Original Research Paper

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Clinical Research

COMPARISON OF ANTIMICROBIAL EFFICACY OF OZONATED WATER, CHLORHEXIDINE AND SODIUM HYPOCHLORITE AGAINST ENTEROCOCCUS FAECALIS IN ROOT CANALS- AN IN-VITRO STUDY.

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ABSTRACT The complete root canal sterilization is the triumph of endodontic treatment so, the choice of root canal irrigant with utmost antimicrobial efficacy is essential. The aim of this in-vitro study was to evaluate the efficacy of ozonated water, 2% CHG and 2.5% NaOCl in extinction of E. Faecalis. **Materials And Method:** The samples include 30 freshly extracted, single-rooted teeth and were randomly divided into 3 groups. Access opening were done and each tooth was instrumented and autoclaved. All the groups were inoculated with E. faecalis, Irrigation was done using 2.5% NaOCl, 2% CHG and ozonated water in their respective groups. Root canal isolate was taken with sterile absorbent point. Samples were collected, cultured on specific media. E. faecalis was counted on CFU. Collected data was statically analyzed. **Results:** NaOCl supersede in maximum elimination of the E. faecalis, ozonated water exhibit a less effect, and lesser effect was seen with CHG.

KEYWORDS : Enterococcus faecalis, ozonated water, chlorhexidine gluconate, sodium hypochlorite.

INTRODUCTION

The success of root canal treatment depends on the complete sterilization of root canals. This objective becomes more difficult to achieve as the root canal system is polymicrobial in nature. Among the various existing causative microorganisms for the failure of the root canal treatment, Enterococcus Faecalis holds an important position and is also used as a biological marker. [1]

One reason for persistent endodontic infection may be the retention of microorganisms in the dentine tissue of the root canal walls. Dentinal tubules present at the wall of the root canal have been shown to harbor microorganisms.[2]

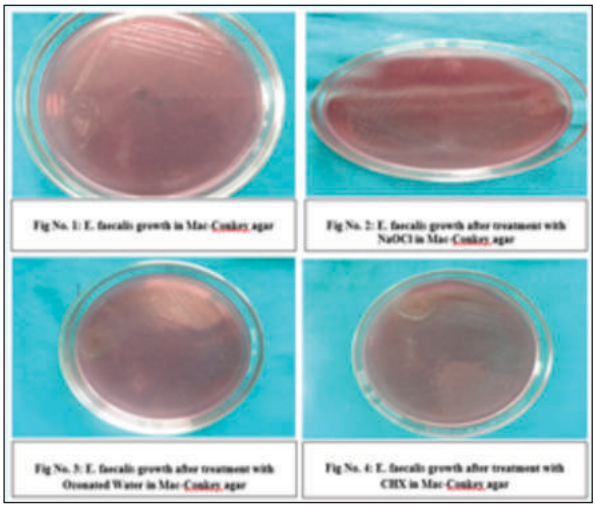
The treatment of apical periodontitis involves the elimination of root canal infection by a combination of mechanical and chemical means. Mechanical instrumentation solely may only reduce the number of bacteria from the root canal system by 50%. This is at least relatively because even in maxillary anterior teeth, the action of files planes only a proportion of the root canal surface. As a result, antibacterial irrigants have to be relied upon to penetrate to the non-instrumented surfaces.[3]

Enterococcus faecalis has long been casuative organism for persistent root canal infections and more recently have been identified as the species most commonly recovered from root canals of teeth with post-treatment disease. 33.3% of cases from which E. faecalis reoccurred at the time of retreatment and failed to heal even after 5 years.[4]

Instead of using various conventional irrigation solutions, chemo-mechanical instrumentation has been the most preferred protocol by most dentists. Flushing the irrigation solution in the canal was reported to be ineffectual in the elimination of bacteria from the canal; therefore, the antibacterial action of the irrigation solution might be the desired property of the solution.[5]

It is well recognized that the primary aim of the treatment of periapical disease comprises the eradication of polymicrobial infections from the involved root canal system. The validity of this has been demonstrated by studies that have shown that the prognosis of successful outcome of treatment is improved by between 10% and 26% when a negative culture test is got prior to obturation. [6]

Ozone whether in the gaseous or aqueous phase, has been shown to be a powerful and reliable antimicrobial agent against various bacteria, fungi, protozoa, and viruses Also, ozone attacks many biomolecules, such as the methionine, cysteine, and histidine residues of proteins. Ozone treatment in endodontics primarily focuses on a high antimicrobial effect as well as helps in minimizing injury to periapical tissues. Its antimicrobial action has been demonstrated against bacterial strains such as Micobacteria, Staphylococcus, Streptococcus, Pseudomonas, Enterococcus, Escherichia coli, and Candida albicans. [7]



AIM & OBJECTIVE

The aim of this study was to investigate the antibacterial effectiveness of ozonated water, sodium hypochlorite and chlorhexidine against Enterococcus faecalis, in order to establish their potential as a root canal disinfectant.

