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IP Indian Journal of Conservative and Endodontics

Journal homepage: https://www.ijce.in/

Original Research Article

An invitro comparison of antimicrobial efficacy of sodium hypochlorite gel with twin kleen irrigating solution against enterococcus feacalis

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ARTICLE INFO

Article history:
Received 07-02-2024
Accepted 06-03-2024
Available online 20-03-2024

Keywords:
Enterococcus faecalis (E faecalis)
Sodium hypochlorite gel
Twin kleen irrigating solutions
Endodontic treatment

ABSTRACT

Background : The Enterococcus faecalis (E. faecalis) is a normal inhabitant of the oral cavity and is the most commonly identified species in post treatment asymptomatic persistent endodontic diseases (both intra-radicular and extra- radicular). Its prevalence ranges from 24% to 77%. To completely eliminate microorganisms from the root canal system, combination of instrumentation and irrigation with antibacterial solutions is necessary. Hence, the purpose of this study was to evaluate and compare the antibacterial efficacy of sodium hypochlorite gel with twin kleen irrigating solution against E. faecalis.
Materials and Method s: 40 non-carious, single-rooted teeth with straight canals were selected and instrumented till F3 (30.06) ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland). E faecalis was prepared and incubated in teeth and were randomly divided into four groups of 10 each, Group A – 5.25% NaOCl gel, Group B – Twin Kleen Irrigating solution, Group C – 3% NaOCl solution and Group D – Saline. Sterile paper points were used to collect the samples after irrigation and then they were transferred to blood agar culture plates to check the presence of E. faecalis.
Results: Significant statistical difference was seen between the four groups. The effect of 5.25% NaOCl gel was superior as compared to Twin Kleen irrigating solution.
Conclusion : Under the limitations of this study, 5.25% NaOCl gel was effective in reducing E. faecalis count followed by Twin kleen irrigating solutions.

1. Introduction

Dental caries is a highly prevalent disease in the world.[1] Endodontic treatment is an option to treat tooth widely destroyed by dental caries. Even though this kind of treatment offers favorable prognosis in most cases, scientific literature suggests there are possibility of failure.[2] Endodontic treatment failure may occur due to different causes such as persistence of bacteria, root canals that are poorly clean and obturated, improper coronal seal (leakage), and untreated canals (missed canals), hence it is essential to remove all pulpal tissues, dentinal debris and viable microorganisms from the root canal system during endodontic treatment.[3] The success of Endodontic treatment primarily depends on successful removal of microbes and their byproducts from the infected root canal system. Failure to effectively eliminate them could lead to impaired healing.[4] The persistence and growth of bacteria in root canal system is the main causative factor for failure of such treatments inspite of proper instrumentation and adequate irrigation.[5]

Enterococcus faecalis (E. faecalis) is most commonly found resistant microorganism in the root canal system

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https://doi.org/10.18231/j.ijce.2024.008

and is the most commonly identified species in post treatment diseases.[6] Therefore, for this reason the principal objective of successful root canal treatment is to eliminate bacteria and their byproducts from the root canal system. In some cases, it is impossible to completely eliminate microorganisms from the root canal system only with instrumentation. In this context, irrigation with antimicrobial solutions is necessary.

Sodium hypochlorite (NaOCl), a halogenated compound, is routinely used to irrigate the root canals during endodontic treatments, due to its excellent nonspecific proteolytic and antibiotic properties.[7] It is used in concentrations from 0.5 to 5.25%, with its antimicrobial activity increasing proportionally along with its toxicity.[8] The main disadvantage of NaOCl is its cytotoxic effects when it is extruded beyond the apex into the periapical tissues.[9] It is expected that using NaOCl gel can reduce the apical extrusion of debris and minimize its side effects. In addition, if the gel and solution forms are equality effective, the benefits of gel form in root canal treatment can be advantageous.[10]

The combination of NaOCl and 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP), or etidronate was proposed to be used as a single irrigant in shaping and cleaning and it lead to the concept of continuous chelation.[11] Twin Kleen (MaarcDental Innovationsendo, India) consist of 9% HEBP. It is a Mild Chelating agent with short term compatibility with NaOCl and eliminates debris impaction in the anatomical irregularity.[12] The combination prevents smear layer formation and can avoid irrigating sequence. It can be used during instrumentation and as a final rinse solution.[13]

