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Comparative Assessment of antimicrobial efficacy of various root canal irrigants against Enterococcus Faecalis in roots of primary molars-An in vitro study

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Abstract

Background and objective: Pulpectomy involve complete removal of necrotic pulp tissue in a primary tooth. The main goal of pulpectomy is to disinfect the root canal completely. Since the most commonly used root canal irrigant is sodium hypochlorite and there have been few studies based on the antimicrobial efficacy of ozone and calcium hypochlorite on the primary teeth, thus the aim of this study is to evaluate the antimicrobial efficacy of three root canal irrigants i.e sodium hypochlorite, calcium hypochlorite and ozone in the primary root canals against E. faecalis which is the most commonly present pathogen in the root canals.

Methodology: The present comparative study was conducted in Department of Pediatric and Preventive Dentistry, V S Dental College and Hospital, in which 60 extracted primary molar teeth were selected. Access opening was done and the canal was enlarged using stainless steel K files up to 40 sizes. Five teeth are selected randomly as negative controls without bacterial contamination and 0.01ml suspension of E. Faecalis in nutrient broath is inoculated into each canal using a Micropipette and the samples are incubated for a week under aerobic conditions by adding 0.01 ml nutrient broath every day. After incubation period, five inoculated teeth were chosen as positive controls and the samples were divided into 5 experimental group of ten specimens each as follows, Group 1- irrigated with 3% sodium hypochlorite; Group 2- irrigated with 2.5% calcium hypochlorite; Group 3 - irrigated with ozone water; Group 4-irrigated with 3% sodium hypochlorite and 2.5% calcium hypochlorite; Group 5- irrigated with 3% sodium hypochlorite and ozone water. The root canals of all the teeth are refilled with normal saline as transfer fluid and kept for one minute and sterile paper points are used to collect the transfer fluid and placed into a test tube containing 2 ml of sterile saline. This saline from each test tube is applied to nutrient agar culture plates and incubated at 37°C for 48 hours. The Colony forming units for each plate was calculated using

bacterial colony counter. The data obtained was tabulated and descriptive analysis was done. ANOVA and students't test was done to compare the antimicrobial efficacy of the groups.

Results: The results showed that there is statistically significant difference in the antimicrobial activity between all the five experimental groups and group 5 showed the best antimicrobial activity as an irrigant in eliminating E. faecalis bacteria in the root canals.

Conclusion: A combination of sodium hypochlorite and calcium hypochlorite showed the best antimicrobial activity which can be due to synergistic effect, however, calcium hypochlorite alone did not eliminate the bacteria when compared to sodium hypochlorite group. Thus, it can be concluded that NaOCl is still the most effective irrigant for elimination of bacteria, however, other irrigants can be used as an adjuvant with NaOCl to produce synergistic effect as an irrigant.

Keywords: Root canal irrigants, Sodium Hypochlorite, Calcium hypochlorite, Aqueous Ozone, E. faecalis.

Introduction

Dental caries is a microbiological disease which is most commonly prevalent among children. Primary teeth aids not only in mastication or esthetics but also helps in preventing malocclusion, development of speech and muscles. Therefore, preservation of the same becomes important. One of the treatment which aims in preserving the primary tooth is pulpectomy.

Pulpectomy involves complete removal of necrotic pulp tissue in a primary tooth. The main goal of pulpectomy is to disinfect the root canal completely. The biomechanical preparation in primary teeth is done, not only to enlarge the pulp canals but also to remove pulpal tissues. It is impossible to remove all the remaining tissue due to the tortuous root canals in the primary teeth. Use of mechanical instrumentation alone cannot clean this tubular network sufficiently. So, irrigants have been used along with mechanical instrumentation to achieve better debridement.[1]

Enterococcus faecalis (E. Faecalis) is an anaerobic facultative microorganism which is most commonly present in the root canals and is highly resistant to conventional chemo- chemical preparation. This microorganism has several virulence factors and is able to withstand prolonged periods of nutrient limitation, persisting as a pathogen in the root canals of both primary and permanent teeth.[1] E. faecalis has been identified as the species most commonly recovered from teeth with failed endodontic treatment in up to 77% of cases using culture or molecule method. Many authors have demonstrated that E. faecalis has the ability to resist intracanal medicaments and to survive as a single microorganism within the canal system.[2]

Sodium hypochlorite (SH) is the most commonly used root canal irrigant because of its broad antimicrobial spectrum and its ability to promote organic tissue dissolution. However, it is highly irritating when in contact with periapical tissues, cytotoxic, reduces the resistance of teeth to fracture and interferes negatively with the bond strength of adhesive restorations to dentin. Because of the adverse effects of this, researchers have developed alternative endodontic irrigants.[2,3]

