



Assessment of Bacterial Micro Colonies Present in Tissue Samples Obtained Using Ozonated Water as an Irrigating Agent from Patients with Periodontitis

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Authors' contributions

This work was carried out in collaboration between both authors. Author NR carried out the study, participated in the sequence alignment, statistical analysis and drafted the manuscript. Author SM conceived the study, participated in its design and coordinated and provided guidance to draft the manuscript. Both authors read and approved the manuscript.

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ABSTRACT

Periodontal diseases are chronic inflammatory diseases that are constantly associated with microorganism, conventionally scaling and root planing followed by flap surgical procedure is done to treat chronic periodontitis condition, irrigating agents are used during both sub gingival scaling and root planing procedure and in flap surgical procedure. Saline and distilled water are the commonly used irrigants and they non specifically reduce the bacterial count, Ozonised water is also used as an irrigating agent. Though it's effect on plaque is known to be effective its effect on tissues is still questionable. The study is conducted to assess the number bacterial micro colonies formed in gingival tissues after irrigating with ozonated water and comparing it with distilled water and saline water which are the commonly used irrigating agents.

The aim of the study is to assess the bacterial micro colonies formed in gingival tissues after irrigating with ozonated water and comparing it with distilled water and saline water which are the commonly used irrigating agents.

A randomized, split mouth study was performed. A total of 10 patients suffering from chronic

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generalised periodontitis were recruited into the study; 20 gingival tissue samples were obtained from the patients at the time of surgery. Both the patient and the clinician were blinded regarding the type of the irrigating agent used. The plaque samples were transferred into carrier medium and were cultured for 24 hours; 24 h after culture, the samples were collected and total colony forming units were calculated. On counting the number of colony forming units formed, it was found that the amount of colonies formed from the samples treated with ozone showed mixed results, wherein it showed both increase and decrease in colony counts.

Keywords: *Periodontitis; microorganism; bacterial count; ozone therapy; irrigating agent; saline; distilled water; ozonated water.*

1. INTRODUCTION

Periodontal diseases are constantly associated with bacterial organisms such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* in subgingival environment, and gingivitis and periodontitis are the common diseases which are affecting the tissues surrounding the tooth [1,2]. Bacteria are the prime etiological agent in periodontal disease, nearly 500 bacterial agents colonise the adult mouth and these bacterial microorganisms are known to cause destructive periodontal diseases [3,4]. Mechanical removal of the biofilm by scaling and root planing followed by flap surgery along with thoroughly irrigating the surgical site and adjunctive use of antibiotics have been the conventional methods of periodontal therapy. The primary purpose of irrigation is to nonspecifically reduce the bacteria and their by-products that lead to the initiation or progression of periodontal diseases.

Many agents like Natural saline, chlorhexidine, iodine are used as irrigating agents during periodontal surgical procedure, which is also known to have antibacterial activity [5]. Ozone is a triatomic molecule it has three oxygen atoms, and it is a gas with extremely pungent odor and it is also an unstable gas that quickly gives up nascent oxygen molecules to form oxygen gas. Ozone is naturally produced by the photodissociation of molecular oxygen into activated oxygen atoms [6–8]. Due to the property of releasing nascent oxygen, it has been used in medicine to kill bacteria and fungi and to control hemorrhages, commercially it is produced in ozone generators [7].

Ozone was first discovered in the year 1840 by a German chemist Christian Friedrich Schonbein it was used in medicine by Lander in 1932. Ozonated water was used as disinfectant by Dr. E.A. Fisch a Swiss dentist. Joachim Hansler and Hans Wolff developed the first ozone

generator for medical use [9,10]. Medical grade ozone is a mixture of pure oxygen and pure ozone in the ratio of 0.05-5% of O₃ and 95-99.5% of O₂. Due to the instability of O₃ molecules, it must be prepared immediately before use and cannot be stored over long periods of time. Hence ozone is administered in the form of ozonated water, ozonated oil or gel and in gaseous form [11]. Ozonated water can be used as mouth rinse and is used to disinfect oral mucosa, and ozone water jet can be used in root canal therapy and in treating gingivitis. Ozone is also used as an immunostimulant, antihypoxic, detoxicating, and antimicrobial agent.

Ozonated water can be used to irrigate the affected area during scaling and root planing, and in non-surgical pocket therapy like curettage and can be used as an irrigant during the surgical procedure and as a final surgical site lavage.

The main use of ozone in dentistry relies on its antimicrobial properties. The antimicrobial effect of ozone is a result of its action on cells by damaging its cytoplasmic membrane due to ozonolysis [12]. It is proved to be effective against both Gram positive and Gram negative bacteria, viruses and fungi.

Though ozone has lots of benefits it has some disadvantages also; Ozone inhalation may be toxic to the pulmonary system. The known side effects of ozone are upper respiratory irritation, rhinitis, cough, headache, nausea, and shortness of breath [13]. But the use of ozone in Periodontology seems to be undismissable. Previously we have worked on plenty of topics in periodontology [14–26]. Considering the advantages and disadvantages of using ozone and its role in periodontics the study was designed and aimed to assess the bacterial micro colonies formed in gingival tissues after irrigating with ozonated water and comparing it

with distilled water and saline water which are the commonly used irrigating agents.