2. Materials and Methods

The study was approved by the Ethical Committee Clearance Board of Maratha Mandal's NGH Institute of Dental Sciences, Belagavi. 40 non-carious, unrestored, matured single-rooted teeth with straight canals were used in this in vitro study. The crowns were then removed with a high-speed hand piece and a diamond bur up to CEJ level. The working length was determined with #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) 1 mm shorter than the apical foramen. Then, rotary instrumentation (ProTaper S1, S2, F1, F2, F3, Dentsply Maillefer, and Ballaigues, Switzerland) was carried out for cleaning and shaping. Each tooth was irrigated with 5 mL of distilled water and sterilized in an autoclave at temperature of 121 °C for 20 minutes. E faecalis was prepared in Brain Heart Infusion (BHI) broth in the Central Research Laboratory of Maratha Mandal's NGH Institute of Dental Sciences, Belagavi. The teeth were inoculated with E.faecalis and incubated for 48 hours in BHI broth at 37 °C. The teeth were randomly divided into 4 groups depending on the irrigating solutions used (Tables 1 and 2):

1. Group A: This group contained 10 teeth in which 5.25% NaOCl gel was used for Irrigation.
2. Group B: This group contained 10 teeth in which Twin Kleen irrigating solution was used for irrigation.
3. Group C: This group contained 9 teeth in which 3% NaOCl solution was used for irrigation. One teeth of this group was lost due to endodontic mishap during the procedure.
4. Group D: This is the control group contained 9 teeth in which Saline was used for irrigation. One teeth of this group was also lost due to endodontic mishap during the procedure.

Table 1: Distribution of teeth in various Groups

Group	Number of teeth
A	10
B	10
C	9
D	9

Table 2: Different irrigating solutions used in all groups

Group	Irrigant (s)
A	5.25% NaOCl gel
B	Twin Kleen Irrigating solution
C	3% NaOCl
D	Saline

After dividing teeth into different groups, a 26 1/2-gauge syringe needle 1 mm shorter than working length was used. Then a number 15 K-file was carried to the working length to assure the complete penetration of all the irrigating solutions in each group. After 1 minute the root canals were irrigated with 1mL normal saline solution. Then, sterile paper points were introduced to the working length of each root canal and remained for 1 minute to collect samples. Then, the paper points were transferred into a tube containing 5mL BHI broth and vortexed for 5 minutes. The presence of broth turbidity was indicative of bacteria remained in the root canal. All the procedures were carried out in a laminar flow chamber with sterile instruments. The samples were transferred to blood agar culture plates to check the presence of E. faecalis.

Growth of E.Faecalis was checked in all the teeth of each group at two different stages.

Stage 1 (Before treatment): E.Faecalis growth was checked before using any kind of irrigating solutions in all teeth among all the groups, including control group. Statistical test applied here was One ANOVA.

Stage 2 (After treatment): E.Faecalis growth was checked after using different irrigating solutions among all the groups, including control group. Kruskal Wallis ANOVA test was applied here.

Results obtained during study were subjected to statistical analysis using SPSS version 24.

3. Results

Table 1 shows distribution of teeth in all groups.Four groups were made, containg 10 teeth in each.

Table 2 shows that 5.25% NaOCl gel was used in Group A, Twin Kleen solution in group B, 3% NaOCl in group C and Saline in group D as irrigating agent manually.

Table 3 shows Mean CFU obtained in all groups before treatment (before irrigation). The results obtained was non-significant (P value= 0.88), which showed that all the teeth among all the groups had equal potential for E.Faecalis growth.

Table 4 shows Mean CFU obtained in all groups after treatment (after irrigation).Group A had no growth of bacteria, hence mean CFU was .000. In Group B, Group C and Group D mean CFU were 32.80, 44.88 and 74.66 respectively. While analysing the results, statistically significant results were obtained when all four groups were compared (P Value<0.05).

Table 3: Before treatment, mean CFU among different group

Group	Mean CFU	Std. Deviation	P value
A	304.9000	77.474666	
B	280.9000	106.58690	0.88
C	308.7778	143.30978	
D	281.4444	43.08164	

Table 4: After treatment, mean CFU among different group

Group	Mean CFU	Std Deviation	P value
A	.0000	.0000	
B	32.8000	35.7356	0.006 HS
C	44.8889	60.50918	
D	74.6667	85.71610	

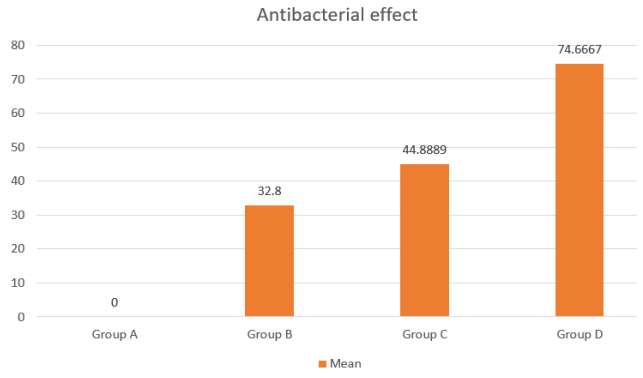

Figure 1: Bar chart representing comparison of mean scores between 4 groups.

