IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 6 Ver. III (June. 2017), PP 13-21
www.iosrjournals.org

Comparing the Effect of Irrigation With 70% Isopropyl Alcohol, Distilled Water and Saline to Remove the Residual Sodium Hypochlorite before Irrigation with Chlorhexidine in Root Canal Therapy – An In Vitro Study

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Abstract:

Aim: *The aim of this study is to compare the effect of different intermediate irrigant in removing residual sodium hypochlorite before irrigating with chlorhexidine by elemental analysis of precipitate formed by the residual sodium hypochlorite using Environmental Scanning Electron Microscopic Energy Dispersive X-Ray Spectroscopy (ESEM-EDS) system.*

Methodology: *Forty extracted single rooted premolars were decoronated and divided into four groups GROUP I (positive control): The irrigation of the canal was done in the sequence 5 ml of 17% EDTA → 5 ml of 5% NaOCl → 5 ml of 2% chlorhexidine (CHX). GROUP II (70% isopropyl alcohol): The irrigation of the canal was done with 5 ml of 17% EDTA → 5 ml of 5% NaOCl, → 5 ml of 70% isopropyl alcohol as intermediate irrigant → 5 ml of 2% CHX. GROUP III (distilled water): The irrigation of the canal was done with 5 ml of 17% EDTA → 5 ml of 5% NaOCl, → 5 ml of distilled water as intermediate irrigant → 5 ml of 2% CHX. GROUP IV (normal saline). The irrigation of the canal was done with 5 ml of 17% EDTA → 5 ml of 5% NaOCl, → 5 ml of 0.9% normal saline as intermediate irrigant → 5 ml of 2% CHX. Using a chisel and mallet, the roots were split longitudinally. One half of the split tooth was selected for the examination of smear layer under SEM. Five samples from the remaining ten halves of each group was taken for ESEM- EDX examination*

Results: *The deposition of the precipitate layer was scored according to the criteria for evaluating smear layer given by* ***Gutmann et al.*** *Kruskal Wallis ANOVA was employed to compare precipitate scores between different groups and teeth levels. There is no statistically significant difference (P>0.05) between the three levels of the Group I. There is a highly significant difference (P<0.001) between the three levels of the teeth of Group II (Isopropyl alcohol group). There is a significant difference (P<0.05) between the levels of the teeth of Group III (Distilled water) and Group IV (Normal saline). There is highly statistically significant difference (P<0.001) in the scores between the irrigants in the coronal and middle third of the root canals. There is a significant difference in between the groups in the apical third (P<0.05). The elements present in the root canal wall of each group recorded and subjected to Analysis of variance (One Way ANOVA) was performed as parametric test to compare different groups as well as different teeth sites. For all statistical evaluations, a two-tailed probability of value, P< 0.05 was considered significant.*

Conclusion: *Isopropyl alcohol removed more residual sodium hypochlorite from the root canal leaving minimum precipitate occluding the dentinal tubules. Elemental analysis of the precipitate showed presence of chlorine. In coronal and middle third contains more chlorine than apical thirds. Isopropyl alcohol removed more chlorine from the root canal.*

Keywords: *Chlorhexidine; intracanal irrigants; parachloroaniline*

I. Introduction

Bacteria in the root canal system provoke the formation of periapical inflammatory lesions (**1**). The aim of root canal treatment is to eliminate bacteria from the infected root canal and to prevent reinfection. Although biomechanical cleaning and shaping of the root canal greatly reduce the number of bacteria, studies have shown that bacteria often persist (**2**). Because of the complexity of the root canal system, mechanical instrumentation cannot completely remove bacteria and tissue from all root canal surfaces and forms a smear layer on the canal surface. Thus, irrigation is required to remove debris, tissue remnants, microbes and the smear layer. Various irrigants have been used for canal disinfection. The most commonly used irrigant is sodium hypochlorite (NaOCl) in concentrations that range from 0.5%–6%. NaOCl is an effective tissue solvent and antimicrobial agent. NaOCl is the most commonly used irrigant during endodontic therapy because of its tissue-dissolving and

antimicrobial properties. Its germicidal ability is related to the formation of hypochlorous acid when in contact with bacteria and organic debris. In high concentration, NaOCl is toxic and can cause injury to periapical tissues. In low concentrations its antimicrobial effect against specific microorganisms is reduced and it has limited antimicrobial effect. It also has several other properties that undermine its clinical value such as discoloration of fabrics on contact, corrosion of endodontic instruments and an unpleasant odour. Therefore, other chemical solutions have also been recommended for irrigation.

