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AN IN VITRO STUDY TO EVALUATE AND PREVENT THE PRECIPITATION FORMED BY THE INTERACTION OF SODIUM HYPOCHLORITE AND CHLORHEXIDINE

Endodontics

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ABSTRACT

Background: As endodontic irrigants, a combination of sodium hypochlorite and chlorhexidine has been advocated to enhance their antimicrobial properties, also known to produce a carcinogenic precipitate, parachloroaniline, making its elimination necessary. **Aim:** To evaluate maximum thickness and composition of the precipitate on root canal wall and its elimination by using various intermediate irrigants. **Materials and Methods:** Working length was determined for the decoronated teeth. Biomechanical preparation was done till F2 Protaper file. The canals were irrigated between each instrumentation with 1 ml 2.5% NaOCl. Irrigation was done with 17% EDTA (5 ml) followed by 2.5% NaOCl (5ml) and final flush with 2.5% CHX (5 ml). Intermediate flushes of 5 ml of saline, distilled water, absolute alcohol, sonic irrigation device and distilled water combination, oxum and acetic acid in between NaOCl and CHX. After drying the teeth were split into two halves for stereomicroscopic observation and grading was done for precipitate. **Statistical analysis:** The data obtained were subjected to Chi square and ANOVA test. **Results:** Maximum precipitation was observed in coronal portion of each group. Absolute alcohol group showed no precipitation, acetic acid group showed orange brown discoloration without forming of any precipitate. **Conclusion:** Formation of parachloroanilinecan be prevented by using absolute alcohol and minimized using different intermediate irrigants.

KEYWORDS

Absolute alcohol, Acetic acid, Chlorhexidine, Parachloroaniline, Sodium hypochlorite.

INTRODUCTION

Cleaning and shaping root canals are essential steps in root canal treatment as the success of root canal treatment relies on thorough chemomechanical procedures. The persistence of residual pulp tissue, infected dentin or bacteria in the root canal system may be responsible for treatment failure. Unfortunately, due to anatomical complexities the mechanical action of instrument is unable to reach areas of the root-canal system. The use of irrigating solutions facilitates debridement of the root canal space and is important for the success of chemo-mechanical preparation as well as root canal treatment.[1,2]

Sodium hypochlorite (NaOCl) is routinely used in nonsurgical endodontic therapy as an effective antimicrobial agent for its chemical dissolution properties and as a lubricant during instrumentation.[3] NaOCl is both an oxidizing and hydrolyzing agent and also bactericidal, virucidal and proteolytic in nature. It has a cytotoxic effect when injected into the periapical tissues, also known to produce allergic reactions. Therefore, an alternative, effective but safer irrigant is desirable.[4]

Chlorhexidine (CHX) is widely used as a mouth rinse in the prevention and periodontal diseases and dental caries, and it has been suggested as an irrigating solution or intracanal dressing in endodontic therapy for its broad-spectrum antimicrobial activity and substantivity.[5] The capacity of chlorhexidine gluconate gel to remove the smear layer is not yet clear and the literature brings ambiguous results of the cleaning ability of CHX as an endodontic irrigant.[6]

A combination of sodium hypochlorite and chlorhexidine has been advocated to enhance their antimicrobial properties. If hypochlorite is still present in the canal, an orange brown precipitate will be observed when the medications interacted. The precipitate parachloroaniline is cytotoxic and carcinogenic, displays abnormal development of cells and pigmentation.[7] Therefore the aim was to use different intermediate irrigation systems like saline, distilled water, absolute alcohol, sonic irrigation device, combination of sonic irrigation device and distilled water, oxum and acetic acid to remove residual NaOCl and thereby eliminating the formation of the parachloroaniline precipitate due to interaction of hypochlorite and chlorhexidine.

MATERIALS & METHODS

The present study was performed in the Department of Conservative Dentistry and Endodontics. One twenty single-rooted, extracted human teeth were used for this study. Teeth were cleaned of soft and hard tissue debris. The teeth were stored refrigerated 0.9% saline and used within 1 month. The crown of each tooth was sectioned at the cementoenamel junction to make all teeth equal length of 15 mm. The coronal portion was removed with a water-cooled diamond disc operated at low speed. Then all the roots were inspected for canal patency with # 10 K –file.A #10 K file was introduced into the root canal to establish the patency of the canal. The working length was determined by passing a #15 K file into the canal until the tip was just visible at the apical foramen and then reducing 1 mm from this length. After each instrument change the root canal was irrigated with 1 ml of 2.5% NaOCl, using a 24-gauge hypodermic needle.

