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Evalution of the Surface Area Covered by the Precipitate Formed on Interaction Between Sodium Hypochlorite and Chlorhexidine: An in Vitro Study

Abstract

AIM: The purpose of this study was to (1) evaluate the surface area covered by the precipitate formed on the interaction between sodium hypochlorite (NaOCl) and chlorhexidine (CHX), and (2) to evaluate effectiveness of absolute alcohol to remove residual NaOCl and thereby prevent the formation of the precipitate. **METHOD:** Forty extracted single rooted human teeth were decoronated and the canals were instrumented, in Group A (Test group) - canals were irrigated with 17% EDTA and 2.5% NaOCl followed by 2% CHX in Group B (Absolute alcohol), Group C (Saline) and Group D (Distilled water), intermediate flushes of absolute alcohol, saline, and distilled water were used between the last two irrigants. Teeth were sectioned longitudinally and they were subjected to stereomicroscopic examination. **RESULTS:** The Group A, samples showed orange – brown precipitate, concentrated more in coronal, middle thirds and apical whereas Group B, showed no evidence of precipitate formation. The Group C, and Group D, showed minimal precipitate in coronal and middle thirds. **CONCLUSION:** The Group A, samples showed orange – brown precipitate, concentrated more in coronal, middle thirds and apical whereas Group B, showed no evidence of precipitate formation. The Group C and Group D, showed minimal precipitate in coronal and middle thirds.

Key Words

Sodium Hypochlorite; CHX; absolute alcohol; precipitate

Basavanagowda[1], Vani Hegde[2], JMV Raghavendra Reddy[3], Pushpa Latha[4], Kalyana Chakravarthi Ponangi[5], Dr. Arpitha Kotha[6]

[1]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Navodaya Dental College & Hospital, Raichur, Karnataka, India
[2]Professor & Head, Department of Conservative Dentistry and Endodontics, AME's Dental College & Hospital, Raichur, Karnataka, India
[3]Reader, Department of Conservative Dentistry and Endodontics, Navodaya Dental College & Hospital, Raichur, Karnataka, India
[4]Senior Lecturer, Department of Conservative Dentistry and Endodontics, The Oxford Dental College and Hospital, Bangalore, Karnataka, India
[5]Post Graduate Student, Department of Conservative Dentistry and Endodontics, Navodaya Dental College & Hospital, Raichur, Karnataka, India
[6]Senior Lecturer, Department of Public Health Dentistry, MNR Dental College and Hospital, Sangareddy, Telangana, India

INTRODUCTION

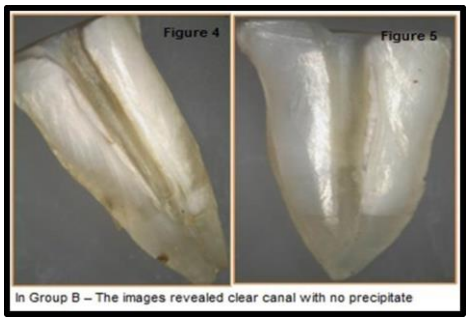
The aim of root canal treatment is to eliminate bacteria from the infected root canal and to prevent reinfection. Biomechanical cleaning and shaping of the root canal greatly reduces the number of bacteria.[1] In root canal cleaning and shaping, mechanical instrumentation alone is insufficient to clean the root canals.[2] Because of the anatomical complexity of the root canal system, organic and inorganic residues and bacteria cannot be completely removed and often persist.[3] In such teeth with complex anatomy such as fins or other irregularities that might be missed by instrumentation, chemical debridement facilitates proper cleaning of the root canal space and is important for successful root canal treatment.[4] Various irrigants have been used during the canal preparation to minimize the residual debris, necrotic tissue, and bacteria, as well as to remove smear layer formed by the mechanical preparation of the dentin.[5,6] Several studies have been conducted for an irrigant that meets four major requirements:

- Antimicrobial activity
- Non-toxicity to Periapical tissues
- Water solubility
- Capacity to dissolve organic matter

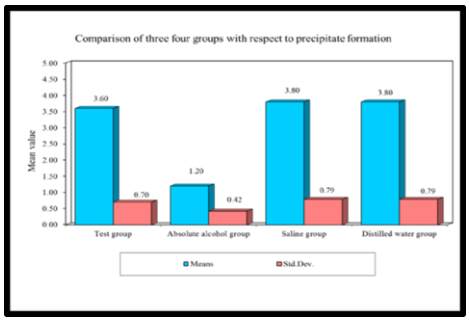
As no irrigant has all the ideal properties, it is mandated to use combinations of irrigants in a

