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Antimicrobial efficacy of 3% sodium hypochlorite, 2%chlorhexidine, ozonated water and photodynamic therapy in root canal disinfection of primary teeth.

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Abstract

Purpose: The aim of the study was to assess the anti-microbial efficacy of NaOCl, 2% ozonated water, Chlorhexi dine and photo dynamic therapy against E. faecalis.

Material and methods: 40 teeth in 3–5-year-old children indicated for pulpectomy in primary maxillary and mandibular anterior teeth were selected. These were randomly assigned into four groups. NaOCl (Group1), 2% Chlorxedine (Group2), Ozonated water (Group3), Photo dynamic therapy (Group 4).

Samples were collected by immersing sterile paper points for 60 seconds into the canal and then transferring into glucose broth(S1), pulp was extirpated using K and H files. Irrigation of canal was done using respective irrigants and then again samples were collected(S2) for evaluation of reduction in E. faecalis count. The CFUs were counted and the data were analyzed statistically.

Result: On intrara group comparison, there was a significant decrease in microbiological count of E. faecalis before and after irrigation in all the groups ($P < 0.05$). On intergroup comparison, although there was statistically nonsignificant decrease in all groups but NaOcl was more effective in reducing E. faecalis count.

Conclusion: Though a reduction in E. faecalis count was observed in all the groups, NaOCl was found to be the most effective amongst the various agents tested for antimicrobial efficacy.

Keywords: sodium hypo chlorite, Chlorhexi dine, ozonated water, photo dynamic therapy.

Introduction

The main objective of pulp therapy in the primary dentition is to retain every primary tooth as a fully functional component in the dental arch to allow for proper mastication, phonation, swallowing, presser vation of the space required for eruption of permanent

teeth and prevention of detrimental psychological effects due to tooth loss.

Pulpectomy is a conservative treatment approach to prevent the premature loss of primary teeth that can result in loss of arch length, insufficient space for erupting permanent teeth, impaction of premolars, and mesial tipping of molar teeth adjacent to the lost primary molar. In addition, pulpectomy is advantageous for retained primary molar teeth.

Bacteria are the first etiologic factor in the development of pulp and periapical diseases. If the microorganisms that remain in the dentinal tubules are not completely eliminated, it may reinfect the root canal. Antimicrobial solutions should have the capability to diffuse the infection, to prevent or terminate microbial development and also possess ability to dissolve organic material during endo dontic treatment.[1]

The success of root canal therapy is accomplished with the reduction or complete removal of the micro-organisms from the root canal system. [2] Instru mentation alone cannot result in complete elimination of microbes as the root canal system is complex.[3]It is recommended that the intracanal irrigants or medica ments have potent antimicrobial property, dissolve organic tissues and debride the root canal system and be nontoxic to the periapical tissues.

Micro-organisms of the infected root canals consist complex flora such as cocci, rods, spiro chaetes, including fungi. Some of the organisms found commonly in endo dontic infections include Entero coccus faecalis, Strep to coccus mutans, Staphyl ococcus aureus, Actino myces, Candida, Treponema, Porphy romonas, Kocuria rhizo phila and Prevo tella**.** [4]

Amongst these Enter ococcus faecalis (E. faecalis), one of the micro-organisms which is resistant against anti-microbial properties of calcium hydroxide and other intracranial medicaments [1,5] Furthermore, E. faecalis has the sub stantial capability to diffuse into the dentinal tubules, [1,6] exhibits powerful adhesion to collagen, [1,7] and presents defiance to irrigation solutions com monly used during the instrumentation of root canals.

NaOCl which is gold standard demonstrates effective antimicrobial efficacy, is a distinguished necrotic tissue solvent, and is the most productive irrigants in removing organic wreck from the root canal system. [1.8]

Chlorhexidine (CHX) can be considered as an alternative irrigation agent because of its substantivity. It has been utilized as an irrigants and intracanal medicament in endodontics.

Ozonated water has emerged as a strong anteri bacterial agent towards bacteria, fungi, viruses and protozoa. [1,9] Ozone in the aqueous phase has advantages that include potency, lack of mutagenicity, rapid microbicidal effects, ease of handling and suitability for use as a soaking solution for dental and medical devices. [1,11]

Aqueous ozone presents no cyto toxicity and is highly bio compatible compared with other antiseptics. In addition, sodium hypochlorite is not as biocompatible as ozone in the aqueous phase for human oral epithelial cells, period ontal cells and gingival fibroblast cells. [1,12]

Another advancement in this respect is the use of laser for photodynamic therapy also known as photo activated disinfection. This uses non-toxic photoactive dye which is activated by light of specific wavelength in presence of oxygen. The activated photo sensitizer transfers energy to available oxygen that gives rise to highly reactive oxygen species which includes free radicals and singlet oxygen. This oxygen is known to have the ability to kill micro-organisms.[13]

Thus the present study was undertaken to compare the anti-microbial efficacy of 3% sodium hypo chlorite, 2%

chlorhexidine, ozonated water and photo dynamic therapy in root canal disinfection of primary teeth.