When used as an irrigant or intracanal medication, antibacterial efficacy of chlorhexidine (CHX) comparable to that of NaOCl and it is effective against certain NaOCl-resistant bacteria strains (**3**). It is a cationic bisbiguanide. Broad-spectrum antimicrobial action that acts by absorbing onto microbial cell walls or disrupting them, causing leakage of intracellular components. In vitro studies have shown CHX to exhibit substantivity in the root canal. However, its inability to dissolve organic matter is a drawback in its clinical use as main irrigant. Both NaOCl and CHX have limitations and although they have reported good antimicrobial effects, they are limited to the removal of bacterial lipopolisacharide(LPS).

A combination of NaOCl and CHX for root canal irrigation has been advocated to enhance their antimicrobial properties. A study by **Kuruvilla** (**4**) suggested that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination was greater than that of either agent used separately. **Zehnder** (**5**) proposed an irrigation regimen in which NaOCl would be used during root canal enlargement followed by irrigation with ethylenediaminetetraacetic acid and CHX as a final flush. However, when NaOCl was present in the canal at the time that CHX was introduced, a precipitation was observed to occur. The formation of this precipitate was observed after mixing NaOCl and 2%CHX in a test tube (**6**). By using x-ray photon spectroscopy (XPS) and time of flight secondary ion mass spectrometry (TOF-SIMS), it has been demonstrated the presence of 4-chloroaniline (PCA) in an amount directly related to the concentration of NaOCl used and how heating CHX can produce this by-product as well. It has been shown that aniline derivatives have different levels of toxicity. **Cathro** (**7**) suggests that this association can form a dense brown flocculate, which is difficult to remove from the root canal and could cause darkening of the dental structures. When studied in rats, rabbits, and cats, the primary toxic effect was methemogloblin formation. Repeated exposures to PCA led to cyanosis and methaemoglobinaemia. In humans, accidental occupational exposure to PCA produced symptoms of increased methemoglobin and sulfhaemoglobin levels, cyanosis, the development of anaemia and systemic changes from anoxia (**8**). While chlorhexidine may spontaneously hydrolyze to PCA over time, it undergoes a chemical reaction when combined with NaOCl and forms a precipitate that contains PCA and tends to stain the walls of pulp chamber which has been reported to be difficult to remove. This precipitate also acts as chemical smear layer and could compromise dentin permeability, the intracanal medication diffusion, and the Obturation sealing. Water or alcohol can be used as an irrigant to flush NaOCl from the canal before chlorhexidine is used, thus minimizing PCA formation (**9, 10**).

II. Materials And Methods

Forty single rooted teeth, fully matured, non-carious permanent premolars extracted for orthodontic purpose were selected for this study. Forty teeth randomly divided into four groups, 10 teeth in each group. The teeth were decoronated at the cementoenamel junction to a standard length of 15mm.The pulp was extirpated using barbed broaches. Working length was determined using 15 size K-file. A no. 10 size K file was inserted into each canal until it was just visible at the apical foramen and 1 mm was subtracted from that point to establish a working length. The root ends of the prepared teeth were inserted into a softened impression material cylinder and allowed to set. This prevented extrusion of the irrigants out of the apex and allowed ease of handling during instrumentation. Chemomechanical preparation was performed with a step-back technique using K files. The canals were enlarged to an ISO size no. 40 K file and step back preparation was done to ISO size no. 60 K file. Gates Glidden drills no. 2-4 were used to enlarge the coronal third of the root canal. Irrigation was performed with 1 ml of 5% of NaOCl solution after each file change.

GROUP I (positive control): The irrigation of the canal was done in the sequence 5 ml of 17% EDTA 5 ml of 5% NaOCl 5 ml of 2% chlorhexidine (CHX).

GROUP II (70% isopropyl alcohol): The irrigation of the canal was done with 5 ml of 17% EDTA 5 ml of 5% NaOCl 5 ml of 70% isopropyl alcohol as intermediate irrigant 5 ml of 2% CHX.

GROUP III (distilled water): The irrigation of the canal was done with 5 ml of 17% EDTA 5 ml of 5% NaOCl 5 ml of distilled water as intermediate irrigant 5 ml of 2% CHX.

GROUP IV (normal saline): The irrigation of the canal was done with 5 ml of 17% EDTA 5 ml of 5% NaOCl 5 ml of 0.9% normal saline as intermediate irrigant 5 ml of 2% CHX.

After preparation of the samples, the canals were dried immediately with absorbent points. The coronal and the apical ends were plugged with pellets of sterile cotton. A thin longitudinal slot was be made along the buccal and lingual aspect of the root using diamond discs, making sure to avoid perforation into the canal. Using a chisel and mallet, the roots were split longitudinally. One half of the split tooth was selected for the examination

of smear layer under SEM. Five samples from the remaining ten halves of each group was taken for ESEM-EDX examination. Photomicrographs were taken at the coronal (10-12 mm from apex), middle (6-7 mm from apex), and apical (1-2 mm from apex) thirds of each specimen.