Given bar chart compare the antibacterial efficacy of all the irrigating solutions used in which 5.25% NaOCl gel showed highest antibacterial efficacy and 3% NaOCl solution showed least antibacterial efficacy among all irrigating solutions used.

4. Discussion

This study compared the in vitro antibacterial activity of 5.25% NaOCl gel, Twin Kleen irrigating solution, 3% NaOCl solution and saline against E. faecalis. E. faecalis was selected in the present study because it is one of the most resistant intracanal bacteria and the most common microorganisms isolated from teeth with persistent apical periodontitis.[6] Several studies have showed that the E. Faecalis was found more in cases of failed endodontic treatment than in cases with primary infection.[14,15] E.faecalis was more likely to be associated with asymptomatic cases than with symptomatic ones in case of primary endodontic infections.[16,17] It is a facultative anaerobic gram-positive coccus that can occur singly, in pairs, or as short chains. They possess the ability to grow in presence or absence of oxygen.[18] Its prevalence in such infections ranges from 24% to 77%. This resistant feature of E.faecalis can be explained by its various survival and virulence factors including its ability to compete with other microorganisms, invade dentinal tubules, survive high pH, tolerate wide range of temperature between 10°C - 60°C and resist nutrional deprivation.[19] An ideal intracanal irrigating solution should be able to do disinfection, have low toxicity, have low surface tension, have ability to remove smear layer and have a broad antimicrobial activity, especially against E.Faecalis.[20]

In literature, numerous irrigating solutions have been proposed. All have few advantages and short-comings as well. Sodium hypochlorite is most effective endodontic irrigant and has been used since 1920. The free chlorine in NaOCl, which is a strong oxidizing agent, dissolves vital and necrotic tissue by splitting proteins.[21] Hypochorous acid exhibits antimicrobial effect by oxidative action on sulphydryl groups of bacterial enzymes, as essential enzymes are inhibited, important metabolic reactions are disrupted, resulting in the killing of the bacterial cells.[22,23]

Several authors in their studies revealed that the heated NaOCl solutions have more antimicrobial efficacy than nonheated solutions.[24,25] NaOCl is used in concentrations ranging from 0.5% to 5.25%.[26] Kozol et al. evaluated the toxic effects of NaOCl and observed that 0.025% was a safe concentration for clinical use, maintaining the antimicrobial action without harmful effects on the periapical tissues.[27] As yet, there is no consensus regarding the correct concentration for use in endodontic therapy, the right concentration lies between its antibacterial activity and its cytotoxicity. The major disadvantages of NaOCl are its cytotoxic effect if injected into the periapical tissues.[6]

Sodium hypochlorite gel was introduced with the aim of providing more controllable and safer irrigation method. It reduces the risk of extrusion, at the same time providing antibacterial efficacy. The advantages also include the increase in contact time. In a study conducted by Zand et al., the gel form of NaOCl 2.5% exhibited significantly lower antimicrobial efficacy in comparison with 2.5% and 5.25% solutions.[2] It may be due to lower concentration and also the viscosity of the gel which prevents flow into anatomical complexities and is a disadvantage. In a study conducted by Shamsi et al., NaOCl 5.25% solution and gel showed the same effectiveness.[3]

This study was the first to evaluate the antibacterial efficacy of Twin Kleen irrigating solution against E. faecalis. Twin kleen irrigating solution contains HEBP and according to the study conducted by Zehnder et al., he found that the persistent presence of HEBP inhibits the formation of smear layer along the walls. Further studies should be carried out to evaluate its efficacy. To determine the most effective root canal irrigator, the efficacy of the irrigating solutions and gels should be further determined with various bacterial species in root canals and with different methods. Further studies are needed to confirm the effect of findings of this study in clinical settings**.**

5. Conclusion

Under the limitations of the study, 5.25% Sodium Hypochlorite gel had significantly highest antibacterial effect against Enterococcus faecalis, whereas Twin Kleen irrigating solution and 3% Sodium hypochlorite solution exhibited lesser antibacterial effect.

6. Conflict of Interest

None.

7. Source of Funding

None.

Acknowledgments

The authors acknowledge all the support and contributions of participators.

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Cite this article: Mirza MS, Uppin VM, Pujar M, Gopeshetti P, Umesh S. An invitro comparison of antimicrobial efficacy of sodium hypochlorite gel with twin kleen irrigating solution against enterococcus feacalis. *IP Indian J Conserv Endod* 2024;9(1):34-38.