Material and methods

This study was randomized single blinded study conducted in the department of Pediatric and preventive dentistry, I. T. S-CDSR, Ghaziabad, Uttar Pradesh, India. Prior ethical approval was taken by ethical committee of I.T.S-CDSR Ghaziabad.

3–5-year-old healthy children who were indicated for pulpectomy in primary maxillary and mandibular anterior teeth and presented themselves to the depart mental clinic for treatment were screened, and those teeth with radio graphic pulpal involvement of pulp, history of spontaneous pain, absence fistula, grossly decayed anterior teeth were included in the study. However, children with medical history external or internal root resorption, mobility, gingival swelling, inadequate bone support, perforated pulpal floor were excluded from the study.

Ul filling the following inclusion criteria were considered for participation in the study. A letter providing all the information of the study was given to the parent/ guardian and they were considered after receiving written consent.

40 primary maxillary and mandibular anterior teeth were randomly assigned into four groups and each group received one of the following root canal disinfection materials

Group 1: Root canal disinfection with 3% sodium hypochlorite

Group 2: Root canal disinfection treatment with 2% chlorhexidine

Group 3: Root canal disinfection treatment with ozonated water

Group 4: Root canal disinfection treatment with photodynamic therapy

After administering local anesthesia, isolation was achieved using rubber dam. Access opening was made and samples were collected by immersing sterile paper points for 60 seconds into the canal which were then tans ferred to glucose broth (S1), followed by extirpation of the pulp and removal of debris using K and H files was done.

After establishing the working length, canals were prepared with H files using pull back motion. Irrigation of canal was done using respective irrigants and then again samples were collected (S2) for evaluation of reduction in E. faecalis count.

For microbiological evaluation

2 microliters of samples were collected before and after disinfection were inoculated on Hi Croma enterococcus faceium agar base for Enterococcus faecalis.

The plates were incubated for 48h in an atmosphere containing 5%`` CO2 and 95% O2 at 37-degree celcius. Colony forming units on plates were counted using colony counter by a blinded assessor.

Statical method

Statistical analysis was done by statistical package for the social sciences (SPSS) software package (SPSS 16 inc, Chicago IL, USA. The data converted into logarithm with base 10 before statistical analysis.

The descriptive statistics mean, standard deviation of E. faecalis count was calculated by One way ANOVA followed by multiple Comparision Bonferroni test for intergroup Comparision and for intragroup Comparision Paired t-test was used.).

The level of significance and confidence interval were 5% and 95% respectively, i.e., $p< 0.05$.).

Results

On intragroup comparison, there was a significant decrease in microbiological count of E. faecalis before and after cavity in all the groups ($P < 0.05$). On

intergroup comparison, although there was statistically nonsignificant decrease in all groups but NaoCl was more effective in reducing E. faecalis count.

Discussion

The major goal of any endodontic treatment is to achieve root canal disinfection. Residual tissue in the root canal may provide enough sustenance for bacteria. Irrigation protocols with various anti-microbial agents allows disinfection of the root canal system, which is unreacha ble by instru mentation.

An effective anti-microbial irrigating agent must possess qualities like the ability to diffuse the infected area to terminate microbial growth as well as have the ability to dissolve organic material, and to avoid the potential growth of resistance to the solutions.[1]

The efficacy of irrigation depends on the ability to bring the irrigants in contact with those elements, materials and structures within the canal system that have to be removed. Root canal irrigants ideally should have a broad anti-microbial spectrum, especially against anaerobic and facultative micro-organisms. The irrigants should dissolve necrotic pulp tissue remnants, inactivate endotoxins and prevent the formation or eliminate the smear layer during instru mentation.

