Original Article

Comparative Evaluation of Antimicrobial Efficiency of Different Concentrations of Sodium Hypochlorite in Root Canals – An *In vitro* Study

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Abstract

Background: The study aimed to assess the efficacy of various concentrations of sodium hypochlorite (NaOCl) solution in *Enterococcus faecalis* reduction in root canals. **Methods:** A total of 100 maxillary central incisors underwent chemomechanical preparation. Immersion in 5.25% NaOCl for 3 min removed smear layer followed by 1 mL of 17% ethylenediaminetetraacetic acid for 3 min. Phosphate-buffered saline solution was the final irrigant used. The samples were then sterilized. *E. faecalis* biofilms formed within the root canals were evaluated after 6 weeks. Samples were divided into five groups in terms of the antibacterial treatment: Group 1: 0.5% NaOCl solution; Group 2: 2.5% NaOCl solution; Group 3: 5.25% NaOCl solution; Group 4: Soaked in saline solution; and Group 5: Negative control. Microbial was quantified by method of colony-forming units followed by data analysis. **Results:** Higher concentrations of NaOCl demonstrated greater antimicrobial reduction as against 2.5% and 0.5% at $P < 0.001$. About 5.25% showed complete inhibition of microbial growth. The highest colonization was in the group irrigated with sterile saline solution. **Conclusion:** The present analysis leaned toward higher antiseptic concentrations to achieve optimum disinfection in the root canals.

Keywords: Disinfection, *Enterococcus faecalis*, Root canal irrigant, Sodium hypochlorite.

Introduction

The bacterial infection of root canal by microorganisms is significant in the cause of periradicular disease such as apical periodontitis.[1] Bacteria and their cytotoxic by-products penetrate into pulp which leads to the formation of biofilm. Biofilm is a sophisticated ecosystem of microorganism creating their own extra-polymeric substance and matrices. The biofilm adheres to the root canal walls, isthmuses, and accessory canals.[2] *Enterococcus faecalis* (*E. faecalis*) is an important microorganism in the biofilm as it is an anaerobic organism. Biofilm encourages inflammatory responses which, in turn, cause pulp necrosis creating an ideal habitat for the anaerobic bacteria. This creates a more resistant environment against antibodies and antimicrobials to take effect.

The main objective of endodontic treatment is to achieve apical healing and preventing infection from occurring.[3] This requires various steps one of the key steps being mechanical instrumentation which involves the physical removal of the biofilm through hand and rotary instrumentation.[4] The study by Peters *et al*., 2001, highlights that 35% root canal walls remain untouched by mechanical instrumentation. Therefore, irrigation with the use of a chemical disinfectant is needed to achieve elimination of bacteria.[5]

Various irrigation solutions have been used as part of the disinfection protocol such as chlorhexidine or sodium hypochlorite (NaOCl) with NaOCl being regarded as the irrigant of choice due to its desirable properties.[6] The elimination of bacteria in endodontic treatment with an outcome of periradicular healing is the key objective (Abbot, 2002). Irrigation with NaOCl is one of the most essential parts of the root canal disinfection protocol as mechanical instrumentation alone is sufficient. NaOCl is the most desired properties of the irrigants available even though it does have some cytotoxic effect on soft tissues.

Access this article online

Website: www.jrad.co.in

DOI: https://doi.org/10.53064/jrad.2021.12.5.67

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Received: 17 Jun, 2021; **Accepted:** 14 Jul, 2021; **Published:** 28 Aug, 2021

How to cite this article: Rai KS, Rai P, Sharma V. Comparative Evaluation of Antimicrobial Efficiency of Different Concentrations of Sodium Hypochlorite in Root Canals – An *In vitro* Study. J Res Adv Dent 2021;12(5):1-265.

At present, there is limited evidence illustrating if a low or a higher concentration of NaOCl is better for root canal treatment outcome with the least amount of cytotoxic effect. Hence, the present study was conducted to answer the research question – "Is a higher concentration of NaOCl more effective in eliminating bacterial growth than lower concentration?"

The objective of this study was to evaluate I which concentration of NaOCl was better effective in eliminating bacterial growth in extracted human permanent teeth so as to facilitate better understanding of the of various determinants influencing NaOCl concentrations.

Methodology

An *in vitro* study design was employed to evaluate the efficacy of low and high concentration of NaOCl on *E. faecalis* in single-rooted teeth. No ethical clearance was required as it was an *in vitro* study. One hundred extracted incisor teeth constituted the sample size. Teeth chosen for the study had intact root without any evidence of resorption, fracture, curved, or any other anomalies of the roots. Teeth were prepared by curetting of remnant masses by a universal curette and then stored in NaOCl solution for 24 h and later in 0.9% saline solution.

The teeth samples were then decoronated at CEJ with the help of a diamond bur leaving behind 10–12 mm root length. Roots shorter than this length were discarded. Pulpal tissue extirpation was done with Hedstrom files. Biomechanical preparation was done by "Step back technique" according to Torabinejad.[7] Any remaining debris was removed by an ultrasonic cleaner, soaked in 17% ethylenediaminetetraacetic acid solution, subsequently stored in 5.25% NaOCl, and then sent for microbiological evaluation.