Stereomicroscopic examination of the specimen revealed orange-brown precipitate deposited all along the canal wall in Group A (figure 1, 2, 3)

Sparse distribution in the Group C and Group D (figure 6, 7, 8)



In Group B – The images revealed clear canal with no precipitate



sequential manner to enhance their antimicrobial effect and dissolution of organic and inorganic tissues. Endodontic irrigants have been well characterized individually; but their combined use is not well-understood. Individually, no irrigant forms precipitate but when irrigants are combined; due to acid-base reaction form a neutral and insoluble substance referred to as precipitate.[7] It is difficult to remove the precipitate from the root canals as it occludes dentinal tubules, preventing the intracanal medicament penetration into dentinal tubules and thus compromises the seal of the obturated root canal.[8] Sodium hypochlorite has effective antibacterial action, organic material dissolution[9] and also removes the necrotic tissue.[10] However, there is a safety concern if sodium hypochlorite is extruded out of the root canal into the periapical tissue resulting in destructive tissue damage. In high concentration NaOCl is toxic and can cause inflammation in the periapical tissues.[10-13] Thus, an alternative irrigant, such as, chlorhexidine, has been advocated. Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that has been advocated as an effective medication in endodontic treatment.[14,15] When used as a root canal irrigant and intracanal medication, it has an antibacterial efficacy comparable to that of NaOCl.[16,17] Prolonged exposure of the root dentin to CHX may result in residual antimicrobial activity of the dentin surface.[18-20] However, the inability of CHX to dissolve organic matter is a perceived drawback.[21] A combination of NaOCl and CHX has been advocated to enhance their antimicrobial properties. Zehnder proposed an irrigation regimen in which NaOCl would be used throughout instrumentation followed by EDTA, and CHX would be used as a final irrigant.[22] If hypochlorite was still present in the canal, a precipitate was observed when the medications interacted.[23] Therefore the purpose of this study was to evaluate the surface area covered by the precipitate formed on the interaction between sodium hypochlorite (NaOCl) and Chlorhexidine (CHX), and to evaluate effectiveness of absolute alcohol to remove residual NaOCl and thereby prevent the formation of the precipitate.

MATERIALS AND METHODS

Forty single rooted extracted human teeth were selected for the study. The external surfaces of the teeth were cleaned of tissue remnants and were stored in 0.9% saline. Teeth were decoronated 1 mm below the cement enamel junction; coronal flaring of the root canals was performed by using Gates Glidden drills #2, #3. The working length was determined with #15 k-file introduced in to the canal until tip of the file was visible at the apical foramen. The root ends of the prepared teeth were inserted into the softened wax and allowed to set, this prevented extrusion of the irrigants out of the apex and allowed care of handling during instrumentation. Canals were instrumented with stainless steel k-file to # 60 at the apex in crown-down technique; recapitulation was performed by using #15 k-file to maintain the apical patency. The specimens were randomly divided into four groups of ten each:

Group A - Test group, the irrigation of the canal was done with 5 ml of 17% EDTA followed by 5 ml of 2.5% NaOCl and a final flush with 5 ml of 2%

Table 1: Comparison of three four groups with respect to precipitate formation by Kruskal Wallis one way ANOVA and Mann-Whitney U test

Groups	Means	Std. Dev.	Median	H-value	P-value
Group A	3.6000	0.6992	3.5	23.8559	0.0000*
Group B	1.2000	0.4216	1.0		
Group C	3.8000	0.7888	4.0		
Group D	3.8000	0.7888	4.0		

Table 2; Pair wise comparison by Mann-Whitney U test

Group B	0.0002*
Group C	0.5967
Group D	0.5967
Group B-Group C	0.0002*
Group B –Group D	0.0002*
Group c-Group D	1.0000

Table 3: Distribution of scores of precipitate formation of four groups

Coronal	Group 1	%	Group 2	%	Group 3	%	Group 4	%	Total
Score 1	0	0.00	8	80.00	0	0.00	0	0.00	8
Score 2	0	0.00	2	20.00	0	0.00	0	0.00	2
Score 3	5	50.00	0	0.00	4	40.00	4	40.00	13
Score 4	4	40.00	0	0.00	4	40.00	4	40.00	12
Score 5	1	10.00	0	0.00	2	20.00	2	20.00	5
Total	10	100.00	10	100.00	10	100.00	10	100.00	40

chlorhexidine (CHX); **Group B** - Absolute alcohol group (Aba), a similar irrigation sequence was followed but received intermediate flushes of 5 ml of absolute alcohol (Isopropyl alcohol); **Group C** - Saline group (sal), received 5 ml of saline and; **Group D** - Distilled water (DW) received 5 ml of distilled water between NaOCl and CHX. The canals were dried immediately with absorbent points. The prepared and irrigated specimens were split longitudinally using a diamond discs, both the halves of the split tooth were examined by stereomicroscopy (Clinical Research Microscope Olympus Japan). Root samples of each group were examined at the coronal, middle and apical third levels were examined. The results obtained were subjected to statistical analysis using Kruskal Wallis one way ANOVA and Mann-Whitney U test.

