Comparative evaluation of antimicrobial efficacy of sodium hypochlorite and Ozone gas & Ozone water as irrigants on enterococcus faecalis an in-vitro study
Ravi Kumar Janga, Madhu Sudhana, M, Harish Tummala

Abstract
Background: Ozone is an effective antimicrobial irrigants which has been accepted by many authors. Aims & Objectives: To evaluate and compare antimicrobial efficacy of ozone gas, ozone water and 2.5% sodium hypochlorite as irrigants against Enterococcus Faecalis. Materials & Methods: Three sets of Six sterile bottles 10 ml of Neutralizing Broth were prepared and group 1 was exposed to ozone gas and group 2 exposed to Ozone water at various time periods and then serial dilutions and subcultures were made to see the reduction in the number of basal colony count. Results: Ozone gas (240s) is more effective than ozone water against Enterococcus faecalis. Conclusion: Based on this study it is suggested that aqueous ozone, ozone gas (5 mg/L) may be used as root canal irrigants.
Key Words: Enterococcus Faecalis; Sodium Hypochlorite ;Ozone Water ; Ozone Gas

Introduction
Antibacterial irrigating solutions are important part of endodontic treatment. The irrigants facilitate removal of necrotic tissue, microorganisms and dentin chips from the root canal by a flushing action. Irrigants can also help prevent packing infected hard and soft tissue apically in the root canal and into the periapical area.(1) The ideal irrigants or combination of irrigants should kill bacteria, dissolves necrotic tissue, lubricate the canal, remove the smear layer, and do not irritate healthy tissue.(2) This paper evaluates and compare antimicrobial efficacy of ozone gas and ozone water as irrigants against Enterococcus Faecalis in an in-vitro study.

Materials and Methods
This in vitro study was conducted to assess the sensitivity of Enterococcus faecalis against ozonated water as well as ozone gas. The Enterococcus faecalis was isolated from samples collected from root canals. Sterile plastic container was used to transport the specimens to the microbiology laboratory within 30minutes to 1hour and incase of delay the samples were preserved in the refrigerator at -2°C to -8°C.

The glucose broth and blood agar medium were prepared and preserved in the refrigerator. It was taken from the refrigerator to reach room temperature and paper points were inoculated into glucose broth in the presence of flame using sterile precaution to avoid contamination. Subcultures were made from the tubes which were showing turbidity on to the blood agar plates by using streak plate method, the inoculated plate were incubated at 37°C in the incubation using candle jar. The growth was observed after 24 to 48 hours of incubation.

The following procedures were used in identification of the isolated organism as Enterococcus Faecalis.

Gram’s Staining is used to see whether a bacterium is gram positive or negative in nature and also to see the size, arrangement and shape of the bacteria. Enterococcus faecalis appears as gram positive cocci arranged in singles, pairs and short chain particles in gram’s staining.

Catalase Test used to see whether an organism produced catalase enzyme or not and Enterococcus faecalis gives negative test.

Bile Esculin agar is a slope agar medium which was used especially for Enterococcus faecalis. Blackening of the medium indicates positive test that is Enterococcus faecalis was present.

The sensitivity of the organism was checked using routine antibiotics in the beginning, for this the blood agar plate were used instead of Mueller-Hinton Agar (MHA) because the isolate was unable to grow on MHA. The methods of sensitivity testing used are penicillin, erythromycin, ampicillin, vancomycin and amoxicillin. Sensitivity is indicated by the formation of inhibition zones surrounding the antibiotic discs. Resistance is indicated by the absence of inhibition zones surrounding the antibiotic discs (Zone of inhibition = Sensitive, No zone of inhibition = Resistant).

Group 1 (Ozone Gas): In this method liquid broth cultures were prepared and exposed to ozone gas at various time periods and then serial dilutions and subcultures were made to see the reduction in the number of basal colony count. The preparation and procedure of the test is as follows. Preparation: Six sterile bottles of 50 ml capacity was taken and 10 ml of neutralizing broth was added to all bottles. Out of the six bottles four bottles are used as test and labeled as ‘T’, one bottles as positive control ‘P’ and one bottles as negative control ‘N’. The test ‘T’ bottles were labeled as T-30 Sec., T-60 Sec., T-120 Sec., T-240 Sec, ‘P’ and ‘N’.
About 5–10 colonies of Enterococcus faecalis from blood agar plate were added to all bottles. The bottles were incubated at 37°C in presence of 5-10% CO₂ for 18 - 24 hours. Two rows of six test tubes with 20 ml. capacity was labeled as Reduced Transport Fluid (RTF30 a, b, RTF60 a, b, RTF120 a, b, RTF 240 a, b, RTF a, b, RTFN a, b and added 9.9 ml of Reduced Transport Fluid to all tubes.