After sterilizing the prepared samples, *E. faecalis* cultures were grown on 10% sheep blood agar (BA) and brain heart infusion plates. Eighty roots were then injected with microorganism while 30 left behind as the negative control. The samples were then incubated in sterile BHI medium for 48 h at 37°C.

E. faecalis presence was assessed in the combined culture of blood heart infusion agar and BA medium to assess the presence of *E. faecalis*. The infected samples were then randomly divided into five experimental groups of 20 based on concentration of the irrigant used, and a positive and a negative control group (Table 1). The root canals first were irrigated with 2 mL of NaOCl solution.

In the positive control group, sterile solution was used as irrigant with no subjection to any disinfection procedure. The root canals were then assessed for *E. faecalis*, and the colony-forming unit (CFU) counts were reported.

Data analysis was done by SPSS version 25.0 (IBM; Chicago). ANOVA with *post hoc* test was performed to identify significant differences between groups for microbiological identification. $P < 0.05$ was considered to be statistically significant.

Results

Table 1 shows different concentration of NaOCl employed in the present study. Comparison of antimicrobial activity of different concentrations of NaOCl was made using ANOVA. Negative control showed no microbial growth after incubation. A significant difference was noted in bacterial concentrations measured as mean CFU with lowest concentration (0.5%) having the highest value followed by 2.5%. Positive control exhibited a mean of 2616 + 86.10 stressing the need of an antiseptic in root canal irrigation (Table 2 and Figure 1).

Discussion

E. faecalis is correlated with persistent endodontic infectious conditions as it is highly resistant to wider range of antimicrobial agents. In addition, this microbe upholds as the best organism to penetrate into dentinal tubules resulting in gross infections. Other characteristics making it the organism of choice are rapid growth, colonizing potential, invasion into dentinal tubules, and interaction with composing cells.[8]

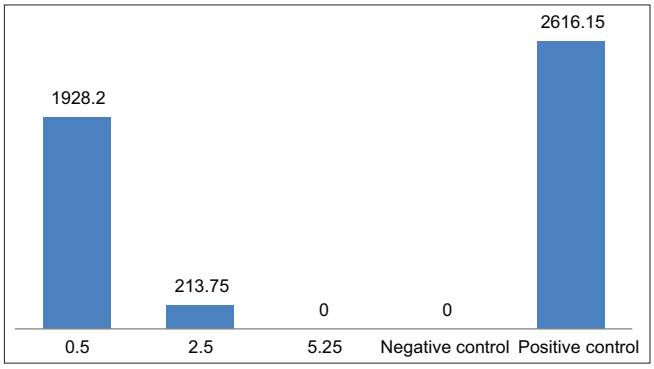

Figure 1: Graphs depicting comparative evaluation of various groups for antimicrobial efficiency

Table 1: Groups evaluated for antimicrobial efficiency

Groups	NaOCl concentration
Group 1	0.5%
Group 2	2.5%
Group 3	5.25%
Group 4	Positive
Group 5	Negative

NaOCl: Sodium hypochlorite

Table 2: Comparative evaluation of various groups for antimicrobial efficiency

Concentration (in %)	Bacterial colony units Mean	Bacterial colony units S.D	*P*-value
0.5	1928.20	154.75	<0.001**
2.5	213.75	19.73	
5.25	0	0	
Positive control	2616.15	86.10	
Negative control	0	0	

** = Highly significant; * = Significant; NS = Nothing significant

Hence, *E. faecalis* infection generally presents a problem with therapy as this bacterium is cumbersome to be removed from the root canal with the help of chemomechanical procedures. The present investigation employed a culture-based approach. It is one of the most reliable methods of detecting viable bacteria, especially when samples are obtained immediately following antimicrobial intervention. Despite this, this manner is particularly difficult, time consuming, and needs certain microbiological facilities and possibly an effective way to evaluate root canal disinfection.

Dakin[9] first used NaOCl in 1915 to disinfect wounds and its utilization was furthered in endodontic by Coolidge in 1919. In 1922, Walker suggested its usage for the irrigating root canals, with Grossman later propagating this procedure.[10,11] In the recent times, this is the solution most expansively employed globally for root canal instrumentation. It is an alkaline solution with an approximate pH of 11–12 with literature evidence backing up its germicidal and antibacterial action. Although NaOCl has been part of the endodontic armamentarium for many decades, investigations are still not able to the volume or the concentration of this solution which can dissolve organic matter without weakening the dental structure in the biomechanical preparation of the root canal.[12]

The antimicrobial efficiency of NaOCl is because of its high pH (hydroxyl ion action) which intercedes with bacterial cytoplasmic membrane and even this solution contains active chlorine which demonstrates its antibacterial action by oxidation of bacterial enzyme and disruption of metabolic function of bacterial cell. Chlorine might even synergize with bacterial cytoplasmic component to form toxic complex that destroys the microorganism. The findings of this investigation demonstrated significant difference among concentrations (0.5%, 2.5%, and 5.25%). This is not in line with the results of other study such as Forghani *et al*. Study of Retamozo *et al*.[13] is in concordance with the present study findings.

Factors to be borne in mind which might alter the study results are the transport media, the culturing conditions, and the choice of culturing medium which warrants assurance in the microbiological analysis.

Conclusion

It can be concluded that NaOCl in higher concentrations (5.25%) is efficient in destroying bacteria. Further studies are recommended which consider different incubation times and to clinical conditions simulating human root canal treatment.

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