ORIGINAL RESEARCH ARTICLE

ANTIMICROBIAL EFFICACY OF OZONATED WATER AND CHLORHEXIDINE MOUTH RINSE ON PORPHYROMONAS GINGIVALIS: AN IN VITRO STUDY

Authors:
Mathew MJ[1],
Jithesh Jain[2],
Peter Simon Sequeira[3],
Vipin Jain K[1],
Sudeep CB[1],
Reshmi JK[4]

[1]Post Graduate Student,
Dept. of Public Health Dentistry,
Coorg Institute of Dental Sciences,
K. K. Campus, Magula, Virajpet
571 218, Karnataka, India.

[2]Professor and Head,
Dept. of Public Health Dentistry,
Coorg Institute of Dental Sciences,
K. K. Campus, Magula, Virajpet
571 218, Karnataka, India.

[3]Professor,
Dept. of Public Health Dentistry,
Coorg Institute of Dental Sciences,
K. K. Campus, Magula, Virajpet
571 218, Karnataka, India.

[4]Senior lecturer,
Dept. of Public Health Dentistry,
Pariyaram Dental College,
Pariyaram Medical College P. O.,
Kannur, 670 503, Kerala, India.

Address for correspondence:
Dr. Mathew MJ,
Post Graduate Student,
Dept. of Public Health Dentistry,
Coorg Institute of Dental Sciences,
K. K. Campus, Magula, Virajpet
571 218, Karnataka, India.

ABSTRACT

Background: *Porphyromonas gingivalis* is an anaerobic bacterium that resides within the biofilm community in the subgingival crevice of the oral cavity and is regarded as a major causative agent in the initiation and progression of severe forms of this disease. The established oral antiseptics for periodontal treatment include Chlorhexidine Gluconate (CHX, 0.2-2%). Regarding side-effects, it is known that Chlorhexidine may cause mucosal desquamation, impaired wound healing and fibroblast attachment to the tooth surfaces, tooth staining and altered taste sensation. The objective of this in-vitro study was to assess the efficacy of ozonated water and 0.2% Chlorhexidine mouth rinse on *Porphyromonas gingivals*..

Methods: The bacterial strains *Porphyromonas gingivalis* (ATCC 33277) were incubated in trypticasein-soy agar supplemented with 5% lamb blood. 100 ml of ozonated water and 0.2% chlorhexidine was poured in the respective well and the plates were incubated at 37^0 for 24 hrs. The zone of inhibition was measured at 1 min and 24 hrs . Independent samples t test was used and p-value <0.05 was considered statistically significant.

Results: After 1 min the effect of ozone on *P.gingivalis* was superior when compared to chlorhexidine.

Conclusion: Ozonated water significantly inhibits the growth of *P.gingivalis* in-vitro.

Key words: Chlorhexidine, Ozone, Bacteria.

J Odontol Res 2013;1(1)11-6.

INTRODUCTION

Actinobacillus actinomycetemcomitans and *Porphyromonus gingivalis* are major putative periodontopathicbacteria.[1] *Actinobacillus actinomycetemcomitans* has been closely associated with periodontitis in young individuals and with cases of refractory adult periodontitis. *Porphyromonus gingivalis* occurs in severe adult periodontitis, failing guided tissue regeneration and acute periodontal abscesses. Other organisms have also been related to advanced periodontitis, although with less evidence.[(1)] *Actinobacillus actinomycetemcomitans* and *Porphyromonus gingivalis* can also seed to and produce severe infections in extraoralsites (gastrointestinal tract, respiratory tract, colon etc.).[(2)]

Removal of dental plaque thus forms an important part of controlling and treating periodontal disease, which brings about qualitative as well as quantitative changes in the subgingival microflora. A number of chemical adjuncts have been used to improve the outcome of mechanical oral hygiene procedures, one of which is chlorhexidine broad spectrum antibiotic with antimicrobial effects on gram positive as well as Gram-negative bacteria, viruses and fungi.[(3,4)]

An alternative approach to conventional antimicrobial or antiseptic agent in the suppression of subgingival bacteria is to inhibit their growth by changing the subgingival environment, which has been shown to be highly anaerobic with a prevailing low oxygen tension.[(5)] First advocated by Dunlop in 1913, various agents such as molecular oxygen,[(6)] hyperbaric oxygenation[(7)] and hydrogen peroxide have been applied.[(8)] It has been shown that repeated sub gingival oxygen irrigation in previously untreated deep periodontal pockets resulted in a significant clinical improvement of the periodontal baseline conditions.[(9)] Recently, ozone therapy is gaining popularity in various treatment modalities in the field of medicine, dentistry, veterinary, food industry, water treatment etc. In dentistry, ozone is being successfully utilized for the treatment of dental caries.[(10)]