RESULTS

Stereomicroscopic examination of the specimen revealed orange-brown precipitate deposited all along the canal wall in Group A (Fig. 1, Fig. 2 & Fig. 3). In Group B: The images revealed clear canals with no evidence of precipitate deposition (Fig. 4 & Fig. 5); whereas there was a more sparse distribution in the Group C and Group D (Fig. 6, Fig. 7 & Fig. 8) groups. The precipitate deposition was concentrated in the coronal and middle thirds of the canals in Group A, B, C, there was no precipitate in the Group D. The results were analysed by using analysis of variance and the (ANOVA) method, analysis of variance revealed no significant difference between the two sections but there is a highly significant difference between the three groups. (Table 1, Table 2 & 3). In Group A - 96% of samples showed precipitate formation all along the canal walls involving coronal, middle and the apical part. In Group B - 2% of samples showed precipitate formation. In Group C - 54-70 % samples showed precipitate formation with a sparse distribution. In Group D - 60-65 % samples showed sparse distribution of precipitate formation.

DISCUSSION

Biomechanical cleaning and shaping of the root canals reduces the bacterial number but does not completely eliminate the bacteria from the canals[1] and, therefore, requires the use of various irrigants in a sequential manner or in combination to enhance their antimicrobial effect.[22] The combination of irrigants was shown to enhance their antimicrobial effect,[11] and their interaction could be detrimental to the outcome of the root canal therapy.[7,24-26] In this study, all specimens of the Ts, Sal, and Dw groups showed immediate formation of an orange-brown precipitate when irrigated with CHX. The precipitate formed was firmly adhered to the canal wall and showed no signs of dislodgement during blot drying of the canals. In the Aba group, in which the canals were irrigated with absolute alcohol before the use of CHX, there was no precipitate evident in all the specimens (Fig. 1B and Fig. F). Stereomicroscopy was used in evaluating the presence of the precipitate and differentiates it from

debris. It also allows the color change in the most natural form to not require any surface treatment of the specimen, permitting accurate measurement of the undisturbed precipitate. The stereomicroscopic examination of specimens in the Ts group showed, thick orange-brown precipitate deposited all along the canal wall. 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 of NaOCl and CHX, with the molecular formula NaC6H4Cl as analyzed by mass spectrometry.[24] When mixed with NaOCl, CHX molecules become hydrolyzed into smaller fragments, each forming a byproduct. The first bonds to be broken in this reaction are between carbon and nitrogen because of the low-bond dissociation energy between the two atoms. The presence of parachloroanaline was confirmed in our study by the Beilstein test for the presence of chlorine and the HCl solubility test for the presence of aniline. The presence of chlorine in the para position of the benzene ring was finally confirmed using the nuclear magnetic resonance imaging technique. Leaching of parachloroanaline from the insoluble precipitate formed is of concern because it has shown to be cytotoxic in rats[27] and possibly carcinogenic in humans International Agency for Research on Cancer.[28, 29] The insoluble precipitate is difficult to remove from the canal and occludes the 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. In this study, the use of absolute alcohol as an intermediate flush between NaOCl and CHX prevented the formation of the precipitate. Because alcohol is a volatile, tensioactive agent, it is highly electronegative and can penetrate deeply to remove the residual NaOCl present in the canals. In an in vitro study, 96% ethyl alcohol was used to remove the NaOCl crystals on the gutta-percha, after its rapid sterilization with 5.25% NaOCl.[30] Alcohol is also volatile so it aids in drying of the canals. However, using absolute alcohol as an endodontic irrigant is not yet well established. The groups Sal and Dw revealed the presence of precipitate in much less thickness compared with the test group. This could be due to the dilution of NaOCl caused by saline and distilled water, respectively. The interaction of NaOCl and CHX forms an insoluble precipitate. The precipitate formed is of clinical relevance with regards to staining, hampering the seal of obturation, and potential leaching of PCA 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 NaOCl or to eliminate its formation by flushing away the remaining NaOCl with absolute alcohol before using CHX 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 PCA leaching out of the canal and periapical tissue response to the use of absolute alcohol as an endodontic irrigant.

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