SEM analysis

Scanning photomicrographs were obtained at 1000X magnification at 15 with JEOL JVM 5600LV Scanning Electron Microscope. Micrographs were taken of the representative areas at the coronal, middle and apical root thirds. The effect of the intermediate irrigants was subjected to detailed analysis.

Score	Criteria
1	Little or no smear layer; covering less than 25% of the specimen; tubules visible and patent
2	Little or moderate or patchy amount of smear layer ; covering between 25 and 50% of the specimen ; many tubules visible and patent
3	Moderate amount of scattered or aggregated smear layer; covering between 50 to 70% of the specimen ; minimal to no tubule visibility or patency
4	Heavy smear layering covering over 75% of the specimen ; no tubule visible or patent

Scoring system for precipitate analysis

Environmental Scanning Electron Microscope And Energy Dispersive X-Ray Microanalysis (Esem-Edx)

The ESEM-EDX does not require the samples to be sputter coated. This reduces the possibility of artefacts. At 500X magnification, representative areas for each third of the root canal were chosen to determine the microstructure of the surface layer and elemental compositions

Statistical Analysis

Data were analyzed using computer software, Statistical Package for Social Sciences (SPSS) version 10. Data are expressed in its as mean, median and standard deviation. Analysis of variance (One Way ANOVA) was performed as parametric test to compare different groups as well as different teeth sites. Kruskal Wallis test was employed to compare smear layer scores between different groups and teeth sites. For all statistical evaluations, a two-tailed probability of value, P< 0.05 was considered significant.

III. Results

Table 1 shows the comparison of scores of the precipitate at various root canal levels with use of the intermediate irrigants. There is highly statistically significant difference (P<0.001) in the scores between the irrigants in the coronal and middle third of the root canals. There is a significant difference in between the groups in the apical third (P<0.05).

Table 2 shows the scores for the precipitate comparing the different levels of the root canal in different groups. There is no statistically significant difference (P>0.05) between the three levels of the Group I. There is a highly significant difference (P<0.001) between the three levels of the teeth of Group II. There is a significant difference (P<0.05) between the levels of the teeth of Group III (Distilled water) and Group IV (Normal saline).

Five samples from each group taken for energy dispersive X-ray spectroscopic analysis under environmental scanning electron microscope (ESEM-EDX) for elemental composition of the precipitate. The EDX confirmed presence of chlorine in the positive group. There is a highly significant difference (P<0.001) in the amount of chlorine in between the groups. The Group I was having significantly high level of chlorine compared to other groups. The Group II contain less amount chlorine compared to other groups.

Table 3 shows the approximate percentage of weight of the elements present on the wall of the root canal dentin.

Table 4 shows comparison of the chlorine content in at various root canal levels with use of the intermediate irrigants. There is a highly statistically difference (P<0.001) in between the groups in different levels of the root canal.

Table1

Root canal level	Group	Mean	Median	± SD	Kruskal Wallis H value	P value
Coronal	Group I	2.60	2.50	0.70	18.315	< 0.001
	Group II	1.20	1.00	0.42		
	Group III	1.70	2.00	0.67		
	Group IV	2.30	2.00	0.67		
Middle	Group I	2.30	2.00	0.48	21.845	< 0.001
	Group II	1.40	1.00	0.52		
	Group III	1.60	2.00	0.52		
	Group IV	2.20	2.50	0.88		
Apical	Group I	2.80	3.00	0.63	11.433	< 0.05
	Group II	2.50	2.50	0.53		
	Group III	2.40	2.00	0.52		
	Group IV	2.70	3.00	0.70		

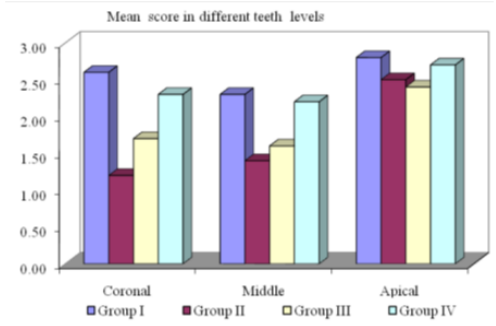

FIG 1

Table 2

Irrigant	Root canal level	Mean	Median	± SD	Kruskal Wallis H value	P value
Group I	Coronal	2.60	2.50	0.70	3.291	> 0.05
	Middle	2.30	2.00	0.48		
	Apical	2.80	3.00	0.63		
Group II	Coronal	1.20	1.00	0.42	17.018	< 0.001
	Middle	1.40	1.00	0.52		
	Apical	2.50	2.50	0.53		
Group III	Coronal	1.70	2.00	0.67	8.569	< 0.05
	Middle	1.60	2.00	0.52		
	Apical	2.40	2.00	0.52		
Group IV	Coronal	2.30	2.00	0.67	8.409	< 0.05
	Middle	2.20	2.50	0.88		
	Apical	2.70	3.00	0.70		