One row of six test tubes with 10ml capacities labeled as RTF30c, RTF60c, RTF120c, RTF240c, RTFPc, RTFNd, and added 1.9 ml of reduced transport fluid to all tubes. The test tubes were arranged in the test tube stands and labeled them. Six sterile blood agar plates were taken and allowed them to dry and labeled as T30, T60, T120, T240, P and N. The ozone generating device, micropipette, tips were kept ready. The ozone generating device, micropipette, tips were kept ready.

Procedure: The Ozone generating device was adjusted to generate 5mg/Liter and separate nozzles were used for each tube. The first step of the procedure was as follows. The first bottle labeled as T30Sec was spurge with ozone for 30 Seconds. Then the second bottle labeled as T60Sec spurge with ozone for 60 Seconds followed by the third bottle labeled as T120Sec spurge with ozone for 120 Seconds. The fourth bottle labeled as T240Sec was spurge with ozone for 240 Seconds. To the positive control bottle labeled as 'P' add 200µl of 2.5% sodium hypochlorite and the cap of negative control bottle labeled as 'N' was kept open and expose to the air for 240seconds and closed.

Group 2 (Ozone Water) In this group liquid broth cultures were prepared and exposed to ozone water at various time periods and then serial dilutions and subcultures were made to see the reduction in the number of basal colony count. The preparation and procedure of the test was as follows.

Preparation: Two sets of six sterile bottles of 50ml capacity were selected. Out of the six bottles in each set, four bottles are used as test and labeled as 'T', one bottle as positive control 'P' and one bottle as negative control 'N'. Label First set of six bottles as T130Sec., T160Sec., T1-120Sec., T1-240 Sec, ‘P1’ and ‘N1’ and 10 ml of distilled water was added to all bottles. Label Second set of six bottles as T2-30Sec., T2-60Sec., T2-120Sec., T2-240Sec, ‘P2’ and ‘N2’ and 10 ml of Neutralizing broth was added and inoculated with 5–10 colonies of Enterococcus faecalis from blood agar plate. Incubate the bottles at 37°C in the presence of 5–10% CO₂ for 18-24 hours. Two rows of six test tubes of 20 ml. capacity and labeled them as RTF – 30a,b, RTF60a,b, RTF120a,b, RTF240a,b, RTFPa, b, RTFNab and added 9.9ml of reduced transport fluid (R. T. F) to all the tubes.

One row of six test tubes of 10 ml. capacities was selected and labeled them as RTF30c, RTF60 c, RTF120c, RTF240c, RTFPc, RTFNd, and added 1ml of Reduced Transport Fluid (R.T.F) to all tubes. The test tubes were arranged in the test tube stands and labeled them. Six sterile Blood agar plates were taken and allowed them to dry and labeled as T30, T60, T120, T240, P and N. The ozone generating device, micropipette, tips were kept ready. The ozone generating device, micropipette, tips were kept ready.

Ozone gas (group 1) showed the best result with five samples, i.e., less number of CFU/ml calculated than followed by ozone water (group 2) (Graph1). The five samples of group 2 showed less number of CFU/ml when compare the 2.5% sodium hypochlorite. The mean CFU/ml determined for test culture before and after exposure to ozone for each experimental group. Scheffes multiple comparison test was used between the groups.

Results

Graph 1: Test statistic: paired t-test. Comparison of groups by: Scheffes multiple comparison

Comparison of 30, 60, 120 and 240 sec with CFU/ml in group - I, group-II

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>946.00</td>
<td>833.40</td>
</tr>
<tr>
<td>60</td>
<td>97.00</td>
<td>466.40</td>
</tr>
<tr>
<td>120</td>
<td>27.80</td>
<td>44.00</td>
</tr>
<tr>
<td>240</td>
<td>83.00</td>
<td></td>
</tr>
</tbody>
</table>

Level of significance = at 5% (p<0.05) (Table 1 and Table 2).
Ozone is currently being discussed as a reliable microbial effect and it is generally accepted that ozone destroys the cell walls and cytoplasmic membrane of bacteria and fungi. After the membrane is damaged by oxidation, its permeability increases and ozone molecules can readily enter the cell causing the microorganism to die. In this study efficacy of ozone gas is compared with ozone water to kill Enterococcus faecalis strain verified that its antibacterial efficacy was not comparable to that of sodium hypochlorite.