After confirming the working length with a#15 K-file, root canals were instrumented up to size #25 K file. Roots having apical diameter greater than a size #25 K file were discarded. The orifice was enlarged with Endo access bur No1 (Dentsply). #SX Protaper (Dentsply) was used for coronal preflaring. Root canals were instrumented 1mm short of the root apex till #F2 Protaper file. #15 K-file was used for recapitulating, to maintain the apical patency. During instrumentation, the canals were irrigated between each instrument change with 1 ml of 2.5% NaOCl, using a 24-gauge hypodermic needle.The root canals were filled with 17% EDTA and left for 3min in order to remove the smear layer. This was followed by irrigation with 5ml of physiologic saline solution.

The biomechanical preparation in all the canals were completed by one operator. After preparation, all the canals were dried with absorbent paper point. The evaluation was recorded for precipitate. The precipitate was measured in three areas (coronal, middle and apical thirds of the root) by means of visual analogue scale.

The specimens were randomly divided into eight groups of fifteen each: (1) the test group, in group 1 the irrigation of the canals was done with 5 ml of 17% EDTA followed by 5 ml of 3% NaOCl and a final flush with 5 ml of 2% CHX; (2) in group 2 a similar irrigation sequence was followed but received intermediate flushes of 5 ml of saline; (3) group 3 samples received 5 ml of distilled water intermittently; (4) group 4 samples received 5 ml of absolute alcohol (5) in group 5 sonic irrigation device was used between NaOCl and CHX.irrigation in this group (6) in group 6 distilled water and sonic irrigation device combination was used between NaOCl and CHX irrigation in this group (7) in group 7 oxum (superoxidized water) irrigation was done intermittently and in the last group (8) in group 8 in between NaOCl and CHX final irrigation, the samples of group 8 received 5 ml of acetic acid as intermediate flush.

A thin longitudinal slot was made along the buccal and lingual aspect of the root using double faced diamond disk operated at low speed, making sure to avoid perforation into the canal. The roots were split longitudinally using a chisel and mallet. Both halves of the split tooth

were examined by stereomicroscopy (OLYMPUS SZX, Japan) after illumination with Nikon 100W optical fiber and under maximum magnification of the microscope (20X) and the image was captured. Root samples of each group were examined at the coronal, middle, and apical third levels separately. The thickness of the precipitate was measured from its outer surface to the inner dentinal wall visually at a uniplanar level and scoring was done.

RESULTS

Scoring was done for the precipitate inside the root canal.

Scoring criteria

Score 0: Clean canal wall, scanty precipitate present, if any. (Fig.1-a)
Score 1: Few small conglomerulations on the canal wall. (Fig.1-b)
Score 2: Many conglomerulations, less than 50% of the canal wall covered. (Fig.1-c)
Score 3: More than 50% of canal wall covered. (Fig.1-d)
Score 4: Completely/ Nearly complete covering of the canal wall. (Fig.1-f)

The Beilstein test and a solubility test with dilute HCl in a 1:1 ratio, and the nuclear magnetic resonance imaging technique were conducted to detect and confirm the composition of the precipitate. For acetic acid group (Group8) a precipitation test was done to rule out presence of any precipitate whereas the orange brown color remained (Fig.1-f). The presence of two identical peaks confirms the presence of aromatic group (Fig.2-a).

The values were obtained and statistical analysis of these tabulated values was done to evaluate the effectiveness of the intermediate irrigants. Chi-square and ANOVA test were done for statistical analysis.

Table 1 shows precipitation in coronal, middle and apical portion.

- In coronal 101 samples shows precipitation of grade 0, 64 samples show precipitation of grade 1, 24 samples show precipitation of grade 2, 15 samples show precipitation of grade 3, 6 samples show precipitation of grade 4.
- In middle portion 110 samples show precipitation of grade 0, 61 samples show precipitation of grade 1, 26 samples show precipitation of grade 2, 12 samples show precipitation of grade 3, 1 sample show precipitation of grade 4.
- In apical portion 147 samples show precipitation of grade 0, 46 samples show precipitation of grade 1, 15 samples show precipitation of grade 2, 2 samples show precipitation of grade 3 and no samples show precipitation of grade 4.
- For grade 0 precipitation Apical portion > Middle portion > Coronal portion.
- For grade 4 precipitation Coronal portion > Middle portion > Apical portion.

significant difference was observed between coronal, middle and apical portion when all specimens with grading of precipitation were taken into consideration.

Table 2 shows precipitation in different groups compared statistically.

- The mean value between groups show least precipitation was seen in group 4 and maximum precipitation was seen in group1.
- As there was no precipitation distinguished in group 8- acetic acid group (fig. 2-a), no table was prepared for group 8.