SH and Calcium hypochlorite (CH) belong to the same family of chemicals and they are primarily used as bleaching agents or disinfectants. In Industry, CH has been traditionally used for disinfection and purification treatment of water and milk. Unlike SH, it can be easily stored in powder form without losing stability. The bactericidal action of these two agents is highly related to the level of available chlorine. CH powder can produce more available chlorine when it comes in contact with water. Furthermore, it can produce calcium

hydroxide which in turn may enhance the antibacterial efficacy of the solution. According to previous studies this substance shows antibacterial properties and the ability to promote organic matter dissolution. The antibacterial activity of these agents can also be relevant to their pH levels, which may affect the cytoplasmic membrane integrity, enzymatic action and cellular metabolism of the microorganisms.

In a preliminary study, no differences were found among the tissue dissolving properties of 5% or 10% CH and 4.65% SH or 1.36% SH solutions after 60 min. Further, it was revealed that similar to 10% SH, 10 and 15% CH could not produce any difference in microleakage when they were used as pre-treatment agents prior to an acetone-based adhesive system. In another study, Gorduysus et al showed that nor SH either CH was effective in removing smear layer and dentinal debris.

There are limited investigations on antibacterial efficacy of CH in endodontic field. Recently, the antibacterial efficacy of 2.5% CH and 2.5% SH in elimination of E. faecalis from the contaminated root canals were investigated. It was found that CH had better antibacterial activity than SH when agitated with ultrasonic energy. In another study, Schmidt et al. indicated that 2.5% CH can effectively be used for decontamination of gutta-percha points.[4,5]

One of the new generations of the disinfectant agents is ozone; a powerful oxidizing agent used to eliminate bacteria in root canals. Recent investigations of aqueous ozone have indicated that it is a powerful antimicrobial agent against oral pathogens. This suggests that aqueous ozone at different doses might eliminate the oral resistant microorganisms too. One of the crucial properties of aqueous ozone is its nontoxicity to oral cells in vitro. On the other hand, it is less toxic than all other known antiseptics. However, the most important disadvantage of aqueous ozone is its unstable concentration in a long time**.** Consequently, aqueous ozone should be used as soon as possible after obtaining from the ozone generator. These properties indicated that aqueous ozone could be beneficial in many branches of dentistry and its use has been recommended by some researchers for the treatment of endodontic infections.[6]

As failure of root canal therapy is mainly caused by microorganisms, it is not surprising that there are enormous advantages in killing these pathogens. Numerous research have proved the antimicrobial effectiveness of ozone as a gas and as ozonated water. Ozone has shown antimicrobial efficacy against resistant pathogens by neutralising them or preventing their growth. Hems examined the antibacterial effect of gaseous and aqueous ozone against E. faecalis in root canals. A significant reduction of the remaining bacteria was observed following the application of aqueous ozone. Naga Yoshi et al advocated that ozonated water had almost the same antibacterial action as 2.5% NaOCl in endodontic therapy, particularly when used with the ultrasonics. Huth et al also informed the possible advantages of employing ozone in root canal management in high concentrations. Another study evaluated the capability of ozone to eradicate an E. faecalis, observed that its antimicrobial effectiveness was not equivalent to that of NaOCl.[7]

The most commonly used root canal irrigant is sodium hypochlorite and there have been few studies based on the antimicrobial efficacy of ozone and calcium hypochlorite on the primary teeth. Thus, the aim of this study is to evaluate the antimicrobial efficacy of three root canal irrigants i.e sodium hypochlorite, calcium hypochlorite and ozone in the primary root canals against E. faecalis which is the most commonly present pathogen in the root canals.

Methodology

Specimen preparation

- 60 extracted primary molar teeth which did not have physiological or pathological resorption more than apical 1/3rd. of the root were selected.
- Crowns were cut at cemento-enamel junction.
- All the teeth were mounted in cold cure acrylic resin blocks
- Access opening was done and the canal was enlarged using stainless steel K files up to 40 size, irrigated with saline and dried using sterile paper points.
- The samples were autoclaved for 15 minutes at 121 deg. C.
- Five teeth were selected randomly as negative controls without bacterial contamination.
- 0.01ml suspension of E. Faecalis in nutrient broath was inoculated into each canal using a Micropipette.
- Samples were incubated for a week under aerobic conditions by adding 0.01 ml nutrient broath every day.
- After incubation period five inoculated teeth were chosen as positive controls.
- Nutrient broath inside the canal was dried out using paper point.
- Samples were divided into 5 experimental group of ten specimens each as follows

Grouping of the specimen

Group 1- irrigated with 3% sodium hypochlorite solution for 5 minutes.

