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EVALUATION OF THE EFFECTIVENESS OF SODIUM HYPO CHLORITE AND EDTA ON REMOVAL OF THE MIXTURE OF CALCIUM HYDROXIDE, CHLORHEXIDINE AND LYCOPENE FROM THE ROOT CANAL: A SEM STUDY

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ABSTRACT: Introduction: The goal of this study was to evaluate the effectiveness of different irrigation solutions on the removal of calcium hydroxide, Chlorehexidine and Lycopene mixture from the root canals by NaOCl, EDTA alone or combination of NaOCl and EDTA. **Materials and Methods:** Thirty eight single rooted teeth were taken. Root canals were prepared by standardized instrumentation up to Master Apical File 45 and irrigated with 2.5% NaOCl after each instrument, followed by 10 mL 17% EDTA as a final rinse. The canals were filled with Calcium hydroxide, Chlorehexidine and Lycopene mixture and stored for 7 days. Specimens were randomly divided into 3 experimental groups (n = 10) according to irrigation protocols. Positive and negative control groups (n = 4) were included. Group 1: the root canals were irrigated with 5 mL 2.5%Naocl, group 2: the root canals were irrigated with 5 mL 17% EDTA, Group 3: the root canals were irrigated with 2.5 ml Naocl and 2.5 ml EDTA. The specimens were longitudinally sectioned and evaluated with scanning electron microscope and scored by 2 examiners. .**Results:** The kappa test showed that there was substantial agreement between the 2 examiners values for scoring the calcium hydroxide dressing in the coronal, middle, and apical thirds in each group. All irrigation regimens left debris on the canal walls. Among all the groups, NaOcl and EDTA combination is efficacious in removal of calcium hydroxide dressing from the root canal followed by EDTA, NaOCl in coronal, middle and apical thirds.($P < 0.05$)**Conclusion:** According to the findings of the present study, combination of NaOCl and EDTA could be used effectively for the removal of Calcium hydroxide, Chlorehexidine and Lycopene mixture from the root canals.

KEYWORDS: Calcium hydroxide, Chlorehexidine, Lycopene, Scanning Electron Microscope.

INTRODUCTION

The major objective of the root canal therapy is to make the root canal system devoid of micro organisms which can be achieved by thorough cleaning and shaping accompanied by debridement and disinfection protocols. Intra canal medication is often advantageous between treatment sessions to reduce the microbial load especially in the lateral, accessory canals, isthmus and apical deltas.[1]

Calcium hydroxide(CH) is widely accepted intra canal medicament as it posseses various properties like anti bacterial activity, anti exudative action, bio compatibility, ability to induce mineralized tissues, ability to dissolve necrotic tissue and ability to inactivate bacterial endotoxin. Its antimicrobial activity is attributed to its alkaline PH.

Chlorhexidine (CHX) is another intra canal medicament which has broad spectrum antibacterial activity, biocompatibility and substantivity. It has been proved that anti microbial efficacy of calcium hydroxide is increased when it is used along with chlorhexidine due to synergistic effect. But this combination might produce free radicals which are detrimental to host tissues.[2]

Antioxidants are necessary to counteract these Reactive Oxygen Species, thereby preventing the harmful effects. Lycopene (LP), a natural antioxidant found in tomato based products, red chillies and watermelons. It has anti bacterial and anti fungal properties. Studies have proven that addition of lycopene to CH, CHX increases the anti microbial efficacy.[1]

However, complete removal of the calcium hydroxide dressing is mandatory before obturation to achieve sealer penetration in to the root canal walls. It has been reported that residual Ca(OH)2 on the root canal walls influences the dentinal bond strength and adversely affect the desirable properties of the sealer.[3]

Several methods have been employed for removal of the calcium hydroxide dressing from the root canal viz, ultrasonics, sonics canal brush and using irrigating agents etc. The most commonly used method for removal of calcium hydroxide is instrumentation along with NaOCl and EDTA.[4]

The aim of the present study was to evaluate the efficacy of irrigants NaOCl, EDTA and combination of NaOCl and EDTA in the removal of Calcium hydroxide, Chlorhexidine and Lycopene mixture from the root canals.

Materials and Methods

Thirty eight freshly extracted human single rooted teeth with straight roots and single canals were used in this study. Scaling was done with ultrasonic instruments, washed with distilled water, and immersed in 10% formalin solution until use. The criteria for tooth selection includes: a single root canal, no visible root caries, fractures, or cracks, no signs of internal or external resorption or calcification and a completely formed apex.