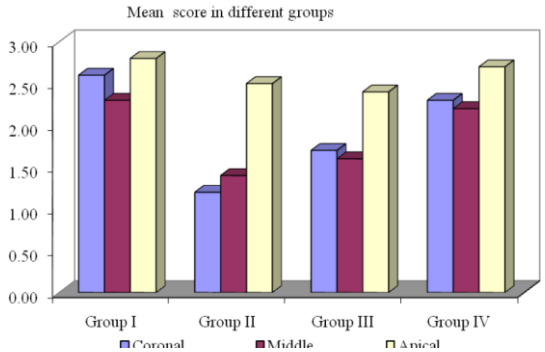

FIG 2

Table 3

Weight %	Group	Mean	± SD	F value	P value
Carbon	Group I	30.64	4.56	0.913	> 0.05
	Group II	29.84	6.84		
	Group III	30.28	3.99		
	Group IV	33.09	7.48		
Oxygen	Group I	38.33	4.83	2.109	> 0.05
	Group II	41.23	6.48		
	Group III	37.87	3.04		
	Group IV	36.70	5.56		
Sodium	Group I	2.13	0.98	1.513	> 0.05
	Group II	2.00	0.49		
	Group III	2.48	0.36		
	Group IV	2.20	0.57		
Magnesium	Group I	1.34	0.47	1.699	> 0.05
	Group II	1.32	0.22		
	Group III	1.25	0.33		
	Group IV	1.08	0.36		
Phosphorous	Group I	9.67	0.48	7.187	< 0.01
	Group II	8.98	1.13		
	Group III	10.36	0.73		
	Group IV	9.91	0.86		
Chlorine	Group I	2.55	1.04	12.661	< 0.001
	Group II	1.06	0.58		
	Group III	1.42	0.34		
	Group IV	1.79	0.61		
Calcium	Group I	15.37	1.36	0.234	> 0.05
	Group II	15.38	1.72		
	Group III	15.62	0.63		
	Group IV	15.10	2.54		

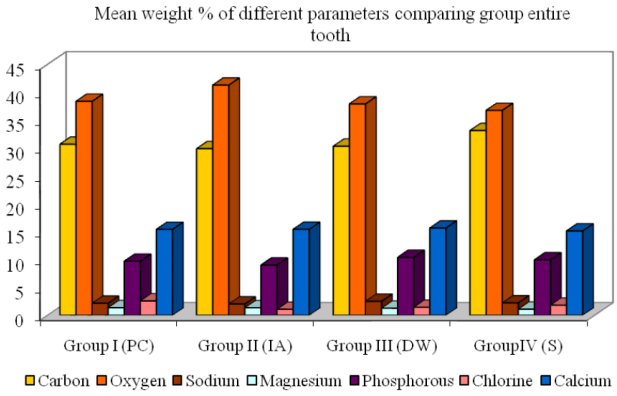

FIG 3

Table 4

Root canal level	Group	Mean	± SD	F value	P value
Coronal	Group I (PC)	2.37	0.41	15.509	< 0.001
	Group II (IA)	1.64	0.20		
	Group III (DW)	1.58	0.14		
	Group IV (S)	2.54	0.29		
Middle	Group I (PC)	3.70	0.82	42.458	< 0.001
	Group II (IA)	1.15	0.14		
	Group III (DW)	1.05	0.21		
	Group IV (S)	1.30	0.14		
Apical	Group I (PC)	1.57	0.25	18.054	< 0.001
	Group II (IA)	0.38	0.34		
	Group III (DW)	1.63	0.30		
	Group IV (S)	1.54	0.36		

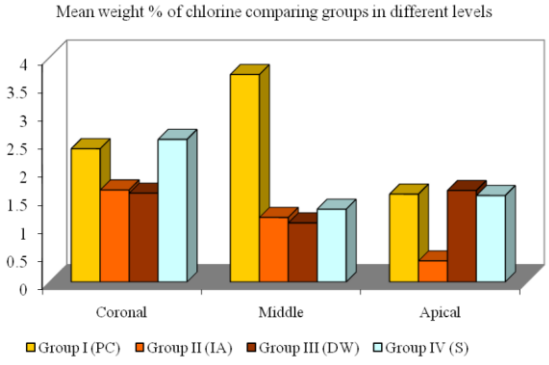

FIG 4

IV. Discussion

Irrigants have an important role in endodontic treatment. They remove the pulp tissue, dentin chips and microbes – necrotic and living. Most solutions have antimicrobial properties that effectively kill residual microbes in the canal. However no irrigant can completely eliminate all organic and inorganic matter at the same time imparts a substantive residual antimicrobial property to root canal dentin. The commonly used antibacterial agents such as sodium hypochlorite, EDTA, chlorhexidine gluconate used for the root canal preparation and final flushes to minimize the necrotic tissue and bacteria. The most common irrigant used in a concentration range 0.5% to 5.25%. It is an effective organic solvent and antimicrobial agent. In low concentrations, it is ineffective against specific microorganisms. The disadvantages of NaOCl are in high concentrations it has low biocompatibility & formation of a smear layer adhered to the dentinal wall.