Group 2- irrigated with 2.5% calcium hypochlorite solution for 4 minutes.2.5% Ca $(OCl)_2$ solution was made up from the granules at the time of experiment. 2.5gm of Ca $(OCl)_2$ was mixed with distilled water for 10 mins.

Group 3- irrigated with ozone water for 3 minutes. Freshly prepared ozone water at the time of experiment from ozone generator was used.

Group 4- irrigated with 3% sodium hypochlorite for 5 minutes and ozone water for 3 minutes.

Group 5- irrigated with 3% sodium hypochlorite for 5 minutes and 2.5% calcium hypochlorite for 4 minutes.

- The root canals of all the teeth were dried with sterile paper points and refilled with normal saline as transfer fluid and kept for one minute.
- Sterile paper points were used to collect the transfer fluid and placed into a test tube containing 2 mL of sterile saline.
- This saline from each test tube was applied to nutrient agar culture plates and incubated at 37°C for 48 hours.
- The Colony forming units for each plate was calculated using bacterial colony counter.
- The data obtained was subjected to statistical analysis using ANOVA and Student's t-test

Figure 1: Armamentarium

Figure 2: sectioning of tooth

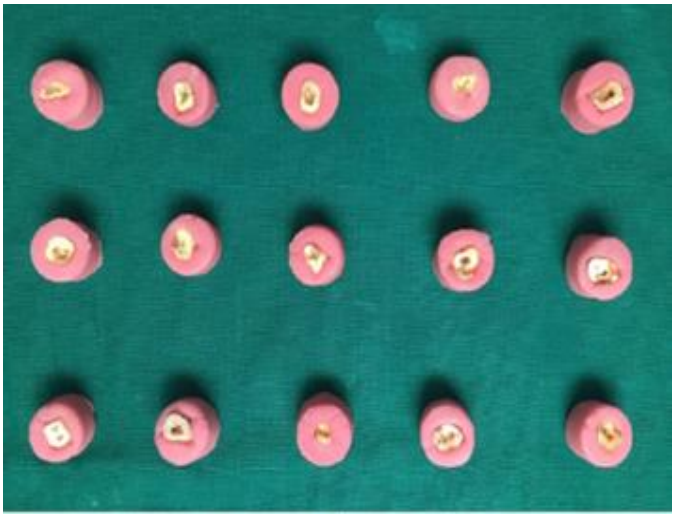


Figure 3: teeth mounted into acrylic resin.

