10.5005/jp-journals-10024-2019

ORIGINAL RESEARCH

Evaluation of 4% Sodium Hypochlorite in eliminating *Enterococcus faecalis* from the Root Canal when Used with Three Irrigation Methods: An *in vitro* Study

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ABSTRACT

Introduction: Endodontic treatment removes all pathogens, such as *Enterococcus faecalis* from pulp and root canals. The aim of this study is to assess the usefulness of sodium hypochlorite (NaOCl) in removing *E. faecalis* from the root canal used with three different irrigation methods.

Materials and methods: This study was conducted on freshly extracted maxillary incisors. After biomechanical preparation, root canals were injected with *E. faecalis*. Three groups were made which contained 30 teeth in each group; 2 mL of NaOCl solution was used for irrigation followed by agitation with K-files in group I; 2 mL of NaOCl solution was used for irrigation and ultrasonic agitation was done in group II. In group III, an alternate irrigation with NaOCl and 3% hydrogen peroxide was done. The fourth group (control) was irrigated with sterile saline solution. *E. faecalis* bacteria were sampled to the root canals with paper points and were transferred to tubes that contained 5 mL of brain heart infusion broth. Tubes were incubated and the presence of broth turbidity was suggestive of bacteria remaining in the root canal.

Results: All three groups showed no statistically significant difference. However, difference existed between experimental groups and control groups.

Conclusion: The author concluded that all three methods of application of NaOCl were effective in disinfecting the root canal than the saline solution.

Clinical significance: No single irrigant has 100% efficiency. Thus by this study, a best irrigating solution with maximum properties can be established.

Keywords: Endodontics, *Enterococcus faecalis*, Irrigation, Sodium hypochlorite.

How to cite this article: Priyank H, Pandey V, Bagul A, Majety KK, Verma P, Choudhury BK. Evaluation of 4% Sodium Hypochlorite in eliminating *Enterococcus faecalis* from the Root Canal when Used with Three Irrigation Methods: An *in vitro* Study. J Contemp Dent Pract 2017;18(3):214-217.

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

The purpose of endodontics is to remove all pathogens from the dentinal tubules and root canal system. It can be obtained by cleaning the root canals by removing contaminated dentin followed by disinfecting the canals using sodium hypochlorite (NaOCl), and using various irrigating solutions.[1] Walker[2] in 1936, introduced NaOCl as an irrigating solution. This solution has the tendency to dissolve the organic tissue and has antimicrobial activity. The process of mechanical cleaning of canals is not a simple procedure. Complexity of root canals in respect to its anatomy, sclerosis of dentinal tubules, and the smear layer poses great difficulty.[3] The presence of *Enterococcus faecalis* and *Candida* species provides resistance to disinfecting agents and leads to failure of endodontic therapy. The penetration power of NaOCl in the dentinal tubules has been reported to be 300 µm, while *E. faecalis* can penetrate the dentinal tubules up to 800 to 1,000 µm deep. The high surface tension of NaOCl affects the tubular penetration and its antibacterial ability.[4]

Thus for successful endodontic therapy, removal of smear layer and disinfection are very important steps. Different protocols have been developed for enhancing the antimicrobial efficacy of disinfecting solutions.[5] Apart from NaOCl, adjuvant irrigation of ethylenediaminetetraacetic acid has also been introduced. Sodium hypochlorite and hydrogen peroxide (H_2O_2) as alternate therapy have been employed as irrigating solutions.[6] This combination causes effervescence, which are useful in debridement and causes disinfection of the root canal.[7] Martin[8] in his study suggested NaOCl irrigation and ultrasonic instrumentation of canals. This combination permits heating of the irrigating substance, ultrasonic waves accelerate chemical reactions, causes exchange of substances in the canal, and removes dentin debris.

The purpose of the study was to compare the effectiveness of NaOCl in eliminating *E. faecalis* from the root canal used with three different irrigation methods.

MATERIALS AND METHODS

This study was conducted at the Department of Endodontics from January 2015 to December 2015. This was an *in vitro* study conducted on freshly extracted 120 maxillary incisors. In all teeth, access opening was made and the biomechanical preparation was performed using K-type files up to size 45, which extended 1 mm beyond the apical foramen and flaring was done. During the procedure, irrigation was done with normal water. Epoxy resin was used for sealing of apical foramen. Plaster blocks were used for mounting and sterilizing at temperature of 121°C for 20 minutes. To contaminate the root canals, pure culture of *E. faecalis* grown in brain heart infusion (BHI) broth was used. The preparation for suspension of *E. faecalis* cells was done in BHI. The plaster blocks were opened in a laminar air flow cabinet; 10 mL of *E. faecalis* suspension was inoculated to contaminate the root canals using sterile 1 mL tuberculin syringes. The stainless steel boxes containing blocks were incubated at 37°C for 24 hours following which three different groups of contaminated root canals were made (Table 1) depending on the irrigating solutions used:

- *Group I*: This group contained 30 teeth in which 2 mL of NaOCl solution was used for irrigation followed by agitation with K-files (Table 2).
- *Group II*: This group contained 30 teeth in which 2 mL of NaOCl solution was used for irrigation and ultrasonic agitation was done (Table 2).
- *Group III*: This group contained 30 teeth in an alternate irrigation with NaOCl and 3% H_2O_2 was done (Table 2).