DISCUSSION

The irrigation plays an important role in endodontic therapy, thereby, choice of an adequate irrigating substance must always consider not only the antimicrobial aspects, but also its biocompatibility to periradicular tissues.[5] A combination of sodium hypochlorite and chlorhexidine for root canal irrigation has been advocated to enhance their antimicrobial properties. A study by Krishnamurthy et al, suggested that the antimicrobial effect of 2.5% sodium hypochlorite and 0.2% chlorhexidine used in combination was greater than that of either agent used separately.[8] However, the presence of sodium hypochlorite in the canals during irrigation with chlorhexidine produces an orange-brown precipitate known as parachloroaniline (PCA). The precipitate is cytotoxic, leading to the concern of it leaching out of the canal.[9]

It was suggested that when mixed with sodium hypochlorite, the chlorhexidine molecules may become hydrolyzed into smaller fragments, each forming a byproduct, resulting in the formation of parachloroaniline and other compounds. The formation of the precipitate could be explained by the acid-base reaction that occur when NaOCl and CHX are mixed. Being a dicationic acid, Chlorhexidine (pH 5.5-6.0) has ability to donate protons. Due to the alkaline nature of NaOCl, it can accept protons from the dicationic CHX. This proton exchange leads to the formation of a neutral, insoluble substance, referred to as “precipitate” that contains parachloroaniline.[10]

Parachloroaniline and its degradation product are toxic and carcinogenic, and its formation when CHX and NaOCl are combined is positively correlated with hypochlorite concentration. The result of agar diffusion test showed a decrease in the inhibition zone formation when higher concentrations of NaOCl were combined with CHX (liquid and gel). The decrease in the inhibition zone as a result of the increased concentration of sodium hypochlorite could be explained by the large quantity of precipitate formed when higher concentrations of hypochlorite were used. This precipitate may create smaller inhibition zone thus presenting difficulty for diffusion through the agar plates. Interestingly, the use of 2% chlorhexidine gel 2.5% sodium hypochlorite showed a smaller inhibition zone than 2% chlorhexidine gel 5.25% sodium hypochlorite. As noted, before, when adding NaOCl to 2% CHX, it dramatically reduces the efficacy of CHX.

Acetic acid solutions in concentrations ranging from 0.2% to 10.0% have been tested. Pandey et al. detected, by scanning electron microscopy (SEM), dentin erosion after the use of 2.25% acetic acid. For this reason, a lower concentration (1%) was suitable. Its activity against E. faecalis was similar to that of sodium hypochlorite and chlorhexidine in both the post-instrumentation and final periods.[11]

Smear layer removal improves disinfection of the canal, but the currently available techniques fail to do so from the entire canal walls. Burleson et al reported that passive sonic and ultrasonic activation for 30 and 60 seconds produces significantly cleaner canals than irrigation alone.[12] Torres et al concluded that Endo Activator did not enhance the removal of smear layer.[13]

In this study the root canals were instrumented till Protaper instrument size F2 with particular irrigation regimen. The objective of this study was to evaluate the precipitate formed between sodium hypochlorite (NaOCl) and chlorhexidine (CHX) and to evaluate effectiveness of different irrigation system to remove residual NaOCl, thereby preventing formation of the precipitate. Samples were observed under stereomicroscope to observe the precipitate.

In this study, all specimens of the group1,2,3,5,7 showed immediate formation of orange brown precipitate when irrigated with chlorhexidine. The precipitate formed firmly adhered to the canal wall which showed no signs of dislodgement during blot drying of the canals. In the group 4, in which the canals were irrigated with absolute alcohol before the use of chlorhexidine, there was no precipitate evident in almost all specimens.[1] A study quoted in Ingle, showed that the precipitate was soluble in 0.1 mol/L acetic acid, but the brown/orange color of the solution remained.[14] Atomic absorption spectrometry indicated that the precipitate contained iron which may be the reason for the color.[14] In this study the root canals were irrigated with acetic acid intermittently in group 8 which shows a orange brown color with absence of any precipitate in the root canal.