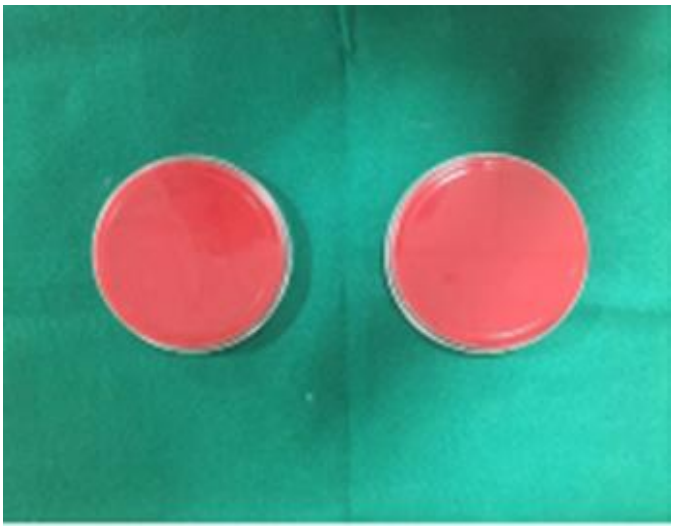


Figure 4: sheep blood agar

Figure 5: samples in the incubator

Figure 6: ozone generator

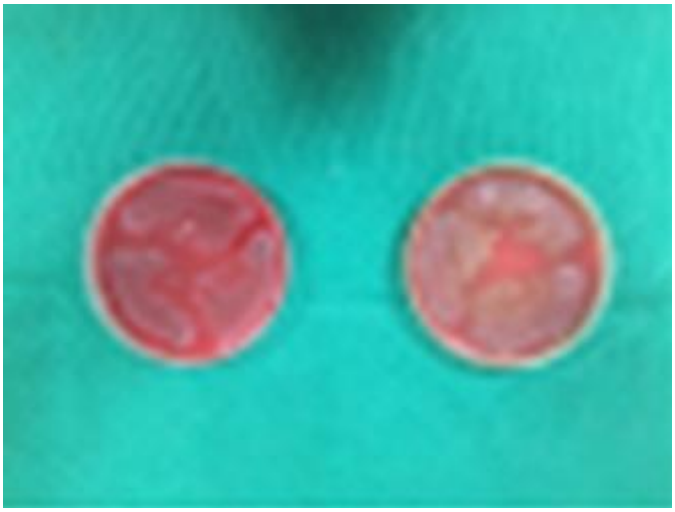


Figure 7: E. faecalis on sheep blood agar

Figure 8: bacterial colony counter

Results

The present study was conducted to assess and compare the antimicrobial efficacy of 3% sodium hypochlorite, 2.5%calcium hypochlorite and ozone water against E. faecalis in roots of primary molars. A total of 60 samples were included in the study and the data obtained was subjected to ANOVA test for statistical significance followed by Student's t- test to compare each group with Positive Control group for antimicrobial efficacy.

The mean antimicrobial activity in the NaOCl group is 9.80×10^2 and the positive control group is 8.50×10^9 with the difference in the mean value of CFU being -8.50×10^9(Graph-1). The difference in mean antimicrobial activity was found to be statistically significant between NaOCl (Group 1) and Positive Control Group with p value < 0.001*.

The mean antimicrobial activity in the $Ca(OCl)_2$ group (Group 2) is 10.20×10^5 and the positive control group is 8.50×10^9 with the difference in the mean value of CFU being -8.49×10^9(Graph-2). The difference in mean antimicrobial activity was found to be statistically significant between $Ca(OCl)_2$ (Group 2) and Positive Control Group with p value < 0.001*.

The mean antimicrobial activity in the ozone group (Group 3) is 13.20×10^7 and the positive control group is 8.50×10^9 with the difference in the mean value of CFU being -8.49×10^9(Graph-3). The difference in mean antimicrobial activity was found to be statistically significant between Group 3 and Positive Control Group with p value < 0.001*.

The mean antimicrobial activity in the NaOCl + Ozone (Group 4) is 7.90×10^4 and the positive control group is 8.50×10^9 with the difference in the mean value of CFU being -8.49×10^9(Graph-4). The difference in mean antimicrobial activity was found to be statistically significant between Group 4 and Positive Control Group with p value < 0.001*.

The mean antimicrobial activity in the NaOCl + $Ca(OCl)_2$(Group 5) is 3.60×10^1 and the positive control group is 8.50×10^9 with the difference in the mean value of CFU being -8.49×10^9(Graph-5). The difference in mean antimicrobial activity was found to be statistically significant between group 5 and Positive Control Group with p value < 0.001*.

The mean value, Standard deviation, and Standard error of mean for all the five experimental groups is depicted in Table-1With the mean antimicrobial activity of NaOCl (Group1) is $9.80 \times 10^{2,}$ Group2 [$Ca(OCl)_2$] is 10.20×10^5, Group 3(ozone) is 13.20×10^7, Group 4(NaOCl+ O_3) is 7.90×10^4 and Group 5(NaOCl+$Ca(OCl)_2$) is 3.60×10^1.The antimicrobial effectiveness reduced in all the 5 groups compared to the positive control group. The antimicrobial effectiveness was found to be higher in Group 3 followed by Group 2, Group 4, Group 1 and Group 5 respectively which is statistically significant.

Intergroup coma parison and intragroup comparison along with the mean value has been depicted in the Table-2 which shows statistically significant difference.

Table 1: intragroup comparision of mean antimicrobial activity

Group	N	Mean	Std Dev	SE of Mean	95% CI for Mean		Min	Max
					Lower Bound	Upper Bound		
Group 1	10	9.80×10^2	2.44×10^2	0.77×10^2	8.05×10^2	11.55×10^2	7.00×10^2	15.00×10^2
Group 2	10	10.20×10^5	2.30×10^5	0.73×10^5	8.55×10^5	11.85×10^5	7.00×10^5	15.00×10^5

Group 3	10	13.20×10^7	3.22×10^7	1.02×10^7	10.89×10^7	15.51×10^7	9.00×10^7	19.00×10^7
Group 4	10	7.90×10^4	3.48×10^4	1.10×10^4	5.41×10^4	10.39×10^4	2.00×10^4	12.00×10^4
Group 5	10	3.60×10^1	2.07×10^1	0.65×10^1	2.12×10^1	5.08×10^1	1.00×10^1	6.00×10^1

The difference mean antimicrobial activity among the groups was found to be statistically significant (P<0.001).