Recent investigations have reported antimicrobial effects on oral pathogens by both gaseous and aqueous forms of ozone, and the effectiveness of ozone in the treatment of oral diseases is currently a subject of intense research.[(11)]

The current study outlines the latest thinking regarding the efficacy of ozone therapy and Chlorhexidine 0.2% on virulent periodontal pathogenic microorganism, *Porphyromonas gingivalis.*

The objectives of this study were to assess the efficacy of ozonated water and 0.2% chlorhexidine mouth rinse on *Porphyromonas gingivalis* and to compare the efficacy of ozonated water and 0.2% Chlorhexidine mouth rinse on *Porphyromonas gingivalis* inhibition.

METHODS

The materials used in this study were:

Test Materials used:

- Ozone
- Chlorhexidine (0.2%)

  Microorganism
- *Porphyromonas gingivalis ATCC 33277*
- The microbial strains selected for the present study were collected from the American Type Culture Collection (ATCC), USA.
- Brain heart infusion agar
- Vernier Caliper.

Ozonated Water Preparation

The wells were subjected inoculated with ozonated water that was released from an irrigation device, “Aqu Ozone”. The device released a single pulsating stream of ozonated water from the nozzle, which could be adjusted for different speeds and pressures ranging from 350 to 500 kPa (kilo pascals) and an ozone output of 0.082 mg/h, at a noise output of <70 dB (decibels) and water outflow of ≥450 ml. A 20-gauge blunt needle was bent and attached to the tip of the nozzle of the ozone dental jet holder and then inoculated into the different wells.

Microbial Assay

The bacterial strains *Porphyromonas gingivalis* (ATCC 33277) was obtained by Dept of Microbiology, Coorg Institute of Dental Sciences, Karnataka. The strains were incubated in trypticasein-soy agar supplemented with 5% lamb blood at 37°C in an oxygen-free atmosphere, and stored in the fridge until use. They were preserved by freezing suspensions at -70°C in skimmed milk supplemented with a cryoprotectant. For the experiments, bacterial suspensions (pure cultures) were inoculated in TS broth and incubated for 7 days at 37^0 C, after which they were placed in the fridge. Wells of 8 mm diameter were cut into solidified trypticasein-soy agar media using a sterilized standard device. 100 ml of ozonated water and 0.2% chlorhexidine was poured in the respective well and the plates were incubated at 37%for 24 hrs. To ensure the consistency of all findings, the experiment was performed and repeated under strict aspetic conditions. The bacterial activity was expressed in terms of the mean of zone of inhibition (in mm) produced by each test product at the end of incubation period. All the measurement of zone of inhibition were carried out by a single examiner. Calibration of the examiner was done prior to and during the study by reexamining 5 % of the samples, to minimize intra examiner variability. Intra examiner agreement was determined using kappas statistics (k) and the score thus obtained (k=0.82) almost perfect, according to Landis and koch, thus meeting the scientific requirement of validity and reliability

Statistical Analysis

The collected data was classified and tabulated in Microsoft Office excel. SPSS for windows version 17 software (Chicago, USA) was employed for statistical analysis. Mean*(X)* and Standard Deviation (SD) were calculated. Independent samples t test was used for and p-value <0.05 was considered statistically significant.

RESULTS

The study was conducted to assess the efficacy of ozonated water and 0.2% Chlorhexidine mouth rinse on *Porphyromonas gingivalis.* Test products (Ozone and chlorhexidine) were tested after 2 intervals. i.e after 1 min and after 24hours. After 1 min the effect of ozone on *Porphyromonas gingivalis* was superior (24CFU/ml) when compared to chlorhexidine (22CFU/ml) as mentioned in Graph I .

DISCUSSION

Ozone is an allotropic form of oxygen. It possesses unique properties which are being defined and applied to biological systems as well as to clinical practice. As a molecule containing a large excess of energy, ozone through incompletely understood mechanisms, manifests bactericidal, viricidal and fungicidal actions which may make it a treatment of choice in certain conditions and an adjunctive treatment in others[12].

Ozone can be considered a potential therapy for periodontal disease due to its wide range of biological effects. Some of these are:

- Inactivation of bacteria, viruses, fungi, yeast and protozoa,
- Enhancement of circulation,
- Stimulation of oxygen metabolism,
- Activation of the immune system.