The insoluble precipitate is difficult to remove from the canal and occludes dentinal tubules preventing the penetration of the intracanal medicaments and compromises the seal of the obturated root canal. Also, its presence imparts color to the canal wall and causes tooth discoloration affecting esthetics.[9]

The use of absolute alcohol as an intermediate flush between NaOCl and CHX prevented the formation of the precipitate, since alcohol is a volatile, tensioactive agent, is highly electronegative which can penetrate deeply for removal of the residual NaOCl present in the canals. The saline and distilled water group revealed presence of precipitate in much lesser thickness compared to test group. This could be due to dilution of NaOCl caused by saline and distilled water respectively.[9]

The interaction of sodium hypochlorite and chlorhexidine forms an insoluble precipitate. The precipitate formed is of clinical relevance

with regards to staining, hampering the seal of obturation, and potential leaching of parachloroaniline into the periapex. It would appear prudent to reduce the precipitate formation by using intermediate flushes of saline or distilled water in greater volumes to enhance the dilution effect on sodium hypochlorite or to eliminate its formation by flushing away the remaining NaOCl with absolute alcohol before using chlorhexidine as the final irrigant; in which case, the biocompatibility of alcohol with the periapical tissues remains a concern. However, further investigation of the NaOCl/CHX precipitate in endodontic situations should address the bioavailability of parachloroaniline leaching out of the canal and periapical tissue response to the use of absolute alcohol as an endodontic irrigant.[9]

The stereomicroscope examination of specimens in the 1st group showed, thick orange-brown precipitate deposited all along the canal wall. The thickness was measured from the outer surface of the precipitate to the inner dentinal wall at a uniplanar level visually and separate numerical evaluation scheme was done in the coronal, middle and apical portion according to the area of precipitation covered.[15] The thickness was found to be maximum in the coronal third and least in 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. It could also be caused by less of the CHX reaching the apical third because it is exhausted in the coronal and middle third because of its interaction with NaOCl.[9]

Nuclear magnetic resonance (NMR) spectroscopy was used in this study to identify the product formed by the reaction because it facilitates structural determinations by exposing a sample to a strong magnetic field and then to radiofrequencies that act on atomic nuclei.[16]

CONCLUSION

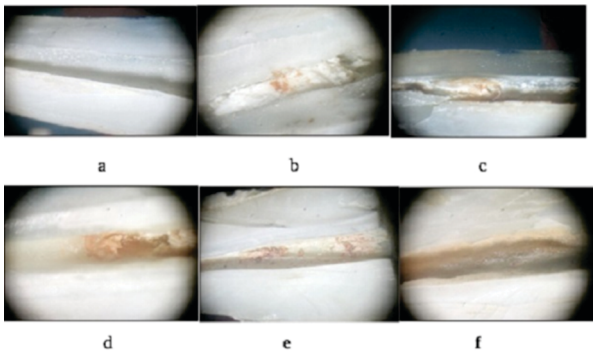
Considering the limitations of this study it may be concluded that:
Maximum amount of precipitate in root canals was formed by the interaction of 2% chlorhexidine and 3% sodium hypochlorite without intermediate irrigation. The use of absolute alcohol as an intermediate flush between 3% sodium hypochlorite and 2% chlorhexidine significantly prevented the formation of the precipitate. Use of different irrigation system intermediately reduced the formation of precipitate.

Table1: Precipitation in coronal, middle and apical portion in all groups

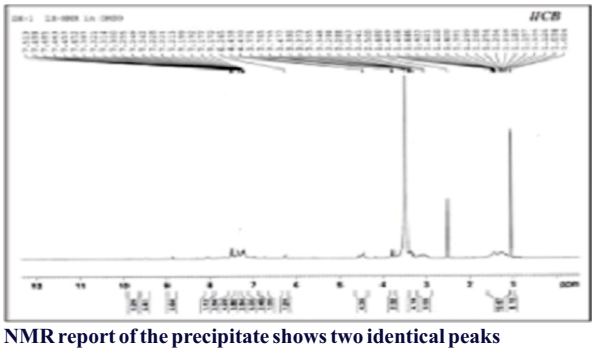
Portion			Grade					Total
			0	1	2	3	4	
1	Group	1	0	1	9	14	6	30
		2	8	19	2	1	0	30
		3	15	13	2	0	0	30
		4	30	0	0	0	0	30
		5	20	8	2	0	0	30
		6	16	10	4	0	0	30
		7	12	13	5	0	0	30
	Total		101	64	24	15	6	210
2	Group	1	0	4	13	12	1	30
		2	9	16	5	0	0	30
		3	19	7	4	0	0	30
		4	28	2	0	0	0	30
		5	21	9	0	0	0	30
		6	19	10	1	0	0	30
		7	14	13	3	0	0	30
	Total		110	61	26	12	1	210
3	Group	1	3	12	13	2		30
		2	15	14	1	0		30
		3	23	6	1	0		30
		4	29	1	0	0		30
		5	27	3	0	0		30
		6	25	5	0	0		30
		7	25	5	0	0		30
	Total		147	46	15	2		210

