Original Article

Comparative evaluation of antifungal activity of Sodium Hypochlorite, Calcium Hypochlorite and modified Salt Solution associated with passive ultrasonic irrigation against *Candida albicans* - An *In-Vitro* study

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Abstract

Aim: The study evaluated the antifungal activity of sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$), and modified salt solution (MSS) assisted with passive ultrasonic irrigation against *Candida albicans*.

Materials and Methods: One hundred and thirty-six single-rooted premolars were decoronated and enlarged up to a file #45, autoclaved, inoculated with *C. albicans,* and incubated for 72 h. The samples were randomly distributed into eight groups ($n = 17$) according to the protocol for decontamination G1: No treatment, G2: Distilled water (DW), G3: 2.5% NaOCl, G4: 2.5% NaOCl + ultrasonic activation (US), G5: 2.5% $Ca(OCl)_2$, G6: 2.5% $Ca(OCl)_2$ + US, G7: MSS, G8: MSS + US. Microbiological testing (Colony forming Unit [CFU] counting) was performed before and after the treatment.

Statistical Analysis: Data were subjected to the one-way analysis of variance followed by the Tukey's *post hoc* test ($P < 0.05$).

Results and Conclusion: Groups 1 and 2 showed the highest mean contamination (5.41 and 4.31 $\log_{10}$ CFU/mL, respectively), which was statistically different from all the other groups ($P < 0.001$). G4 showed the lowest mean contamination (0.24 $\log_{10}$ CFU/mL) with statistically significant value ($P < 0.001$). 2.5% NaOCl with ultrasonic activation can aid in significant fungal reduction. Ultrasonic activation of 2.5% NaOCl, 2.5% $Ca(OCl)_2$, and MSS was also found to have improved antifungal activity against *C. albicans*.

Keywords: Calcium hypochlorite; *Candida albicans;* colony-forming units; modified salt solution; passive ultrasonic irrigation; sodium hypochlorite

INTRODUCTION

The main objective of endodontic treatment is to completely clean and disinfect the root canal system to eliminate the microorganisms.[1] One of the major failures of root canal treatment is the presence of resistant microbial species such as variety of anaerobes, aerobes, and fungi.[2] Among fungi, Candida species were found in infected root canals with a prevalence ranging from 0.5% to 55% and possess virulence factors that may play a role in the onset of endodontic pathologies.[3]

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Biomechanical procedures are the important steps in root canal treatment for eliminating bacteria and irritants, but it often fails to eliminate the microorganisms completely due to the anatomical complexities of the root canal system.[4]

Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant due to its effective tissue-dissolving ability and antimicrobial activity.[5] However, NaOCl has been found to be highly irritating to tissues when extruded periapically,[6] is known to decrease the fracture resistance of teeth[7] and bond strength of adhesive restoration to dentine.[8,9] Due to which newer alternative irrigants are needed that promotes optimal decontamination of root canals with no damage to the related involved tissues.

Calcium hypochlorite ($Ca(OCl)_2$) is a stable chemical substance used for industrial sterilization, bleaching, and water purification treatments.[10] It also found to have antibacterial and pulp tissue-dissolving properties without interfering the mechanical properties of dentin. It is biocompatible, found to have least cytotoxicity compared to NaOCl and chemically stable during storage.[11-13]

Hypertonic salt solution has the ability to inactivate bacterial biofilm which led to the development of modified salt solution (MSS). It contains sodium chloride and potassium sorbate, and its mode of action is based on a multiple-hurdle strategy that combines a series of stress factors (hurdles) that the microorganisms are unable to withstand. It has the ability to reach the complex root canal structures behind the main canal lumen thus enhancing disinfection of the root canal system.[14] However, its antimicrobial potential against *C. albicans* has not been evaluated. MSS was found to have disinfecting properties against *Enterococcus faecalis* in a root canal model.[15] In addition, it has been suggested that the action of irrigant can be improved by passive ultrasonic irrigation (PUI) resulting in a significant reduction of bacteria as compared to manual irrigation.[16]

The aim of the present study was to compare *in vitro* efficacy of NaOCl, $Ca(OCl)_2$, and MSS associated with PUI in the root canal infected with *C. albicans*.

The null hypothesis states that there is no difference in the antifungal activity of three endodontic irrigants with manual and PUI against *C. albicans*.