In control group, 2 mL of 0.85% sterile saline solution was used for irrigation of root canals.

After dividing the teeth into different groups, irrigation of teeth was done with 1 mL saline solution and size no. 40 paper points were used for sampling the bacteria from the root canals. After 1 minute, they were transferred to tubes containing 5 mL of BHI broth. Tubes were vortexed for 5 minutes and incubated for 4 days at a temperature of 37°C. The presence of broth turbidity was indicative of bacteria remaining in the root canal.

Results obtained during study were subjected to statistical analysis using chi-square test; $p < 0.05$ was considered significant.

Table 1: Distribution of teeth in various groups

Group	*Number of teeth*
I	30
II	30
III	30
Control	30

Table 2: Different irrigants and irrigating methods in all groups

Group	*Irrigant(s)*	*Irrigating method*
I	4% NaOCl	Manual
II	4% NaOCl	Manual and ultrasonic agitation
III	4% NaOCl and 3% H_2O_2	Manual: Alternate use
Control	Saline	Manual

RESULTS

Table 1 shows distribution of teeth in all groups. Four groups were made. Groups I, II, and III contained 30 teeth each. Fourth group was control group, which also contained 30 teeth.

Table 2 shows that 4% NaOCl was used as an irrigant manually in group I. In group II, 4% NaOCl and ultrasonic agitation was used together. In group III, alternate use of 4% NaOCl and 3% H_2O_2 was used manually. In control group, normal saline was used as an irrigating agent.

Table 3 shows number of positive cultures in all groups. In group I, 18 positive cultures were found. In group II, 16 positive cultures were found. In group III, 18 cultures were positive, while in the control group, all cultures were positive for bacterial growth. The results obtained in all three experimental groups were non-significant ($p > 0.05$), whereas in the control group, all 30 teeth were irrigated with normal saline showed 100% positive culture and the difference ($p < 0.05$) was statistically significant.

Table 3: Number of positive cultures in all groups

Group	*No. of Positive culture*
I	18
II	16
III	18
Control	30

DISCUSSION

The purpose of successful endodontics is removal of microorganisms found in infected root canals and formation of effective seal to prevent recolonization with bacteria. The role of irrigating solution in achieving root results is desirable. An ideal irrigating solution should be able to do disinfection, have low toxicity, have low surface tension, have the ability to remove smear layer, and have a broad antimicrobial activity, especially against *E. faecalis*.[9]

In the literature, numerous irrigating solutions have been proposed. All have few advantages and shortcomings as well. Sodium hypochlorite is the most effective endodontic irrigant. The free chlorine in NaOCl, which is a strong oxidizing agent, dissolves vital and necrotic tissue by splitting proteins.[10] Chlorine causes irreversible oxidation of –SH groups of essential enzymes, disrupting the metabolic functions of the bacterial cell.[11]

Several authors in their studies revealed that heated NaOCl solutions have more antimicrobial efficacy in dissolved organic tissues than nonheated solutions.[12,13]

This study was conducted on freshly extracted 120 maxillary incisors. After performing biomechanical preparation, teeth were divided into three groups depending on methods of irrigation (Table 1). In group I, 30 teeth were irrigated with 2 mL of a 4.0% NaOCl solution. Result of our study showed that 18 out of 30 teeth contaminated with *E. faecalis* showed positive cultures. In group II, 30 teeth were irrigated with 2 mL of 4.0% NaOCl solution. After that, a 15 number ultrasonic file was placed in the canal. The unit was activated with the file unconstrained and the solution was ultrasonicated for 1 minute (Table 2). In this group, 16 teeth contaminated with *E. faecalis* showed positive cultures. In group III, 30 teeth were irrigated initially with 1 mL of 4.0% NaOCl and then with 1 mL of 3% H_2O_2, followed by 1 mL of NaOCl again (Table 3). The results obtained in all three groups were nonsignificant. The control group, in which all 30 teeth were irrigated with normal saline, showed 100% positive culture. The difference in experimental group and control group was statistically significant.

Enterococcus faecalis is an anaerobic bacteria. It leads to apical periodontitis. It is resistant to a wide range of antimicrobial agents. It is difficult to remove this from root canals, hence, endodontic treatment becomes challenging.[14]

In this study, we also used ultrasonic irrigation. Ultrasonic irrigation is more effective in removing debris, and it is suggested that passive ultrasonic irrigation is more efficient than sonic activation.[15] After inserting files in the canals, they are passively activated with ultrasound energy, the acoustic cut causes cleaning of the canals more effectively in comparison to the use of manual instrumentation alone. Ultrasonic vibration is more effective when it touches the handle of a manual file inserted in the canal.[16,17]

We also used H_2O_2 as an irrigating solution which releases nascent oxygen. It produces bactericidal effect by interfering with bacterial metabolism when it comes in contact with tissue enzymes.[18] Hence, we recommend the usage of 3 to 5% of H_2O_2 in endodontics.[19-21]

CONCLUSION

The success of root canal therapy depends on the ability to remove smear layer and elimination of *E. faecalis* from root canals. Sodium hypochlorite has been found to be more effective in disinfecting the canals than sterile solution.

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