CHX is a cationic bisbiguanide. Its Broad-spectrum antimicrobial action that acts by absorbing onto microbial cell walls or disrupting them, causing leakage of intracellular components. In vitro studies have shown CHX to exhibit substantivity in the root canal for some time after being used as an endodontic irrigant solution (**12, 13**). However it has got no tissue dissolution ability. To combine the desirable effect of irrigants, they have been used in combination and in sequence. Hence, studies on the possible effects of the interaction between them have gained importance. **Kuruvilla *et al*** (**4**) suggested that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination was better than that of either component. A suggested clinical protocol by **Zehnder M** (**5**) for treating the dentin before root filling consists of irrigation with sodium hypochlorite to dissolve the organic component, irrigation with EDTA to eliminate the smear layer and irrigation with chlorhexidine to impart substantive antibacterial activity.

Basrani *et al* (**6**) found a brown colour precipitate when chlorhexidine was mixed with sodium hypochlorite in the test tube and parachloraniline (PCA) was found when this precipitate subjected to gas chromatography (**14, 15**). This precipitate is an insoluble neutral salt formed by the acid base reaction between NaOCl and CHX. Para-chloroaniline is the main product of the interaction of NaOCl and CHX, with molecular formula NaC6H4Cl as analysed by mass spectrometry. When mixed with NaOCl, CHX molecules become hydrolysed into smaller fragments, each forming a by product. The first bond to be broken in this reaction is between carbon and nitrogen because of low bond dissociation energy between two atoms.

Relevance of para-chloroaniline

Toxicologic studies in animals have shown that the hematopoietic system is the major target for PCA toxicity. The primary toxic effect is the formation of methemogloblin and the development of haemolytic anaemia. Studies have shown that this occurs after 90 days of exposure to PCA. A carcinogenic effect by an increase in sarcoma formation in the spleen has also been reported (**8**). Another reported adverse effect of PCA was a delay in egg hatching and an increased rate of abnormal development and pigmentation in the hatchlings of zebra fish (**18**). Adverse effects have also been shown to occur in the embryos of Daphnia magna (**19**). It has been shown that aniline derivatives cause ultrastructural alterations in the livers of rainbow trout and zebra fish 38 and hemosiderin deposition in the spleen as well as epithelioma formation in the gas bladder of Japanese killifish 37. In humans, there have been reports of severe methaemoglobinaemia in neonates exposed to PCA as a result of the breakdown of CHX to PCA by the humidifier heater in neonatal incubators (**20**).

Matheus Souza *et al* (**16**) found there was visible colour change when 2% chlorhexidine gluconate with 5.25% of sodium hypochlorite and concluded that if the combined use of 2% chlorhexidine with 5.25% NaOCl is chosen, the latter should be introduced first in the root canal and be completely removed by an irrigating intermediary, such as physiological saline solution, before the placement of any formulation of chlorhexidine.

Kanchanakaew C *et al* (**17**) observed colour change when canal irrigated with 2.5% NaOCl followed by 2% CHX and formation of dark brown precipitate and concluded that this precipitate might have interfered with the root canal filling by forming gaps between Epiphany sealer and dentin.

Relevance of the study

Scientific work is lacking on the various characteristics and effects of this precipitate. Its effect of on antibacterial properties is not known. As this precipitate is sticky in nature and cannot be flushed out from the root canal wall it can affect the patency of the dentinal tubule and the sealability. Use of intermediate irrigants may be an effective method to avoid or minimize its formation.

The present *in vitro* study used three intermediate irrigants – isopropyl alcohol, distilled water and normal saline to remove the residual sodium hypochlorite from the canal before irrigating with chlorhexidine. As there was eno visible precipitate or colour change in the other groups, the samples studied under the scanning electron microscope (SEM) for the presence or absence of the precipitate, to assign scores based on the Gutmann scoring system (**11**) and elemental analysis of the precipitate done using ESEM-EDX. The selection of the parameters for precipitate covering the dentinal tubule for the present study can be explained on the basis of

study conducted by **Akisue *et al*** (**21**) who showed that the combination of 1% NaOCl and 2% CHX solutions results in the formation of a flocculate precipitate that acts as a chemical smear layer.