MATERIALS AND METHODS

Sample collection and preparation

One hundred and thirty-six extracted, single-rooted premolars were obtained from patients affected by dental caries or severe periodontal disease. The teeth were standardized to a length of 14 mm with a diamond bur. The working length (WL) was set at 1 mm short of the anatomical apex as 13 mm. The patency of each canal was established with a size 15 K-file (Dentsply Maillefer, Ballaigues, and Switzerland). Size 3 and 4 Gates Glidden Burs (Dentsply Maillefer, Ballaigues, and Switzerland) were used to flare the coronal aspect of each canal and the root canal preparations were performed with a MTWO (VDW. Munich, Germany) rotary system #40.04 file size at the WL according to the manufacturer's instructions. During root canal instrumentation, irrigation was performed with 5 mL of 2.5% NaOCl (Parcan, Septodont, India) using side-vented needles (Neoendo, 30 guage, 25 mm). After instrumentation, the root canals were irrigated with 3 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Prevest Denpro, Jammu, India) and then filled with 1 mL of 17% EDTA for 3 min to remove the smear layer. Two milliliters of distilled water was used for final irrigation. Paper points were used to dry the root canals, apex was sealed using composite resin (3M, Saint Paul, MN, USA) and two layers of nail polish were applied around the root surface to prevent bacterial leakage. Each root was fixed with Putty-C Silicone (3M, Saint Paul, MN, USA) in an Eppendorf tube. The tubes were placed in plastic carrier boxes, placed in autoclave sachets (Getinge group), and then autoclaved for 30 min at 120°C.

Contamination of the specimens

C. albicans (ATCC 90028) was adjusted to 0.5 turbidity on densitometer (1.5×108 fungi mL^{-1}) and experimental teeth were inoculated with 0.3 mL. The samples were incubated at 36°C and 91% humidity for 72 h. Every 24 h, freshly made suspension of *C. albicans* were added. At 48 h, 100 µL aliquots were taken from each tooth using a calibrated micropipette and plated on Sabouraud 4% dextrose agar plates to verify the growth and colony-forming units (CFUs) were checked.

Preparation of irrigation solutions

NaOCl solution was prepared at 2.5% concentration using DW. $Ca(OCl)_2$ solution was prepared at 2.5% concentration using DW (weight/volume ratio) and mixed with a magnetic stirrer for 30 min. MSS was prepared by dissolution of 3M NaCl and 1M potassium sorbate in demineralized water.

Classification of treatment groups

The study groups were divided into 8 ($n = 17$) and subjected to one of the following irrigations:

- G1: NT-No treatment
- G2: DW
- G3: 2.5%NaOCl
- G4: 2.5%NaOCl + US (Ultrasonic activation)
- G5: 2.5% $Ca(OCl)_2$
- G6: 2.5% $Ca(OCl)_2$ + US
- G7: MSS
- G8: MSS + US.

For manual irrigation groups, 2 mL of irrigation solution was delivered in the canal for 1 min followed by 2 ml for 30 s and again 2 mL for 30 s at 1 mm from the WL.

For US groups, 2 mL of irrigation solution was delivered and activated for 1 min, followed by 2 mL solution with activation for 30 s and again 2 mL activation for 30 s at 1 mm from the WL with piezoelectrical ultrasonic unit by using ultrasonic file # 15. The scale power 2 was used to promote ultrasonic activation. A total of 6 mL was used for entire procedure. To inactivate the residual effect of these irrigants, all experimental teeth were flushed with 3 mL sterile DW for 1 min.

Microbiologic analysis

Sterile paper points were used to dry the root canals. 0.5 mL of sterile saline result was introduced into the canal and an endodontic hand file (K-file# 40) was used to the WL. A calibrated micropipette of 100 µL was used to remove the aliquots in the root canal. The aliquots were cultured on Sabouraud 4% dextrose agar and the plates were incubated at 36°C and 91% humidity for 24 h.

The number of CFU of *C. albicans* was recorded before and after the irrigation protocols to estimate the antifungal activity. All the procedures were conducted under the aseptic conditions and in duplication.

Statistical analysis

Descriptive analysis includes the expression of *C. albicans,* CFUs in terms of mean and standard deviation for each study group. One-way ANOVA test followed by Tukey *post hoc* test was used to compare the mean CFU counts and percentage reduction between the groups. Student's paired *t*-test ($P < 0.05$) was used to compare the mean, CFU counts before and after decontamination procedures. Data were analyzed using the SPSS version 22.0, Armonk, NY, USA: IBM Corp.