The crowns of the teeth were decoronated using low speed diamond disc and the roots were adjusted to have a length of 12mm. The working length was established 1 mm short of the apical foramen. The roots were subjected to standardized root canal instrumentation (step-back technique) up to #45 MAF and were irrigated with 2.5% NaOCl after each instrument, followed by 10 mL 17% EDTA as a final rinse. Cleaning and shaping of all teeth were done by the same operator. All root canals were dried with paper points. 1.5gr of Ca(OH)2 powder, 1ml of CHX and 1ml of 5% Lycopene were mixed, and the canals were filled with CH, CHX and Lycopene mixture. The access cavities were sealed with a temporary filling material. They were then stored at 37°C and 100% relative humidity for 7 days to simulate the clinical situation.

After 7 days, the teeth were reopened and the Ca(OH)2 medication was initially removed using 10 mL saline solution and reinstrumentation with MAF using a circumferential filing action. The patency of the apical foramen was obtained by introducing a 10 K-file until it was visible at the apical foramen several times during the procedure. The specimens were randomly divided into the following 4 experimental groups (n = 10) according to the final irrigating solution used for the removal of Ca(OH)2 residues:

Group 1: The root canals were irrigated with 5 mL 2.5%Naocl.

Group 2: The root canals were irrigated with 5 mL 17% EDTA

Group 3: The root canals were irrigated with 2.5 ml Naocl and 2.5 ml EDTA

Eight of the specimens were divided into negative and positive control groups. In the negative control group (n = 4), teeth were not filled with Ca(OH)2 dressing. In the positive control group (n = 4), teeth were filled with Ca(OH)2 dressing, but no removal procedure was attempted. The volume of irrigant and application time were standardized for all groups. Longitudinal grooves were then prepared on the buccal and lingual surfaces of each root with the use of a diamond disk at a slow speed without penetrating the canal. The roots were then split into 2 halves with a chisel and stored in deionized water at 37°C until Scanning electron microscope (SEM) analysis.

The samples were then mounted on metallic stubs, gold sputtered and examined under a Scanning electron microscope. The selected dentinal surfaces of the cervical, middle and apical thirds (9, 6, and 3 mm from the apex, respectively), equidistant from the lateral walls were examined by SEM at 1,000x magnification. Two calibrated examiners analyzed the cleanliness of dentinal walls with the use of a graded scale independently in a blind manner. A scoring system that was used in the study of Kuga et al was defined to assess the quantity of the remnants on the canal walls.[5] The scores used were as follows **(Fig. 1, Fig. 2, Fig. 3 and Fig. 4).**

score 0: Absence of residues

score 1: small amount of residues (up to 20% of the surface covered)

score 2: moderate amount of residues (20%–60% of the surface covered)

score 3: large amount of residues (more than 60% of the surface covered).

The interexaminer reliability was verified using the kappa test. Statistical analysis was performed using the Kruskal-Wallis test at the 5% level of significance.

Results

The kappa test showed that there was substantial agreement between the 2 examiners values for scoring the calcium hydroxide dressing in the coronal, middle, and apical thirds in each group. All irrigation regimens left debris on the canal walls. All the groups were significantly different from the positive and negative control groups ($P < 0.05$). Kruskall- wallis test showed that there was statistically significant difference between all groups (**Fig. 6 and Fig. 7).** Among all the groups, NaOCl and EDTA combination is efficacious in removal of calcium hydroxide dressing from the root canal followed by EDTA, NaOCl in coronal, middle and apical thirds.