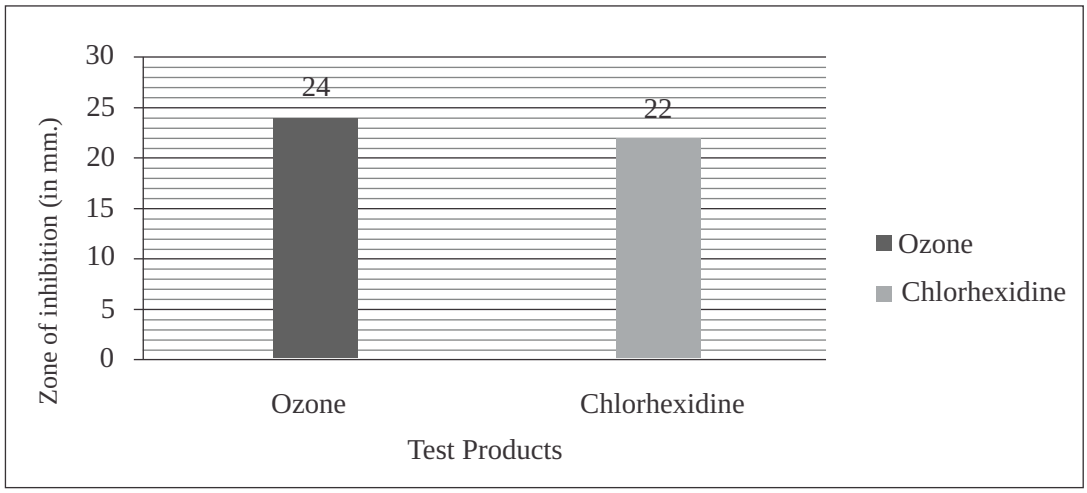
Each of these actions is without doubt beneficial in management of inflammatory periodontal disease[12].

The results of the present study showed that inhibition of the *P.gingivalis,* the specific strain selected by both ozone therapy and chlorhexidine were comparable. The difference between the inhibitory effects of both the test materials was statistically significant ($p < 0.05$).

The results of this study were thus in line with a previous study conducted by Kammer et al to examine the effect of ozonated water on oral microorganisms including *Porphyromonas gingivalis*. The viable count of the organism was found to decrease when treated with ozonated water.

This particular study had studied the effect of ozonated water on several other microorganisms implicated in the causation of periodontal disease.

Graph I : Zone of inhibition (in mm.) of the test products against *P. gingivalis* after 1 minute



After 24hrs the effect of ozone on *Porphyromonas gingivalis* was low (6CFU/ml) when compared to chlorhexidine (22CFU/ml) as mentioned in Graph II, which indicates that residual effect of chlorhexidine remains the same whereas, the effect of Ozone was depleted after 24hrs.

Graph II : Zone of inhibition (in mm.) of the test products against *P. gingivalis* after 24 hours

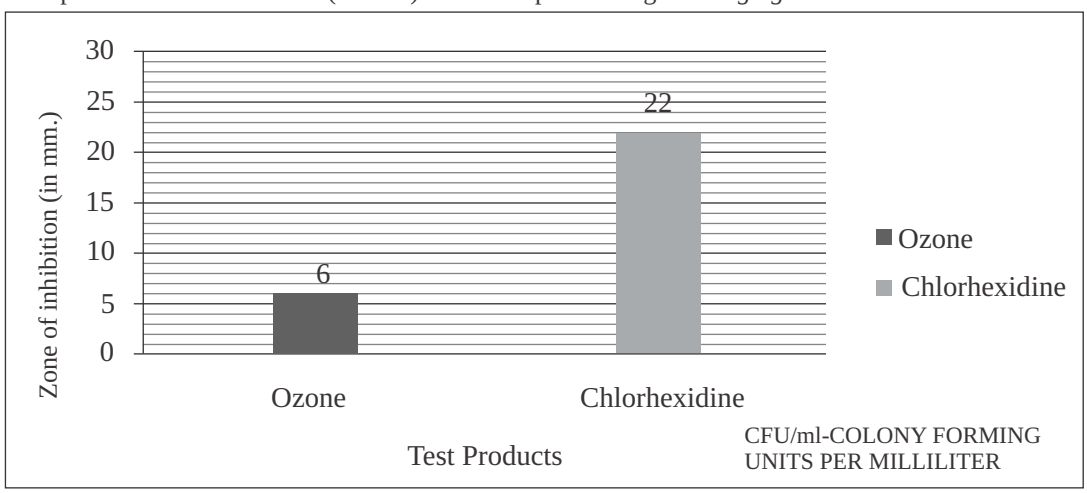


Table 1 shows antimicrobial activity of the extracts against *Porphyromonas gingivalis* after 1 min and 24 hours. Ozone showed a lowest inhibition rate against *Porphyromonas gingivalis* compared with the chlorhexidine and this finding was found to be statistically significant.