Source of variation between and within the groups has been depicted in Table 2 which denotes statistically significant difference.

Table 2: comparison of mean within and between the groups

Source of Variation	Df	Sum of Squares (SS)	Mean SS	F	P-Value
Between Groups	4	138.82×10^{15}	34.70×10^{15}	166.841	<0.001 *
Within Groups	45	9.36×10^{15}	0.21×10^{15}	---	---
Total	49	148.18×10^{15}	---	---	---

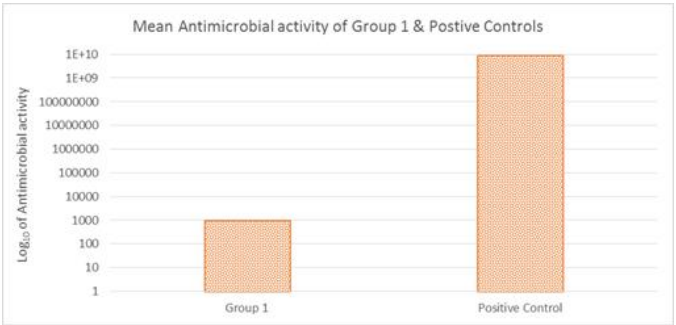
*Denotes significant difference

The difference in mean antimicrobial activity was found to be statistically significant between Group 1 & Group 3 (P<0.001), Group 2 & Group 3 (P<0.001)

Group 3 & Group 4 (P<0.001) as well as between Group 3 & Group 5 (P<0.001).

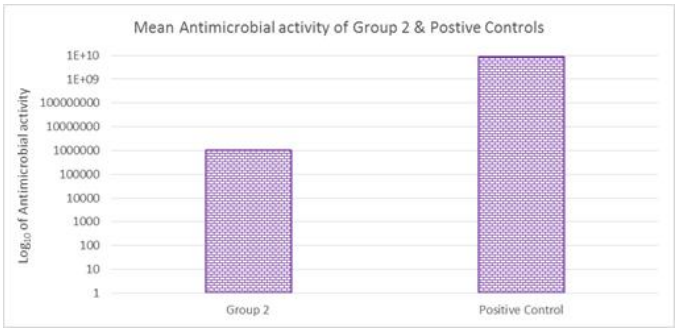
The difference in mean antimicrobial activity was found to be statistically significant between Group 1 & Group 3 (P<0.001), Group 2 & Group 3 (P<0.001),

Group 3 & Group 4 (P<0.001) as well as between Group 3 & Group 5 (P<0.001).