RESULTS

The logarithmic mean and standard deviation of fungal counts between different treatment protocols are expressed in log10 CFU/mL. Neither group was suitable to promote a complete decontamination of the root canal system.

After the irrigation protocols, groups 1 and 2 showed the highest mean contamination (5.41 log $_{10}$CFU/mL and 4.31 $\log_{10}$ CFU/mL, respectively), which was statistically different from all the other groups ($P < 0.001$). G4 (2.5% NaOCl with ultrasonic activation) showed the lowest mean contamination (0.24 $\log_{10}$ CFU/mL) with statistically significant value ($P < 0.001$). However, there was no statistically significant difference found when G3 (NaOCl, 0.39 $\log_{10}$ CFU/mL), G5 ($Ca(OCl)_2$, 0.45 $\log_{10}$ CFU/mL), and G6 ($Ca(OCl)_2$ + US, 0.41 $\log_{10}$ CFU/mL) ($P = 1.00$) were compared. Manual and PUI of MSS were found to have lesser antifungal activity compared to manual and PUI of NaOCl and $Ca(OCl)_2$ [Table 1 and Graph 1].

DISCUSSION

The results of the present study showed that G4 (2.5%NaOCl with US) was the most highly effective irrigant against *C. albicans* with the significantly lowest mean contamination value (0.24 $\log_{10}$ CFU/mL) ($P < 0.001$), which is in concurrence with the previous study.[17] The antibacterial effect of NaOCl as an irrigant along with PUI has been previously studied and PUI contributed in significant reduction of microbial content.[11,17]

Our goal was to investigate the influence of PUI to deliver $Ca(OCl)_2$ and MSS in the root canal space and compare NaOCl, $Ca(OCl)_2$, and MSS for its effect over *C. albicans* to evaluate the antifungal effect, as it is not been studied so far.

According to the present study, there was no statistically significant difference found when G3(NaOCl, 0.39 $\log_{10}$ CFU/mL), G5($Ca(OCl)_2$, 0.45 $\log_{10}$ CFU/mL), and G6($Ca(OCl)_2$ + US,0.41 $\log_{10}$ CFU/Ml) ($P = 1.00$) were compared. These findings of G3 and G5 are in accordance with the previous study.[18]

In our study, NaOCl group performed better than $Ca(OCl)_2$ group in terms of CFU against *C. albicans* with no statistically significant difference between their antibacterial efficacy. The action of NaOCl is a potent irrigant with a broad-spectrum antimicrobial activity that causes the breakdown of proteins and amino acids through its "free chlorine release." It has the potential to dissolve the necrotic pulp tissue and organic debris.[2,19]

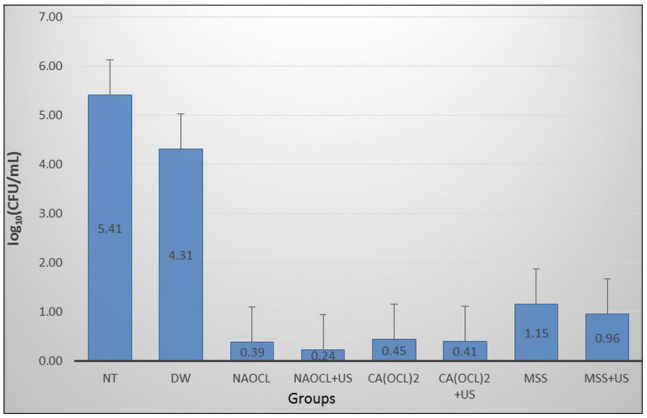
The action of $Ca(OCl)_2$ can be attributed to the generation of hypochlorous acid when mixed with water[11] and is available in granules and the formation of hypochlorous acid occurs when dissolved in aqueous solution. In this way, this compound is released which penetrates bacterial cell walls and neutralizes the bacteria.[11]