When irrigants come in contact with vital tissues, they should be systemically non-toxic, non-caustic to periodontal tissues and have minimal potential for an anaphylactic reaction.[14]

Infections in primary root canal are associated with a wide variety of microorganisms. Cogulu et al. [15] found that the most prevalent species of bacteria in deciduous root canal were E. faecalis, Porphyromonas gingival is and Treponema denticola. In this study E. faecalis was studied as it is a Gram-positive facultative anaerobic coccus, which is a well-known endodontic pathogen, for the infection of the root canals and also it is resistant against disinfecting agents and antibiotics and can be effectively colonized; it forms a biofilm on root canal walls and invades dentinal tubules.

A variety of irrigants solutions have been used in endodontics to eliminate or reduce the number of bacteria before obturation of the canal systems. In the present study, the efficacy of 2% Chlorhexidine gluconate, ozonated water, 3% sodium hypochlorite (NaOCl) and photodynamic therapy as irrigants was analyzed.

NaOCl is a widely used irrigants in root canal treatment. It can create large zones of inhibition against E. faecalis. In the present study 3% NaOCl was effective in reducing the bacterial counts in root canal.

The result of the present study is consistent with those done by Baum Gartner and Mader [16] that confirmed 2.5% NaOCl is extremely effective in removing vital pulp tissue from dentinal walls. Siqueira et al. [17] reported that instru mentation and irrigation by using 2.5% NaOCl provided a decrease of 99.9% in the count of viable bacteria in the root canal.

However, some authors emphasized that the antibacterial effectiveness of 2.5% concentrations of NaOCI might be improved by usage of larger volumes of solution and continuous exchange of agent.

Ozone has been studied as a new alternative disinfectant agent in root canals. It has shown antimicrobial efficacy against resistant pathogens by neutralizing them or preventing their growth interesting features; debriding action, bactericidal effect, angiogenesis stimulation capa bility and high oxidizing power.

In our study, ozonated water had no residual effect which may be correlated to poor diffusion ability of these substances to deeper areas of the dentinal tubules. Also, irregularity of primary root canal system may resist antibacterial activity of ozonated water. Also rapid

deterioration of the ozone just after contact with organic compounds, such as culture media, which is one of its environmental disadvantages, may cause a decrease in antimicrobial effectiveness of ozonated water. Naga Yoshi et al. [19]

concluded that ozonated water had almost the equal antimicrobial effectiveness as 2.5% NaOCl for endodontic irrigation. They also showed a lower grade of toxicity against bacterial cells. However, Hems et al. [20] evaluated the capability of ozone to terminate an E. faecalis strain and confirmed that its antibacterial effectiveness was not comparable to NaOCl which is in accordance to our study.

Furthermore, Cardoso et al. [21] reported that the ozonated water was efficient against E. faecalis and C. albicans but had no effect. Naga Yoshi et al. [18] reported that after irrigation with O_3aq, the viability of Streptococcus mutans and E. faecalis, invading dentinal tubules significantly reduced.

Haas and Kaymak [22] reported that the antimicrobial effect of ozone depends on varied factors, such as ozone concentration and quantity of bacteria, exposure period, and variables in the bacterial permeability that verified the occurrence of diverse effects on each microorganism.

Chlorhexidine gluconate has a broad spectrum of antibacterial effect. It permeates into the cell wall and causes osmotic imbalance, resulting in leakage of intracellular components.

Two percent liquid con centration is effective at reducing or completely eliminating E. faecalis from the canal space and dentinal tubules. In our study, Chlorhexidine reduced bacterial counts after instrumentation probably due to its ability to adsorb to dentin, acting on bacterial cell walls and cytoplasmic membrane, resulting in the loss of osmotic balance and leakage of intracellular material.[23]

Its antimicrobial activity has residual effects ranging from 7 days [24]to 12 weeks. Substantivity of chlorhexidine is facilitated by its viscosity, which keeps the solution in contact with the canal walls and dentinal tubules.[25] According to Estrela et al.,[26] the antimicrobial activity of chlorhexidine may be explained by the interaction between its cationic nature and the anionic compound on the bacterial surface (phosphatase groups of teichoic acids in Gram-positive bacteria and lipopolysaccharides in Gram-negative bacteria).

Leonardo et al. [27] concluded that CHX gluconate could be recommended as an irrigation solution because of its antibacterial effectiveness, substantivity and lower cytotoxicity compared with NaOCl. In our study 2% CHX showed antibacterial activity but was not as effective as NaOCl.

In the present study, NaOCl showed greater reduction followed by CHX, photodynamic therapy and ozonated water. Onçag et al. [28] reported that after the irrigation procedures for 5 minutes 2% CHX had a faster and more effective on E. faecalis compared NaOCl. Ruiz-Esparza et al. [29] reported that 2% CHX showed a greater reduction as compared to intracanal bacterial loading and suggested that this irrigating solution is an alternative for pulpectomy treatment of necrotic primary teeth.