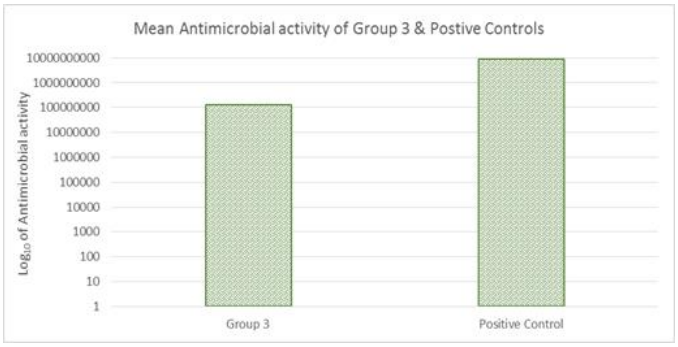
Group 1	980
Positive C	8500000000

Graph 1: mean antimicrobial activity of group 1 and p0sitive controls



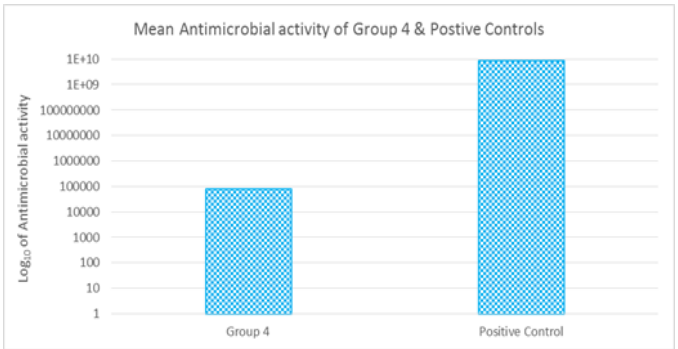
Group 2	1020000
Positive C	8500000000

Graph 2: mean antimicrobial activity of group 2 and p0sitive control



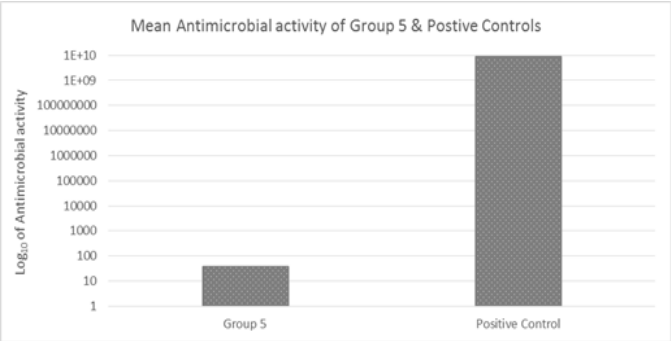
Group 3	132000000
Positive C	8500000000

Graph 3: mean antimicrobial activity of group 3 and p0sitive controls



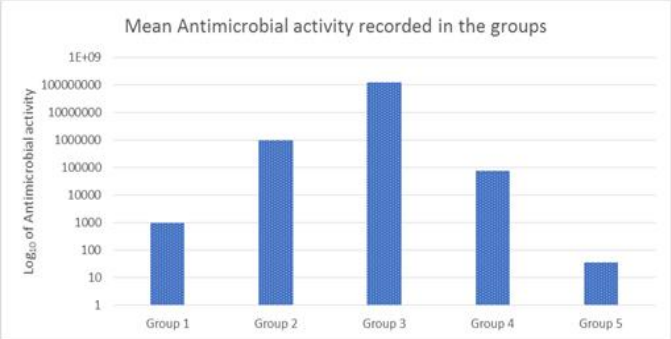
Group 4	79000
Positive C	8500000000

Graph 4: mean antimicrobial activity of group 4 and p0sitive controls



Group 5	36
Positive C	8500000000

Graph 5: mean antimicrobial activity of group 5 and p0sitive controls



Group 1	980
Group 2	1020000
Group 3	132000000
Group 4	79000
Group 5	36

Graph 6: mean antimicrobial activity recorded in the groups.

Discussion

The major goal of endodontic treatment is to disinfect the root canal system before canal obturation. Residual tissue in the root canal may supply enough sustenance for bacteria. Irrigation procedures with antimicrobial agents allow disinfection of places of the root canal system, which is unreachable by instrumentation. Antimicrobial irrigating agents must have qualities like the ability to diffuse the infected place, to terminate microbial growth as well as possessing the ability to dissolve organic material, and to avoid the potential growth of resistance to the solutions.

Deciduous teeth may present unusual internal morphology of the pulp chamber, such as connections, including horizontal anastomoses and furcation, presence of inaccessible areas. Therefore, endodontic treatment of primary teeth is considered extremely sophisticated. In the root canal system with such a complicated and active microbial circumference, choice of an efficacious antibacterial agent is critical during treatment. This in vitro study was designed to assess the antimicrobial efficacy of NaOCl, and Ca $(OCl)_2$, O_3, in the endodontic therapies of primary molars.[3]

One of the main aims of root canal treatment is to eliminate the bacteria, their byproducts and the substrate from the root canal system. The use of irrigation solution in this process is essential to ensure bacterial elimination and eradication of organic tissue remnants. Maximum antibacterial effect, maximum tissue dissolving effect on the necrotic tissues and the least toxic effect on the peripheral tissues are some important features of an ideal root canal irrigant. The complex morphology and the irregularity of root canals of primary teeth negatively affect the success of chemo-mechanical endodontic treatment. Sodium hypochlorite is, till date, the most commonly employed root canal irrigant. The organism E. faecalis was selected in this study because it is most commonly isolated in endodontic retreatment and has been identified to be resistant to currently used chemicals such as sodium hypochlorite and has been found to survive as a mono infection in root canals.[12]

In order to evaluate the bacterial count, CFUs were expressed in log CFU/mL in the present study. This method was chosen based on previous studies and it allows, in an acceptable way, bacterial quantification from the root canal. The plate culture method is the most

commonly used methodology in bacterial reduction studies. E. faecalis is a facultative anaerobic microorganism that is highly resistant to antimicrobial strategies and is usually found in cases of failure of endodontic therapy. It has shown several virulence factors and an ability to persist for long periods in environments with nutrients limitation and survival starvation. These characteristics can help to explain the E. faecalis survival in the root canals in the present study.[5,24]

