the use of different irrigants in a sequential manner or in combination is required, especially in areas untouched during chemomechanical preparation which amount to almost 40%–60% of the total surface area.[5]The combination of irrigants was shown to enhance their antimicrobial effect[17], and their interaction could be unfavorable to the outcome of the root canal therapy.[6,7]

The extracted teeth were stored ina solution of 0.5% Chloramine T to prevent dehydration and provide adequate disinfection while having no adverse effect on the organic phase (collagen) of dentin. Instrumentation was done up to size 30 and 0.09 taper to allow the irrigating solution to penetrate deeper into the apical portion of the root canal.[18]

The beneficial effects of the mutually exclusive properties of NaOCl and CHX (tissue dissolution and solidity respectively), were maximized by the alternate use of these solutions. The antimicrobial effect of a combination of NaOCl and CHX has been shown to be superior to either of the components individually.[4]

The present study intended to evaluate the inhibition of precipitate formation specifically on the radicular dentinal surface. Thus, the prepared specimens were evaluated using Scanning Electron Microscope at a magnification setting of ×140, which efficiently provided a well magnified two‐dimensional view for evaluating the precipitate formed.[4]
In this study, all specimens of Group 1 showed immediate formation of an orange-brown precipitate when irrigated with CHX. The precipitate formed adhered resolutely to the canal wall and showed no signs of shedding during blot drying of the canals.[19] The precipitate is an insoluble neutral salt formed by the acid-base reaction between NaOCl and CHX. Parachloroanaline is the main product of the interaction between NaOCl and CHX, with the molecular formula NaC6H4Cl as analysed by mass spectrometry.[6] When CHX is mixed with NaOCl, its molecules become hydrolysed into smaller fragments and each fragment will form a by-product. Due to the low bond dissociation energy between the two atoms, the first bond to bebroken in this reaction is between carbon and nitrogen.[8]

Leaching of parachloroanaline from the insoluble precipitate formed is of concern because it has shown to be cytotoxic in rats[20] and may be carcinogenic to humans (International Agency for Research on Cancer group 2B).[21,22] The insoluble precipitate is hard to remove from the canal and occludes the dentinal tubules[7] preventing the penetration of the intracanal medicaments and compromises the seal of the obturated root canal. Moreover, its incidence also imparts colour to the canal wall and causes staining of the tooth, which affects its esthetics.

Group 3, where 10% Citric acid (an organic acid) was used asintermediate rinse, showed little 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 as an exothermic reaction which takes place when citric acid, an organic acid reacts with sodium hypochlorite, an oxidising agent leading to formation of bubbles formed on release of chlorine gas (Cl_2) rendering sodium hypochlorite ineffective.[5,23,24]

The superior performance exhibited by Sodium metabisulfite when used as an intermediate irrigating solution (Group 2) in minimalizing the precipitate formation could be associated to its effectiveness in neutralizing NaOCl by removing free chlorine ions. The reaction between NaOCl and Sodium metabisulfite (weak acid salt) leads to the formation of hypochlorous acid which then undergoes an oxidation reaction, which converts sulfite group to sulfate group.[25]

CONCLUSION

Within the limitations of this study, it can be concluded that 6.25% Sodium hypochloriteshowed maximum elimination of residual sodium hypochlorite when compared to 10% Citric acid. Further studies are needed to evaluate the effect of Sodium metabisulfite on the structural properties of radicular dentin.

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