Samples	Mean zone of inhibition (in mm) ±S.D		p value
	After 1 min	After 24hrs	0.0482*
Ozone	24	6	
Chlorhexidine (0.2%)	22	22	

*- statistically significant

Nevertheless, the results for the bacterial strain *Porphyromonas gingivalis* can be compared to the present study.

Our findings are similar to that obtained by Ripolles de ramon et al.[13], who found a significant decrease in the microbiological periodontal parameters.

To compare the effectiveness of ozonated water, the present study used a positive control, namely chlorhexidine which is the standard adjunctive therapy for treatment of periodontitis.

The outcomes in both the groups (ozonated water and Chlorhexidine) with regard to the inhibition of the bacterial count were similar at 1 min after treatment. However, assessment after 24 hours showed that microbial count in the ozonated group had increased.

This differed significantly from that of Chlorhexidine group. The substantivity property of Chlorhexidine is well known and would have accounted for this difference found after 24 hours. Hence, this could be one drawback of ozonated water when compared to Chlorhexidine.

Our study considered only a single strain of a single species of microorganism known to a periodontal pathogen. The effect of ozone therapy at different concentrations on other microorganisms implicated in the red complex of periodontal disease, at various time intervals need to be determined. The inherent limitations of an in-vitro study need to kept in mind while interpreting the results.

Effectiveness trials in-vivo where the effect of other confounders like saliva, which could dilute the concentration of ozone, a number of other aerobic and anaerobic microorganisms in and around the gingival pocket, the mode and dose of administration, the cost of the therapy need to be evaluated thoroughly before making generalizing statements.

Ozone therapy significantly inhibits growth of the *Porphyromonas gingivalis* which was the selected strain in the present study. One major drawback of ozone is its unstable nature. The absorbance of water in the ozone increased almost linearly with time from 5 to approximately 60 seconds. The stability of ozone in the water is low and ozone dissipated very quickly in the ozone demand free water at room temperature over 5 mins, as described by Shechter.[14,15]

CONCLUSION

Ozonated water significantly inhibits the growth of *Porphyromonas gingivalis* in vitro. However, the inhibitory effect was pronounced at 1 min and was low at 24hours.

Ozone may be considered as an alternative management strategy due to its powerful ability to inactivate microorganisms. Also, there is growing evidence that ozone can be employed as a useful therapeutic agent in both dentistry and medicine.

REFERENCES

1. Slots J, Chen C. The oral microbiota and human periodontal disease of the normal microflora. London: Kluwer Academic Publishers, 1999:101-127.
2. Van Winkelhoff AJ, Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in nonoral infections.Periodonto12000 1999: 20: 122-135.
3. Bernstein D, Schiff G. IN vitro viricidal effectiveness of a 0-12% chlorhexidine Gluconate mouth rinse. J Dent Res 1990;69:874-6.
4. Ellepola AN, Samaranayaka LP. Adjunctive use of chlorhexidine in oral Candidoses.A review Oral Dis 2001;7:11-7
5. Mettraux GR, Gusberti FA. Oygen tension in untreated human periodontal pockets. J Clin Periodontol 1984;55:516-21.
6. Hirsch RS, Clarke NG, Townsend GC. The effect of locally released oxygen on the debelopment of plaque and gingivitis in man. J Clin Periodontol 1981;8:21-8.
7. Guenterman RH, Bishop JG, Collings KC, Dorman HL. The effect of increased blood oxygen tensions on periodontal disease. J Periodontol 1972;43:233-6.

8. Wennstrom J, Lindhe J . Effect of hygrogen peroxide on developing plaque and gingivitis in man. J Clin Periodontol 1979;6:115-30.

9. Schlagenhauf U,Horlacher V, Netuschel L, Breck M. The effect of repeated subgingival oxygen irrigations in previously untreated periodontal pockets. J Clin Periodontol 1994;21:48-50.

10. Rickard GD, Richardson R, Johnson T, McColl D, Hooper L. Ozone therapy for treatment of dental caries. Cochrane Database Syst Rev 2004;(3):CD004153.

11. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, Hickel R, Brand K. E ect of ozone on oral cells compared with established antimicrobials. Eur J Oral Sci 2006;114: 435-440

12. Durga Kshitish, Vandana K Laxman. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients.A clinical and microbiological study.Indian Journal of Dental Research 2010;3:341-348.

13. Ripolles de ramon, J., Colmenero ruiz, C., Gallut ruiz, J. et al. Clinical, microbiological and inmunological evaluation of the ozone's therapy in patients with moderate-severe periodontal pockets. AvPeriodon Implantol. 2004;16,1:63-72.

14. Wilson M. Suceptability of oral bacterial biofilms to antimicrobial agents. J Med Microbiol 1996;44:79-87.

15. Scheter H. spectrophotometric method of determination of ozone in aqueous solution.Water Res 1973;7:729-39.