According to results of present study, the tested decontamination protocols were not able to promote the complete elimination of E. faecalis from root canals. These findings are in accordance with previous studies where E. faecalis was not completely eliminated from the root canals using hypochlorite solutions as decontamination strategy. [24]

In the present study, 3% NaOCl has been used as an irrigant, which showed significant growth reduction, this is in accordance with a study conducted by A. Michael Ringel et. al. NaOCl is a broad-spectrum antimicrobial agent with vital tissue dissolving capacity. It is a predentine solvent and hence it increases dentine permeability by opening up dentinal tubules. Sodium hypochlorite remains one of the most widely used irrigants in endodontic therapy but its toxicity to periapical tissues remains a principle concern.[2,28]

Sodium hypochlorite acts by various mechanisms that is, it neutralizes amino acids forming water and salt. With the exit of hydroxyl ions, there is a reduction of ph. Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as a solvent, releases chlorine that, combined with the protein amino group, forms chloramines. Another mechanism of action is that the hypochlorous acid and hypochlorite ions lead to amino acid degradation and hydrolysis. The chlorination reaction between chlorine and the amino group forms chloramines that interfere in cell metabolism. Chlorine (strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of NaOCl groups (sulphydryl group) of essential bacterial enzymes.[29]

In this study NaOCl showed significant reduction in bacterial growth which is similar to a study conducted by Rupali et al.[2]

Mohammad Frough-Reyhani et al evaluated the antimicrobial efficacy of 2.5% NaOCl and concluded that NaOCl completely eliminated E. faecalis contradicting to the results in the present study.[30]

The results of the present study are in accordance with the findings of Portenier et al, Ghoddusi et al and Davis et al while studies done by Dun Avant et al, Baumgartner et al, Krause et al are in disagreement with these results. The disparity in the results may be caused by differences in methodology and variance in strains tested.[12]

Recently Ca $(OCl)_2$ was tested as an endodontic irrigant by Dutta and Saunders. It is one of the chlorine solutions which is widely used for several disinfection purposes particularly for water purification treatment. The Ca $(OCl)_2$ solution has reasonably low cost. It is also safer for clinical use than NaOCl because the initial rate of tissue dissolution by Ca $(OCl)_2$ is lower than NaOCl and less tissue irritant. It also has greater available chlorine than NaOCl (up to 65% available chlorine) and its byproducts in freshly prepared aqueous solution have both antimicrobial and tissue dissolving effect.[25]

The alkaline property of hypochlorite can improve the bactericidal efficacy against the anaerobic bacteria. In addition, Ca $(OCl)_2$ granules partially dissolve in an aqueous solution and liberate both hypochlorous acid

and Calcium Hypochlorite (CH) and result in an increased pH level. The hypochlorous acid disrupts the vital functions of the organism resulting in death of the cell. It also has been found that exposure of E. faecalis to CH at pH 11.1 for 30 min results in 0.4% cell survival and a relatively small increase in alkalinity (pH 11.5) which can per se increase the level of antibacterial activity.[4,5]

Despite all desirable properties its liberation of chlorine limits its usage; because of its effect on coronal seal and resulting symptoms. Dutta and Saunders concluded that Ca $(OCl)_2$ has the potential of being a root canal irrigant. Studies also demonstrated that tissue dissolution of NaOCl (4.65%) was faster than the Ca $(OCl)_2$ solutions (5 and 10 %) over the first 35 min, but there were no significant differences among the solutions thereafter. [25]

Ca $(OCl)_2$ is available in granules and releasing of two molecules of hypochlorous acid occurs when this substance is dissolved in aqueous solution. Therefore, a higher amount of chlorine is released when compared to NaOCl, where only one molecule of hypochlorous acid is released. However, the results of present study showed significant difference in the reduction of E. faecalis between groups where NaOCl and Ca $(OCl)_2$ solutions were tested. These findings are in accordance to results of previous study of Almeida et al. The higher superficial tension of Ca $(OCl)_2$ can explain the results of present study, since this characteristic can affect the penetration of substance in the root canal walls. In addition to a similar antimicrobial activity, the Ca $(OCl)_2$ has also ability to promote tissue dissolution, it is a biocompatible substance, it maintains more stable the active chlorine content and does not promote reduction of the mechanical properties of dentin as reported by Clarkson et al.[24,27]

Mahadi et al in their study concluded that the antimicrobial efficacy of Ca $(OCl)_2$ and NaOCl in eliminating E. faecalis was similar contradicting to the results of the present study.[4]

According to the present study the group 5 [NaOCl and Ca $(OCl)_2$] showed the highest antimicrobial activity with nearest eradication of the bacteria which may be due to synergistic effect which was in accordance with the study done by Ana Paula et al.