Table 2: Mean value of precipitation of different groups

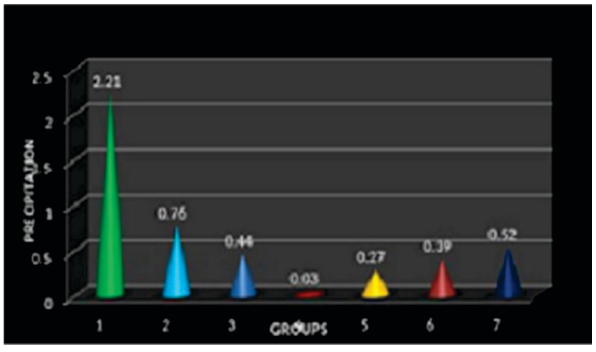
	N	Mean	Std. Deviation	Std. Error
1	90	2.21	.954	.101
2	90	.76	.659	.069
3	90	.44	.638	.067
4	90	.03	.181	.019
5	90	.27	.493	.052
6	90	.39	.594	.063
7	90	.52	.657	.069
Total	630	.66	.917	.037



(a) Score 0 precipitation in root canal
(b) Score1 precipitation in root canal
(c) Score 2 precipitation in root canal
(d) Score 3 precipitation in root canal
(e) Score 4 precipitation in root canal
(f) No precipitation but discoloration in acetic acid group



NMR report of the precipitate shows two identical peaks



Graph showing comparison of precipitation of different groups (According to the amount of formation of precipitate in different groups) group1> group2> group7> group3> group6> group5> group4.

REFERENCES

1) Gomes NV, Ferraz CCR, Gomes BPFA, Zaia AA, Teixeira FB, Filho FJS. Influence of irrigants on the coronal micro leakage of laterally condensed gutta-percha root fillings. International Endodontic Journal 2002; 35: 791-95.
2) Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JAP. Dissolution of pulp tissue by aquous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. International Endodontic Journal 2004; 37: 38-41.
3) Essner MD, Javed A, Eleazer PD. Effect of sodium hypochlorite on human pulp cells: an in vitro study. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2011, 112: 662-666.
4) White RR, Hays GL, Janar LR Residual antimicrobial activity after canal irrigation with chlorhexidine, J Endod 1997, 23:229-31.
5) Oliviera DP, Barbizam JVB, Trope M, Teixeira FB. In vitro antibacterial efficacy of endodontic irrigants against Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2007,103: 702-6.
6) Vasconcelos BC, Cruz SML, Deus GD, Moraes IG, Ferreira CMF, Filho EDG. Cleaning ability of chlorhexidine gel and sodium hypochlorite associated or not with EDTA as root canal irrigants: a scanning electron microscope study. J Appl Oral Sci. 2007, 15(5): 387-91.
7) Gomes NV, Ferrez CC, Gomes BP, Zaia AA, Teixeira FB, Souza FFJ. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root fillings.

Int Endod J 2002; 35: 791-5.
8) Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine, Journal of Endodontics 2010; 36:1154-1157.
9) Morgental RD, Tanomaru JMG, Faria NB, Duarte MAH, Kuga MC, Filho MT. Antibacterial efficacy of endodontic irrigating solutions and their combinations in root canals contaminated with Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2011; 112: 396-400.
10) Barsani R, Manek Sheela, Mathers Dan, Fillery E, Sodhi RNS. Determination of 4-Chloroaniline and its Derivatives formed in the interaction of sodium hypochlorite and chlorhexidine by using gas chromatography. Journal of Endodontics 2010; 36:312-314.
11) Pandey PK, Koushariya M, Shukla S, Das S. Outcomes of superoxide solution dressings in surgical wounds: a randomized case control trial. Int Biol Med Res 2011; 2(4): 965-968.
12) Burleson A, Nusstein J, Reader A, Beck M. The in vivo evaluation of hand/rotary/ultrasound instrumentation in necrotic human mandibular molars. J Endod 2007, 33:782-87.
13) Torres DU, Rodriguez MPG, Luque CMF et al. Effectiveness of the endo activator system in removing the smear layer after root canal instrumentation. Journal of Endodontics 2010; 36(2): 308-11.
14) Ingle, Bakland, Baumgartner. Endodontics. Irrigants and IntracanalMedicaments. : Edition 6th, 2007, Page no 1005.
15) Smita Singh, Niraj Nigam. Comparative evaluation of surface characteristics of dentinal walls with and without using plastic finishing file. J Conserv Dent 2010; 13(2): 89-93
16) Jiang LM, Verhaagen B, Versluis M. Evaluation of a sonic device designed to activate irrigant in the root canal. Journal of Endodontics 2009.