In recent years photodynamic therapy has been employed to target micro-organisms in root canals suggesting its usefulness as an adjunct to current endo dontic disinfection techniques.

Irrigation with 3% NaOCl was more effective in eliminating E. faecalis compared to PDT in our study. Combined use of NaOCl and PDT is known to provide most strong antibacterial efficacy.[30]

Though numerous dyes have been used for PDT, blue dyes have shown better results in reducing bacterial and fungal infection. Methylene blue (MB) was used as

photosensitizer in this study because of its superior hydrophilicity along with its low molecular weight and positive charge which allows passage across porin-protein channels in outer membrane.

MB is known to predo minantly interact with anionic macromolecule lipo polysaccharides and results in formation of dimers which participate in photo sensitization process. In our study 0.01% methylene blue activated by diode laser with wavelength of 940 nm was effective in reducing the count. Our study was in accordance with study by Komine and Tsuji moto [31] who evaluated the relation between the amount of singlet oxygen generated by different concentrations of activated methylene blue and the bactericidal effect of PDT in suspensions of E. faecalis. They concluded that methylene blue at a concentration of 0.01%, when activated by diode LASER with a wavelength of 660 nm and 200 mW of power, was able to generate the greatest amount of singlet oxygen and consequently result in a large reduction in the number of colony-forming units of the micro-organism.

Bergmans et al [32] tested the bactericidal effect of PDT on strains of Streptococcus anginosus, E. faecalis and mixed cultures containing E. faecalis and Fusobacterium nucleatum inoculated in root canals of extracted teeth. The authors verified that when microorganisms were organized in individual cells or monolayers, PDT easily eliminated them.

However, when microorganisms were arranged in biofilm, the bacterial eradication was substantially reduced in the deeper layers.

Conclusion

Thus, the present study concluded that irrigation with3% sodium hypochlorite was effective in reducing E. faecalis count in root canal of primary teeth compared to 2% chlorhexidine, ozonated water and photodynamic therapy.

Hence, we recommend use of 3%sodium hypochlorite over other irrigants because of its broad antimicrobial spectrum and ability to dissolve necrotic tissue remnants.

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Legend Tables and Figures

Table 1:

Group	Pre	Post		
	Mean±SD	Mean±SD	Diff mean±SD	P value
Group 1	1.78±0.15	0.29±0.30	1.4856±0.26	0.000
Group 2	1.53±0.16	0.21±0.20	1.3141±0.28	0.001
Group 3	1.56±0.28	1.08±0.57	0.4814±0.29	0.022
Group 4	1.65±0.16	0.98±0.23	0.6708±0.13	0.000

Table 2:

P value	0.236	0.002
[b]Grp 1 vs grp 2	0.376	1.00
Grp 1 vs grp 3	0.659	0.020
Grp 1 vs grp 4	1.000	0.053
Grp 2 vs grp 3	1.000	0.010
Grp 2 vs grp 4	1.000	0.025
Grp 3 vs grp 4	1.000	1.000

a Intergroup comparison by One way ANOVA

b Multiple comparison by Bonneferroni test

c Intragroup comparison by Paired t-test

NS-Not Significant

*Significant p<0.05

**Highly significant P<0.01

Table 3: Comparison of % reduction bacterial count

Group	Mean±SD	P value
Group 1	1.477±0.26	0.000
Group 2	1.314±0.28	
Group 3	0.481±0.29707	
Group 4	0.6708±0.13048	
Grp 1 vs grp 2	0.163±0.16	1.000
Grp 1 vs grp 3	0.996±0.16	0.000
Grp 1 vs grp 4	0.806±0.16	0.001
Grp 2 vs grp 3	0.832±0.16	0.001
Grp 2 vs grp 4	0.643±0.16	0.006
Grp 3 vs grp 4	0.18936+0.16175	1.000

Figure 1: Pre and Post E. faecalis count in NaOCl group

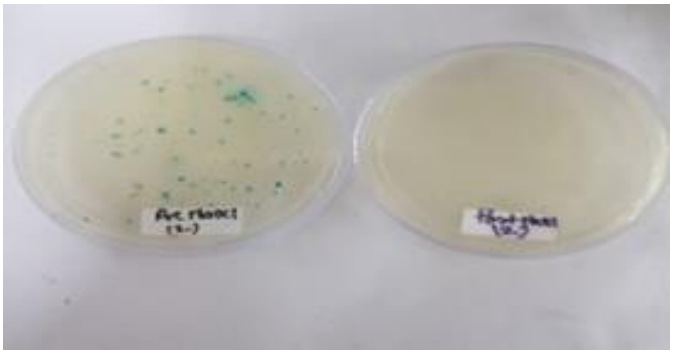


Figure 2: Pre and Post E. faecalis count in Chlorhexidine group.