Ozone presents antimicrobial properties that are used for water treatment, the food industry, and treatment of diseases, such as arthritis, otitis, and ulcers.

The antimicrobial effect of the ozone applied to dentistry is rarely discussed. Ozone showed effectiveness on several microbial species found in the oral cavity, for the treatment of lesions and in prosthesis disinfection. Earlier studies showed the antimicrobial effect in different dental areas, such as in periodontology, restorative dentistry and endodontics.[10]

It has been reported that ozone, in the gaseous or aqueous phase, has a strong oxidizing power with a reliable microbicidal effect and it is generally accepted that oxidation mediated by ozone destroys the cell walls and cytoplasmic membranes of bacteria and fungi. After the membrane is damaged by oxidation, its permeability increases and ozone molecules can readily enter the cells causing the death of the microorganism.[8]

In the present study ozone group exhibited the least antimicrobial activity but is statistically significant compared to positive group.

Gaseous ozone was bubbled through distilled water for 20 mins and the same was irrigated into the canals. Ozonated water exhibited antimicrobial activity which was statistically significant and the data is in accordance with a study done by Faria et al. Hems et al evaluated

the antimicrobial effect on E. faecalis, and observed significant bacterial reduction after 4 mins of exposure.

Naga Yoshi et al conducted an in-vitro study on the effect of ozonated water on E. faecalis and verified that the ability of those microorganisms to invade dentinal tubules decreased significantly after irrigation with ozonated water.[10]

Buck et al, Hems et al evaluated ozone potential as an antibacterial agent and the results showed that biofilms incubated with ozonated water showed no significant reduction in cell viability attributable to ozone alone, whereas no viable cells were detected with NaOCl over the same time.[6]

Naga Yoshi et al reported a significant reduction but not complete elimination with NaOCl 2.5%, which is in accordance with the present results. One study, evaluated the efficacy of aqueous ozone against E. faecalis in bovines and considerable decrease in the amount of remaining bacteria was reported. [10]

In another study, Hems et al examined the antibacterial effect of gaseous and aqueous ozone against E. faecalis in root canals. A significant reduction of the remaining bacteria was observed following the application of aqueous ozone.

Cardoso et al investigated the effectiveness of aqueous ozone to eradicate E. faecalis and Candida albicans from root canals. They demonstrated that aqueous ozone can eliminate bacteria. Furthermore, Estrela et al evaluated the antimicrobial efficacy of aqueous ozone and NaOCl in root canals inoculated with E. faecalis and concluded that aqueous ozone did not achieve complete elimination of E. faecalis which is in accordance with the present study. [19]

D. Savitri et al in her study concluded that ozone water showed least effectiveness in eradicating E. faecalis which was similar to the present study.[31]

In a recent study, Zan et al also investigated the antibacterial effect of 4 mg/L aqueous ozone against E. faecalis in root canals and it was concluded that aqueous ozone showed a remarkable antibacterial effect, it did not show equal efficacy to that of the traditional NaOCl against E. faecalis as seen in the present study.[19]

To conclude a combination of NaOCl and Ca $(OCl)_2$ has been shown to be a better irrigant in eliminating the bacteria, this could be due to the synergistic action of hypochlorous ions, however no group was completely able to eliminate the bacteria. Previous studies have also shown difficulty in the same which could be due to the survival rate of E. faecalis or the treatment protocols in the present study.

Conclusion

The present in vitro study was conducted to assess the antimicrobial efficacy of three root canal irrigants against E. faecalis in roots of primary molars. The root canal irrigants used included 3% sodium hypochlorite, 2.5%calcium hypochlorite and ozone water.

Within the limitations of the study neither group was able to completely eliminate the bacteria from the root canals, however all the irrigants used in the study showed significant decrease in the bacterial count compared to the positive group.

A combination of sodium hypochlorite and calcium hypochlorite showed the best antimicrobial activity which can be due to synergistic effect, however calcium hypochlorite alone did not eliminate the bacteria when compared to sodium hypochlorite group. Thus it can be concluded that NaOCl is still the most effective irrigant for elimination of bacteria, however other irrigants can be used as an adjuvant with NaOCl to produce synergistic effect as an irrigant.

Further long-term studies are required to prove calcium hypochlorite and ozone as an effective root canal irrigant individually.

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