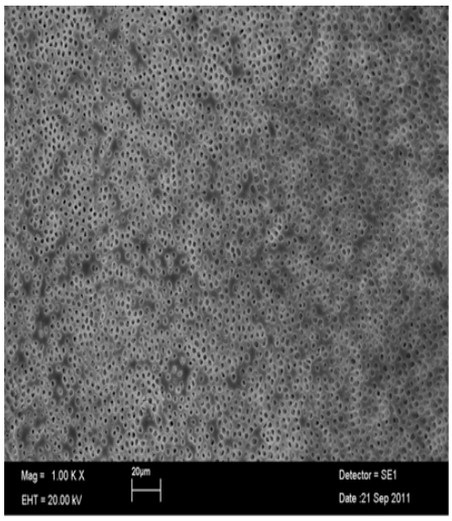

Fig. 1: Score 0

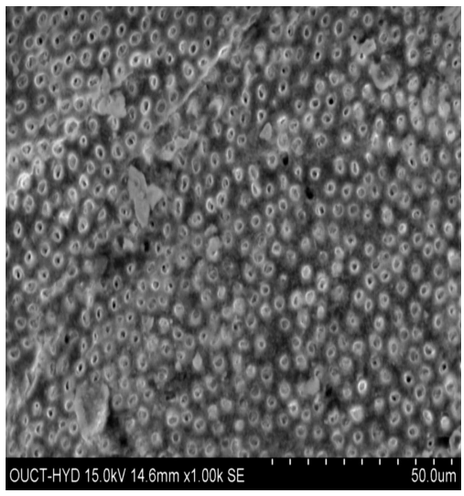

Fig. 2: Score 1

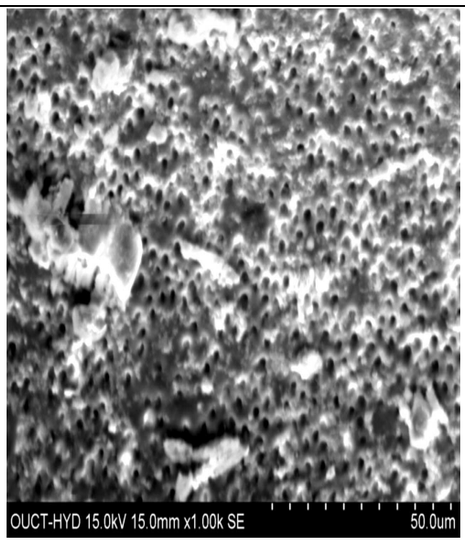

Fig. 3: Score 2

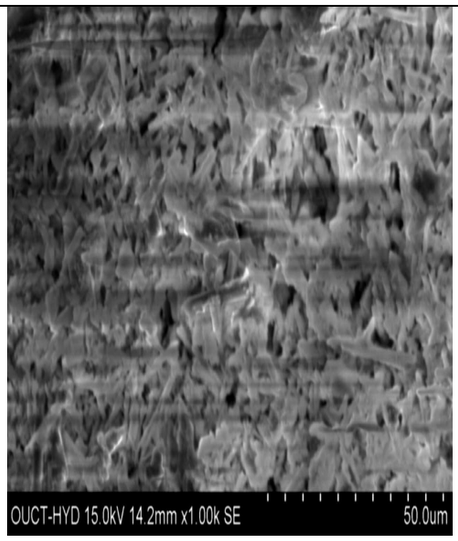

Fig. 4: Score 3

Scanning Electron Microscopic images of scores

Discussion

Because Endodontic infections are polymicrobial, many microorganisms take part in constitution of biofilms and become more resistant when they are together. Consequently, medicaments can be combined to improve their activity against polymicrobial infections. Using this idea, different vehicles have been added to CH to enhance its properties. In addition, the type of vehicle may have significant effect on the antimicrobial performance of CH.[6]

Even though CH has good antimicrobial properties, studies have reported that it was unable to eliminate

E.faecalis. Tuck et al in an in vitro study reported that CHX-CH combination showed more antimicrobial efficacy against E.faecalis. In addition, the antimicrobial activity of CH against C.albicans was also increased to some extent when manipulated with CHX. In vitro studies have demonstrated that CHX might induce Reactive Oxygen Species (ROS) in an alkaline environment. It has been reported that ROS, in addition to having antimicrobial efficacy also pocess detrimental effects on host tissues. Anti oxidants like Lycopene is necessary to counteract the effect of ROS. It was hypothesized that addition of natural