SEM results

SEM micrographs of Group I showed the root canal lumen to be coated extensively with the precipitate and a subjective change in the morphology of the root canal surface. SEM examination of all the groups with the intermediate irrigants – isopropyl alcohol, distilled water and saline - revealed substantial reduction in the area covered by the precipitate. Analysis using one way ANOVA showed a statistically significant difference in the precipitate formation and patency of the dentinal tubules between the positive control group and other groups. There was no statistical difference in the scores in the coronal, middle and apical third of the positive control group. The mean score of the group I was high in the all three root canal levels. That indicates the nearly uniform formation of precipitate all over the canal. Group IV using normal saline shows least efficiency in removal of the precipitate; hence it is possible that it is to be avoided as intermediate irrigant between NaOCl and CHX Group II using 70% isopropyl alcohol removes more residual sodium hypochlorite and leaves least amount of precipitate compared to distilled water and normal saline respectively in coronal, middle third of the teeth. This is in accordance with a previous study by **Krishnamurthy** and **Sudhakaran** (**10**).
In apical third distilled water removed more sodium hypochlorite and leaves least amount of precipitate followed by isopropyl alcohol and normal saline.

In order to find out the chemical nature of the precipitate EDX was done with ESEM. In ESEM the samples does not required gold coating to avoid the artefacts. Previous studies evaluated the chemical nature of the precipitate in test tubes only. Present study examined the surface of the root canal as well as the amount of the chlorine in the root canal. Along with the normal constituents of tooth like calcium, phosphorous, carbon, oxygen, sodium and magnesium, chlorine also found in significant amount which is not a normal constituent. **Krishnnamurthy** and **Sudhakaran** confirmed this finding in their study.

The amount of chlorine present recorded in wt% and subjected to analysis. From the statistical analysis it was noted that there is a significant difference between the chlorine values in all four groups. There was also significant difference between the groups in the coronal third. The coronal third of the teeth left less chlorine when compared to 70% isopropyl alcohol. The chlorine content is more throughout the teeth when chlorhexidine irrigated with sodium hypochlorite without any intermediate irrigant. In coronal middle third chlorine content was less when distilled water used as intermediate irrigant (1.58±0.14, 1.15±0.14) which is comparable to the chlorine values after irrigating with isopropyl alcohol (1.64±0.20, 1.15±0.14) for coronal and middle third respectively. When comparing the different intermediate irrigant with respect to entire tooth 70% isopropyl alcohol leaves least amount of chlorine in the root canal.

This indicates that the precipitate that occluded the dentinal tubule, which contains chlorine in significant amount. In apical third of all groups the scoring for precipitate significantly more as there was reduced patency of dentinal tubule compared to the coronal and middle third. But according to the elemental analysis of the same shows less amount of chlorine in the apical third. This might be due to the uninstrumentation of the apical third as this part is difficult to irrigate. This result at the apex might have been different if irrigation was supplemented with ultrasonic, sonic or negative pressure irrigation.

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 53. Alcohol is also volatile so it aids in drying of the canals. However, using isopropyl alcohol as an endodontic irrigant is not yet well established. The groups distilled water and Normal saline revealed the precipitate layer with reduced amount of chlorine compared with the positive control group. This could be due to the dilution of NaOCl caused by distilled water and saline, respectively.

It can be concluded from the present study that 70% isopropyl alcohol effectively removes residual sodium hypochlorite from the root canal. When there was comparison in between groups at different levels the mean scores of 70% isopropyl alcohol can be comparable to the mean score of distilled water group. Normal saline removes least residual sodium hypochlorite from the root canal. This contradicts the findings of **Krishnnamurthy** and **Sudhakaran (**10) where normal saline removed more NaOCl from the canal compared to distilled water.

V. Conclusion

The in vitro set up used in the present study might not mimic the conditions existing in the oral cavity and this would result in variation of the results in an in vivo study. Hence further studies required to quantify the precipitate formed using more precise tools and techniques, and assess the effect of the precipitate on periapical tissue.

The result showed the significant reduction of the mean score (P <0.001) and reduced amount of chlorine (P< 0.001) when isopropyl alcohol used as intermediate irrigant in between sodium hypochlorite and chlorhexidine. Within limitations of this study, the following conclusions were drawn:
i) Isopropyl alcohol removed more residual sodium hypochlorite from the root canal leaving minimum precipitate occluding the dentinal tubules. ii) Elemental analysis of the precipitate showed presence of chlorine. iii) In coronal and middle third contains more chlorine than apical thirds.