The result of our study showed that manual and PUI of MSS found to have an antifungal activity; however, it was lesser than compared to NaOCl and $Ca(OCl)_2$ groups ($P < 0.001$). MSS found to eliminate *E. faecalis* in a biofilm depending on the varying contact time;[20] however, there are no studies related to the evaluation of antifungal activity against *C. albicans* for our reference. MSS is a powerful disinfectant found to have a strong antimicrobial property against *E. faecalis* preventing regrowth of residual bacteria (*E. faecalis*) in about 50% of root canals when compared to $Ca(OH)_2$ as in interappointment root canal dressing.[14,21]

Table 1: Fungal reduction and count of *Candida albicans* (log_{10} colony forming unit/mL) after and before decontamination procedures

Groups	Before irrigation		After irrigation		Fungal reduction		Percentage reduction	*P*
	Mean	SD	Mean	SD	Mean	SD		
NT	5.41	0.23	5.41	0.22	0.00	0.00	-	1.00
DW	5.40	0.22	4.31	0.08	1.09	0.22	20.15	<0.001
NaOCl	5.44	0.26	0.39	0.09	5.05	0.29	92.83	<0.001
NaOCl + US	5.43	0.22	0.24	0.12	5.19	0.23	95.68	<0.001
$Ca(OCl)_2$	5.44	0.21	0.45	0.13	4.99	0.20	91.80	<0.001
$Ca(OCl)_2$ + US	5.43	0.23	0.41	0.10	5.02	0.25	92.51	<0.001
MSS	5.41	0.23	1.15	0.13	4.25	0.23	78.67	<0.001
MSS + US	5.45	0.19	0.96	0.12	4.49	0.21	82.39	<0.001

NT: No treatment, DW: Distilled water, NaOCl: 2.5% sodium hypochlorite, NaOCl + US: NaOCl + ultrasonic activation, $Ca(OCl)_2$: Calcium hypochlorite, $Ca(OCl)_2$ + US: $Ca(OCl)_2$ + ultrasonic activation, MSS: Modified salt solution, MSS+US: MSS+ultrasonic activation, SD: Standard deviation



Graph 1: Microbiological analysis - A chart representing the mean and standard deviation of CFUs (log10 CFU/mL) of *Candida albicans* after irrigation protocols between different Groups. NT: No Treatment, DW: Distilled Water, NaOCl: 2.5% Sodium Hypochlorite, NaOCl + US: 2.5% Sodium Hypochlorite + Ultrasonic Activation, $Ca(OCl)_2$: Calcium Hypochlorite, $Ca(OCl)_2$ + US: Calcium Hypochlorite + Ultrasonic Activation, MSS: Modified salt solution, MSS + US: Modified salt solution + Ultrasonic Activation, CFU: Colony-forming unit

The result of this study also showed that the use of an ultrasonic device can increase the decontamination potential of NaOCl, $Ca(OCl)_2$, and MSS in the elimination of *C. albicans* from the root canal space. This can be correlated to the proposed ability of ultrasonic and Vibringe sonic irrigating devices, causing sonic waves in irrigating solutions deposited inside the root canal[22] that might aid in the adherence of these endodontic irrigants to the microbial cellular wall providing better delivery along all the areas of the root canal space.[11]

In this study after irrigation protocols, sampling was accomplished with a sterile paper point. This scraping sampling technique helps to collect the fungi from the smear layer, biofilm remnants, and noninstrumented areas.[11]

The counting of CFUs expressed in log CFU/mL which is been widely used in endodontic research methodologies to evaluate the bacterial load reduction.[23] This method has two advantages, first the capacity for counts of any number of bacteria using dilutions and secondly counting of viable bacteria excluding the dead bacteria and debris.[24,25]

Endodontic infections are polymicrobial in nature, interactions between multiple organisms could potentially have different dynamics than which was demonstrated by our study. One limitation of this study is that a single organism was used to infect the root canal. Another limitation lies in the scarce literature found about $Ca(OCl)_2$ and MSS as an irrigator during endodontic treatment. Further, investigations are required to assess the efficacy and efficiency of $Ca(OCl)_2$ and MSS with varying contact time and dentinal tubular penetration in the root canal on a biofilm model to reflect its clinical efficacy.

Considering the result of the present study, it can be concluded that 2.5% NaOCl with ultrasonic activation can aid in the chemomechanical preparation and effective decontamination of root canal infected with *C. albicans*. Ultrasonic activation of 2.5% NaOCl, 2.5% $Ca(OCl)_2$, and MSS were also found to have improved antifungal activity.

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Conflicts of interest
There are no conflicts of interest.

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