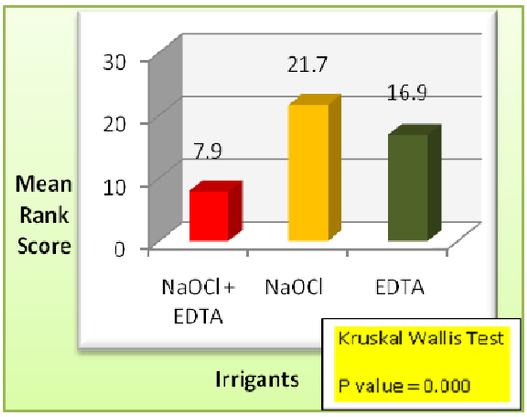

Fig. 5: Comparison of three irrigants at coronal part of roots

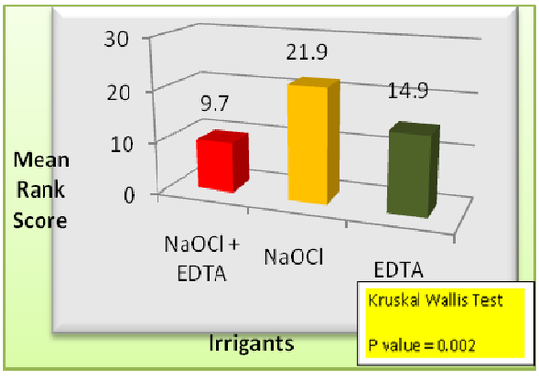

Fig. 6: Comparison of three irrigants at Middle part of roots

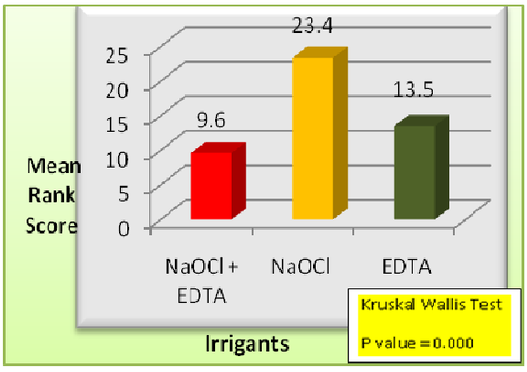

Fig. 7: Comparison of three irrigants at apical part of roots

antioxidants like lycopene to the CH-CHX mixture increases the antibacterial efficacy of the CH-CHX mixture and also decreases damage to the host tissue by lowering ROS formation.[2]

However, removal of the calcium hydroxide dressing is mandatory prior to obturation of the root canal to enhance the success of endodontic therapy. Remnants of Ca(OH)2 influences the setting mechanism of various types of root canal sealers .[7,8] Setting reaction of the sealer occurs more rapidly that may prevent the placement of gutta-percha . Moreover reaction with Ca(OH)2 remaining on the canal wall can cause considerable changes to the physical properties and the sealing ability of various sealers. It has been reported that a layer of the ZOE sealer in contact with the residual calcium hydroxide is rapidly set due to a reaction of eugenol with calcium hydroxide. spectroscopic characterization of the interaction mechanism reveals that ZOE cements in contact with calcium hydroxide are completely disorganized to a certain depth as the zinc oxide-eugenol reaction is compensated for the preferential interaction of calcium hydroxide with eugenol. The set sealer cement exhibited the granular appearance, which implies the destruction of the sealer layer in contact with calcium hydroxide. The extent of reaction further depends on the surface area of the canal walls covered by residual calcium hydroxide. The presence of such remnants at critical areas, like the apical region, may adversely affect the clinical performance of the sealer and possibly the long-term prognosis of root canal therapy.[9]

In the present study irrigants like NaOCl, EDTA are used along with Master Apical File to remove Calcium hydroxide dressing form the root canal. None of the irrigants used in this study removed the CH completely from the root canals. This is in agreement with results of the previous studies, which showed the presence of CH debris on the root canal walls, regardless of the removal technique.[10,11,12] The present study showed the worst results for calcium hydroxide removal in the NaOCl group, with significant differences compared with all of the other groups. Literature shows that NaOCl alone is inadequate for the removal of Ca(OH)2 from the root canals because of its limited ability to dissolve inorganic materials. The conclusion reached by many authors is that the use of NaOCl during or after instrumentation produces superficially clean canal walls with the smear layer remaining.

The calcium present in the hydroxyapatite crystals constitutes one of the important inorganic elements in dentine. The chelating agents have been used in Dentistry for their direct action on calcium ions. One of the most preferred chelating agents is EDTA .[13] It is used in combination with NaOCl for the removal of the smear layer. It also demineralizes dentine, opens the dentinal tubules, and facilitates an easy preparation. In the present study irrigation only with 17% EDTA performed significantly gave better results than those of 2.5% NaOCl. This can be explained by the ability of EDTA to dissolve inorganic substances such as calcium. Additionally, treatment with EDTA may neutralize calcium hydroxide residues. [14,15]

Compared with the NaOCl and EDTA only groups, the combined use of NaOCl and EDTA with MAF improved the removal efficiency. This is in agreement with the results of a previous study, which showed the importance of recapitulation using MAF to improve the removal of CH. Probably the EDTA treatment may chelate residual calcium hydroxide, which then is easily removed by NaOCl irrigation. How ever, there is no evidence that EDTA along with NaOCl can completely remove CH from the root canal. This difference could be a result of the variable dimensions of the root canal system and their subsequent preparation size and taper, which affects the irrigant penetration.

Regardless of the irrigants used in the present study, coronal and middle third regions are significantly cleaner than the apical third region which may be attributed to the presence of complex anatomical variations including additional canals, anastomoses, ramifications and lateral canals in the apical third and accessibility of irrigants is comparatively higher in coronal and middle thirds.[16]

CONCLUSION

Within the limitations of the present study, it can be concluded that combination of Naocl and EDTA is efficacious in the removal of Calcium hydroxide, Chlorehexidine and Lycopene mixture from the root canals. It was also observed that apical third of the root canals showed more remnants compared to coronal and middle thirds.

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