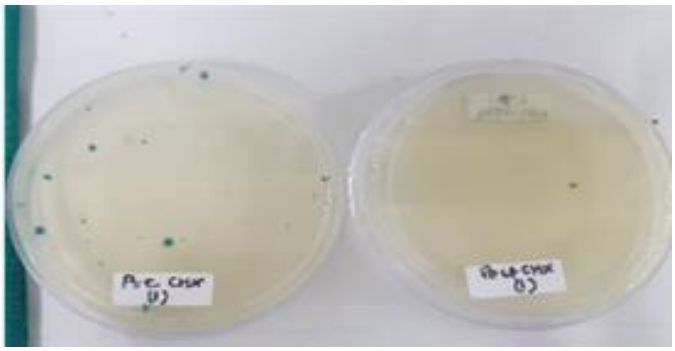


Figure 3: Pre and Post E. faecalis count in photodynamic therapy group.

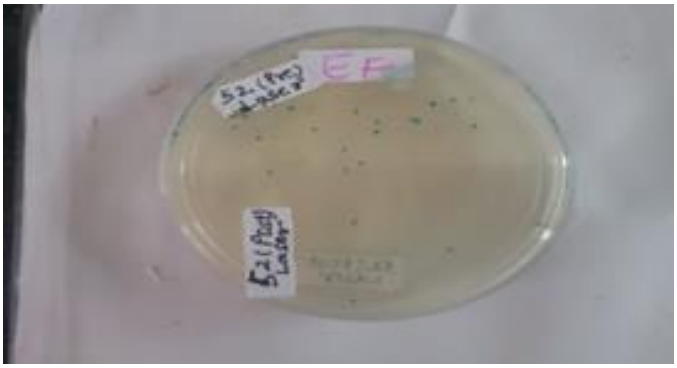
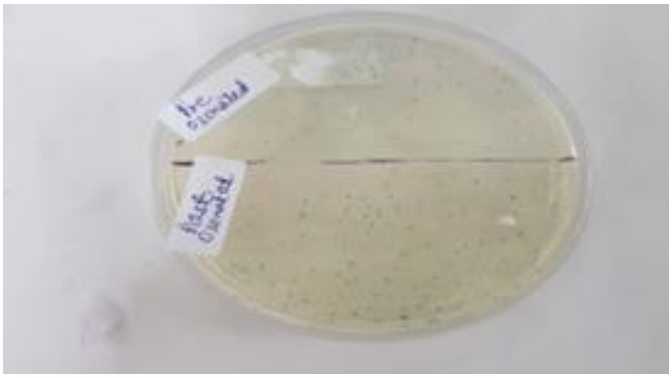
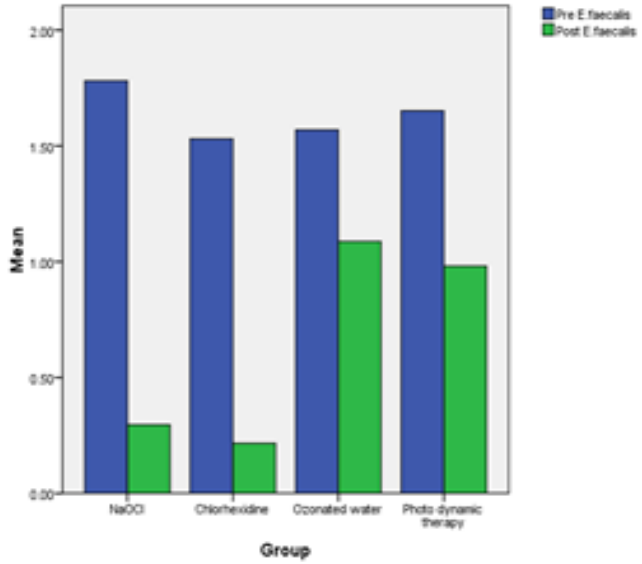


Figure 4: Pre and Post E. faecalis count in ozonated water group.



Graph 1: Intragroup Comparision



Graph 2: Intergroup Comparision