Ozone properties are expected to be useful in dentistry although more studies are required to assess its benefits as an effective antimicrobial agent. Ozone is blue gas containing three oxygen atoms. It is irritant, toxic, and unstable it is also very reactive. Ozone is being discussed as a gas that can be used clinically for endodontic treatment. However results of studies into its efficacy against endodontic pathogens has been inconsistent, and there is little information regarding the most appropriate application time and concentration to use.

Enterococcus faecalis a gram positive facultative anaerobe, was chosen as the test microorganism because it has significant implication in treatment resistant cases and is difficult to kill.

The hard nature of this bacterium is such that it can grow and survive as a monocolure under diverse conditions including in nutrition depleted root canal system. Enterococci possess a number of virulent factors that permit adherence to host cells and extracellular matrix, facilitate tissue invasion, effect immunomodulation and cause toxin-mediated damage.

Ozone is currently being discussed as a possible alternative antiseptic agent in dentistry because of its reported high antimicrobial power without the development of drug resistance. Ozone gas is already being used clinically for endodontic treatment.
mentioned above 240 s was effective to kill the Enterococcus faecalis. C. Estrela et al utilized 1.2% of ozone gas and water for a time period of 20 minutes and was not effective against Enterococcus faecalis. In this study the concentration of ozone water and gas is higher than that of C. Estrela et al study and the results suggests that the higher concentration and less time were effective.

K.C. Huth et al conducted a similar study in which the growth of microorganisms was attained within overnight at 37°C, 10 ml of BHI. (3) In this study the growth of organism attained after 24 hours and the growth of organism mentioned in K.C. Huth et al. study were similar. (3) In the present study the efficacy of ozone water and gas against Enterococcus facials is differed in time that is 15 minutes. As per this study increased higher concentration of ozone for less time period that is 240 s, the concentration of 5mg/Ltr was effective against Enterococcus facials.

M Nagayoshi et al reported that ozone in gaseous or aqueous phase has strong oxidizing power and reliable microbial effect. In his study ozonated water was effective against the bacteria in the infected dentinal tubules so he concluded that ozonated water shows strong bactericidal effect against bacteria invading in the dentinal tubules of root canals. (6, 9) R.S. Hems et al stated ozone had an antimicrobial effect on plank tonic Enterococcus faecalis cells and those suspended in fluid, but little effect when embedded in biofilms. (1) The antimicrobial efficacy was not comparable with that of 2.5% NaOCl in his test conditions. In this study Enterococcus faecalis was collected from re treatment canals. The sample collected was not plank tonic. The results revealed that ozone is effective in gaseous and water form against Enterococcus faecalis used in liquid broth culture. (8) While comparing with R.S. Hems study we found that efficacy of ozone was similar to that of our study. (1)

The effectiveness of ozone in liquid broth, was better due to the penetrability, was easier when compared to biofilms. Ozone was effective in this study due to isolated strain. This should be a major factor contributing to the healing or maintenance of an infection involves the post immunological response.

Acknowledgements

The author thanks Sangam dairy water plant for giving the opportunity for utilization of ozone water and gas. This work was supported by micro labs Dr. Harish Tummala.

Authors Affiliations: 1. Dr. Ravi kumar Janga, M.D.S., Dr.Sudha and Nageswararao Institute of Dental Sciences, Chinouptalli, Gannavaram, Krishna Dist, 2. Dr. Madhusudhana M. M.D.S., Sibar Institute of Dental Sciences, Takkellapadu, Peddakakani, 3. Dr. Harish Tummala M.Sc, Katuri medical college, Chinakondrupadu, Guntur Dist, A.P, India.

References


Address for Correspondence

Dr. Ravi kumar Janga, M.D.S., Dr. S.N Institute of Dental Sciences, Chinaoutpalli, Gannavaram, Krishna Dist., Andhra Pradesh, India, Ph: 919440250244, Email: drkumarjanga@gmail.com

Source of Support: Nil, Conflict of Interest: None Declared