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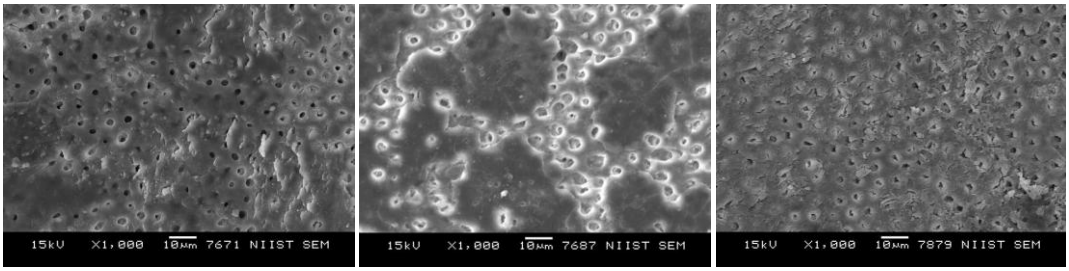
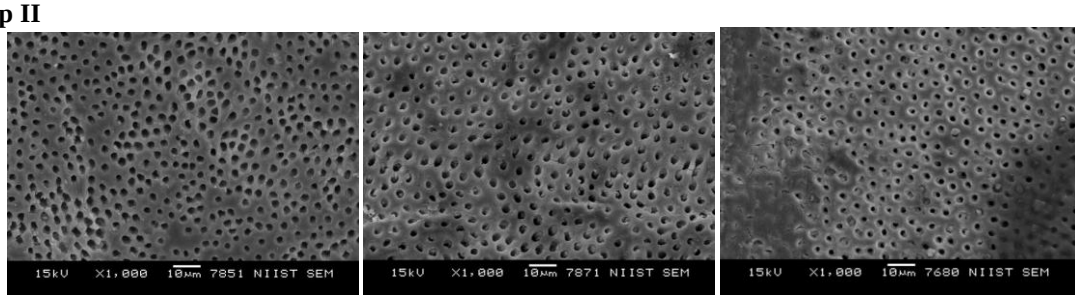
SEM Photographs (Group I, Group II, Group III, Group IV)