MATERIALS AND METHOD

In this in vitro experimental study, 45 single-rooted teeth were randomly divided into 3 groups of 15 samples each, the teeth with intact crown, no restoration, no cracks and no caries were included for the study, and teeth with calcified or sclerosed canals, teeth with internal or external root resorption, and teeth with open apices was excluded.

Each tooth was cleaned with the help of ultrasonic scalar and stored in normal saline. Accesses opening were done and each tooth was instrumented, sealed and autoclaved. Then, all the groups were inoculated with *E. faecalis*, Irrigation was done using 2.5% sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate and freshly prepared ozonated water in their respective groups. Root canal isolate was taken with the help of sterile absorbent points, and carried to the laboratory in nutrient broth Samples were inoculated on culture plate of Mc-conkey medium and incubated at 35 ± 2°C for 18-24 hours and up to 48 hours. Viable bacterial counts were determined.

Statistical analysis was performed by the SPSS 18 software. Statistical differences among solutions were determined by one-way ANOVA. To compare several groups, Tukey post-hoc test was applied and the mean differences with $p<0.05$ were considered statistically significant.

RESULTS

NaOCl succeeded in maximum elimination of the E. Faecalis, ozonated water produced a less effect, and a lesser effect was seen with CHX (Table 1). The difference of mean value of NaOCl is 3.3×10^5, Ozonated water is 4.08×10^5 and CHX is 4.74×10^5 (Table1) and NaOCl shows significant difference (p-0.033) with CHX (Table2).

Table I: Depicts The Mean, And Range Of Reduction Of CFUs Observed For All Groups (CFU/ml x 10^5).

Groups	N	Mean	Std. deviation	Std. Error	Minimum	Maximum
NaOCl	15	3.3×10^5	1.83×10^5	0.47×10^5	0	5.51×10^5
Ozonated water	15	4.08×10^5	1.76×10^5	0.45×10^5	1.33×10^5	6.31×10^5
CHX	15	4.74×10^5	1.81×10^5	0.46×10^5	2.13×10^5	7.24×10^5

Table II: Mean Difference Between CFU Of *E. faecalis* Under The Effect Of Different Groups (CFU/ml x 10^5).

Groups	Within the groups	Mean difference	Std. Error	Sig.
NaOCl	Ozonated water	-1.05×10^5	0.65×10^5	0.255
	CHX	-1.71×10^5	0.65×10^5	0.033
Ozonated water	NaOCl	1.05×10^5	0.65×10^5	0.255
	CHX	-0.66×10^5	0.65×10^5	0.578
CHX	NaOCl	1.71×10^5	0.65×10^5	0.033
	Ozonated water	0.66×10^5	0.65×10^5	0.578

DISCUSSION

This study was undertaken to compare the antimicrobial effect of 3 different (2.5% NaOCl, Ozonated Water And 2% CHG) root canal irrigants against E. faecalis bacteria.

Sundqvist *et al. (1992)* recovered numerous species of anaerobic bacteria from failed root canal systems. Results of the study showed that 38% of failed root canal treated teeth were contaminated by the bacteria Enterococcus faecalis.

Many studies have evaluated the antimicrobial effects of NaOCl and CHX in endodontic treatment, thus they can be considered as a reference to evaluate new irrigants ozonated water. The effectiveness of the irrigant is dependent on the contact time, concentration, and the nature of the microorganism. In present study NaOCl is most effective agent against *E. faecalis* and this result were similar to study done by **Spratt *et al.*** *(2001)*. It was impossible to make direct comparisons between ozonated water and NaOCl because the concentration of ozone changes constantly during irrigation.

In present study results shows that ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl and this result were similar to study done by **Nagayoshi et al. (2004) Gulabivala *et al.* & Estrela *et al. (2007).***

In present study NaOCl is more effective than chlorhexidine and the results were similar to study done by **Vahdaty *et al.* (1993)** in a 'bovine root dentine infection' model but no differences were shown by **Heling & Chandler (1998) or Siqueira *et al*. (1998).** Moreover, in present study CHX is the least effective irrigant but the study done by **Oncag *et al. (2003)*,** showed that CHG, whether alone in a concentration of 2% or in a concentration of 0.2% and combined with cetrimide, was more effective than NaOCl.

However different authors suggested different irrigant on the basis of their efficacy that's why to establish the most appropriate irrigant with antimicrobial efficacy, we need a broad-spectrum study along with large sample size.

CONCLUSION

In the limitation of this study, the following conclusion may be drawn about these 3-root canal irrigants: The result of this research suggests that NaOCl, Aqueous Ozone and CHG has ability to eliminate E. *faecalis*. And the NaOCl and Aqueous Ozone are more effective in the elimination of *E. faecalis*. And CHG is the least effective among these three-root canal irrigants. However, the antimicrobial effectiveness of these agents can be enhanced by increasing the contact time, flow rate, agitation, concentration, or the addition of other agents to increase the antimicrobial property.

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