Coronal **Middle** **Apical**

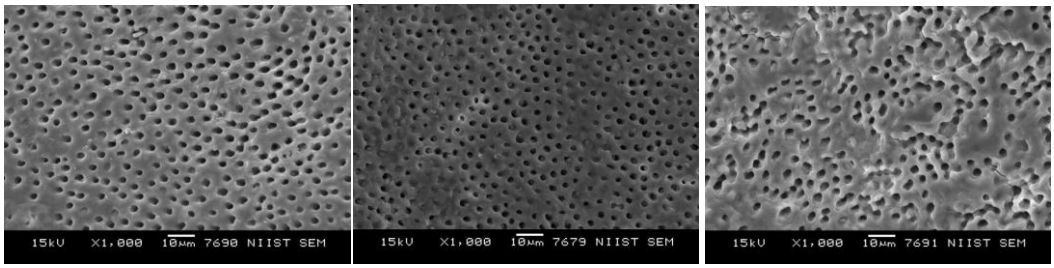
Group I



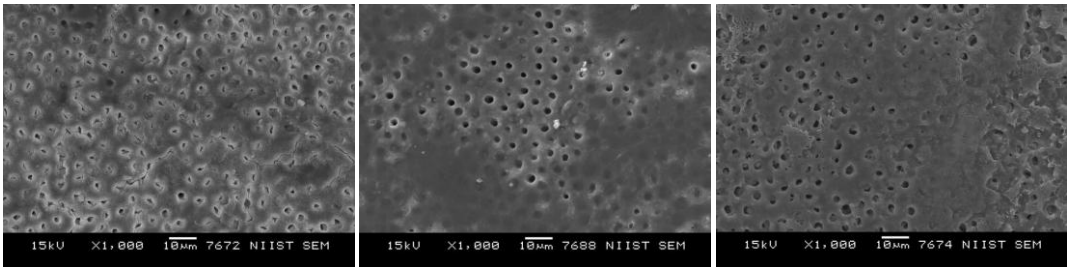
Group II



Group III



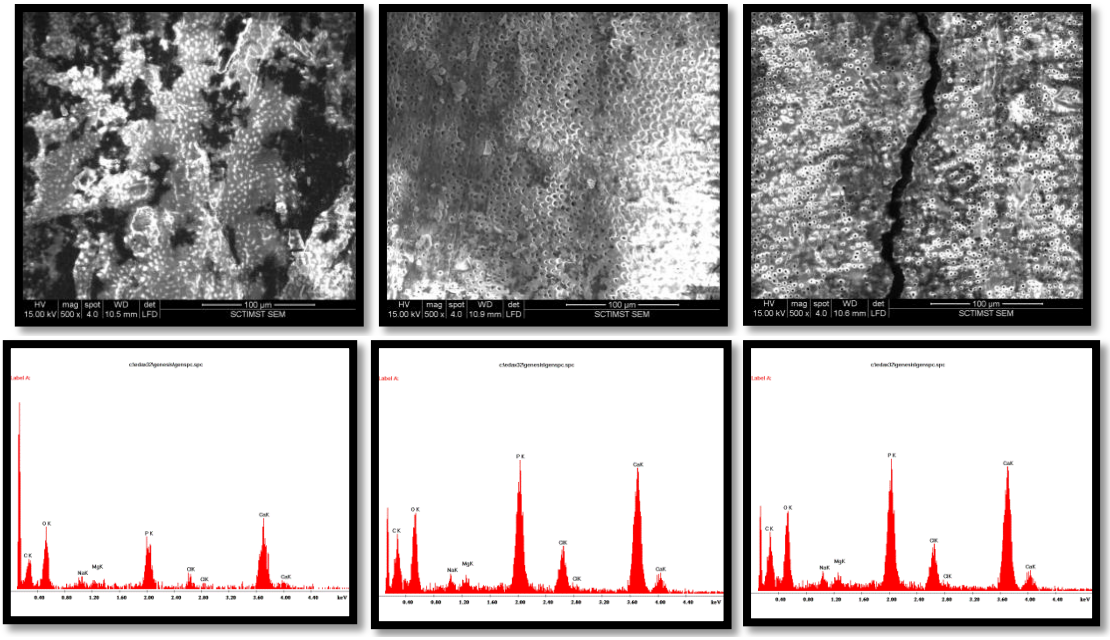
Group IV



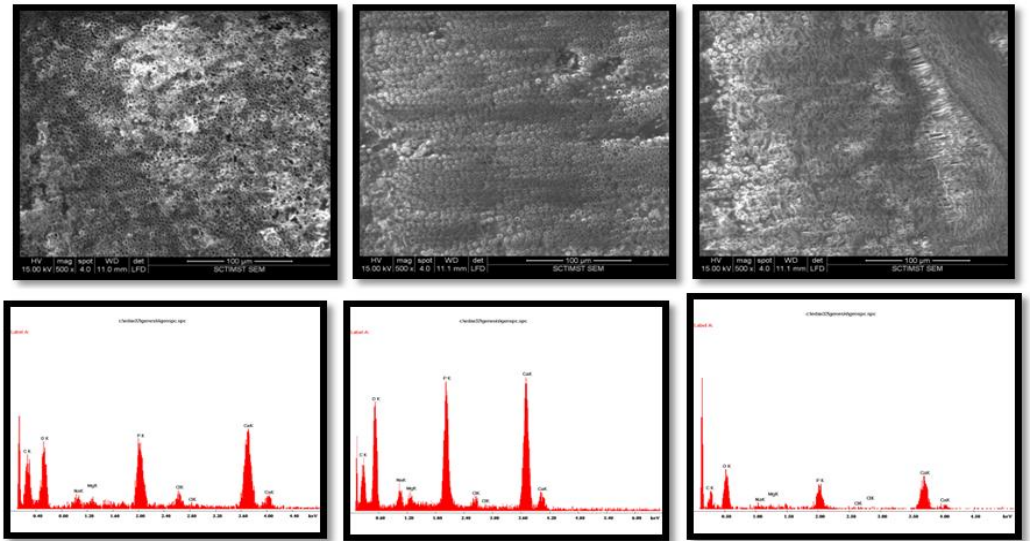
ESEM-EDX images

Group I

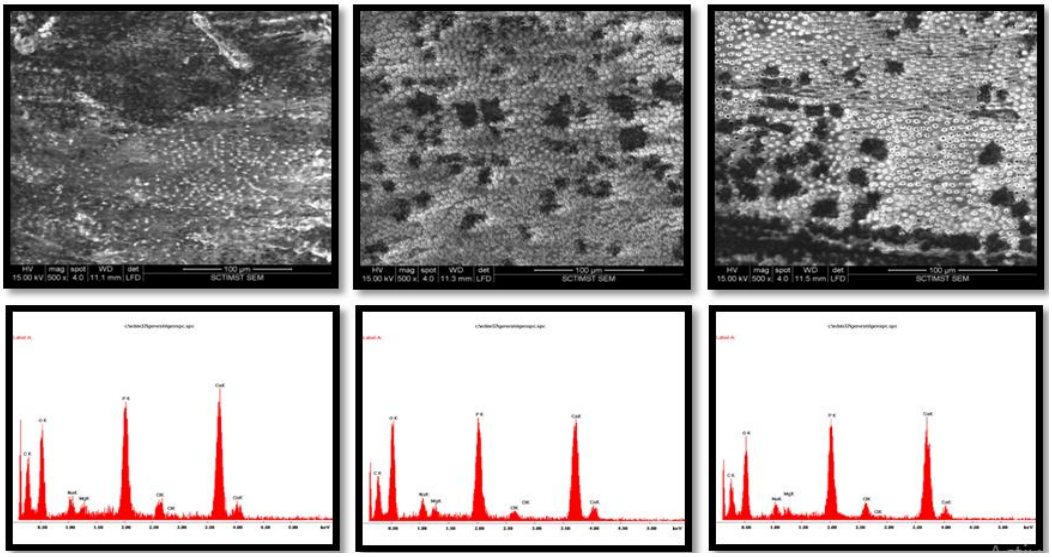
Coronal **Middle** **Apical**



Group II



Group III



Group IV