2. MATERIALS AND METHODS

The participants of the study were selected from the outpatient department of periodontics, Saveetha Dental College and Hospitals, Chennai India. A split mouth clinical study was performed. 10 patients who were suffering from chronic generalized periodontitis were selected for the study based on convenience sampling ; Randomisation was performed using a lot method , the patient was asked to pick up a slip wherein the type of irrigating agent to be administered and the site of application has been enclosed in an envelope. The test site was irrigated using ozonated water and the control site was treated using either natural saline or distilled water; Ozone and Saline (group 1),Ozone and distilled water (group 2).

The patient was blinded regarding the type of the irrigating agent used. Patients who have respiratory tract disorders, lactating mothers, patients suffering from systemic disorders, and patients under antibiotic therapy were excluded from the study and healthy patients with chronic periodontitis were included in the study.

All the patients involved in the study underwent periodontal flap surgery. Gingival tissue was collected during flap surgery using a sharp-tipped instrument (Gracey curettes). The collected gingival tissue samples were immediately transferred into an Eppendorf tube containing 1 ml of brain–heart infusion broth.

Patients were subjected to irrigation with water ozonated by zerodis portable ozone generator which delivers ozone with ozone density of 300 mg/h (+/-10%) with a power of 13 W for 15–30 min. The water gets ozonized, and the ozonated water was used as an irrigating agent in the test site. Similarly the control site was also treated and was irrigated using either of Natural Saline or distilled water post irrigation the gingival tissue sample was collected and was transferred into eppendorf tube the tissue samples were microbiologically analysed for the amount of micro colonies it formed.

Microbiological analysis: Bacteria were isolated from tissue sample, Nutrient agar medium was prepared and 100 mu litre of individual sample was spread into the solidified agar medium using

L-rod, the plates were then incubated at 37 degree Celsius for 24 hours and the microbial colonies formed were counted.

Procedure of Isolation of Bacteria from tissue sample: All the contents were dissolved and autoclaved at 121°C, 15 lbs for 15 minutes. After sterilisation media was poured into sterile petri plates and allowed to solidify for 30 minutes (Table 1).

Spread plate method was used 100 µl of individual samples was spread into the solidified agar medium using L-rod. The plates were then incubated at 37°C for 24 hours. The colonies were studied for its count.

Table 1. The Nutrient agar medium contains the above ingredients

Peptone	5 gms
Beef extract	1.5 gms
Yeast extract	1.5 gms
Sodium chloride	5 gms
Agar	20 gms
Distilled Water	1000 ml
pH	7.4

3. RESULTS AND DISCUSSION

A total of twenty samples were collected from the patients and was subjected to microbial colony counting.

In group 1 microbial colony counts from tissue samples treated with ozonated water and saline was assessed (Figs. 1,3,4) five sites were treated with ozonated water and other five sites were treated with saline. It was seen that the number of microbial colony counts were higher in tissue samples treated with saline, it was also observed that in two samples out of the five tissue samples, the number of colonies formed in sites treated with ozone was higher.

In group 2 microbial colony counts from tissue samples treated with ozonated water and distilled water was assessed (Figs. 2,5,6) five sites were treated with ozonated water and other five sites were treated with distilled water. It was seen that the number of microbial colony counts were higher in sites treated with ozonated water but one tissue sample showed reduction of bacterial microcolonies in ozonated water.

On counting the number of colony forming units formed, it was found that the amount of colonies formed from the samples treated

with ozone showed mixed results, wherein it showed both increase and decrease in colony counts.

From the results obtained it has been seen that compared to saline and distilled water ozonated water did not show any suggestive difference in the number of colonies formed. Ozonated water showed both increase and decrease in the microbial colony counts. The results of this study is in concordance with few other studies where scaling and root planing with ozone water irrigation has no additional effect in improving the periodontal conditions of patients [27] but other studies indicate that there is a significant improvement in both clinical and microbiological parameters when ozonated water is used for sub gingival irrigation and could be an efficient adjunct to scaling and root planing in the treatment of chronic periodontitis [28]. Brauner et al has demonstrated that the combination of professional tooth cleaning and daily rinsing of the mouth with ozone water can improve clinical findings in cases of gingivitis and periodontitis.

Also Carinci et al stated that The topical use of ozone therapy along with mechanotherapy improves clinical results. Local ozone therapy into the pocket achieves a greater reduction of bacterial loading, proving bactericidal for most perio pathogens. It was seen that in results of previous study done by Habashneh et al where scaling and root planing done along with ozonated water as irrigating agent has no significant effect as compared to scaling and root planing done along with distilled water as irrigating agent [29]. But in our previous study it where it was found that there was reduction in microbial colony counts from samples treated with ozonated water compared to that of distilled water [30].

Ramzy et al in their study recommended the use of ozonised water as irrigating agent in the treatment of aggressive periodontitis [31]. Nagayoshi et al tested the efficacy of ozonated water on survival oral microorganisms. Gram negative bacteria were more sensitive to ozonated water than gram positive bacteria.

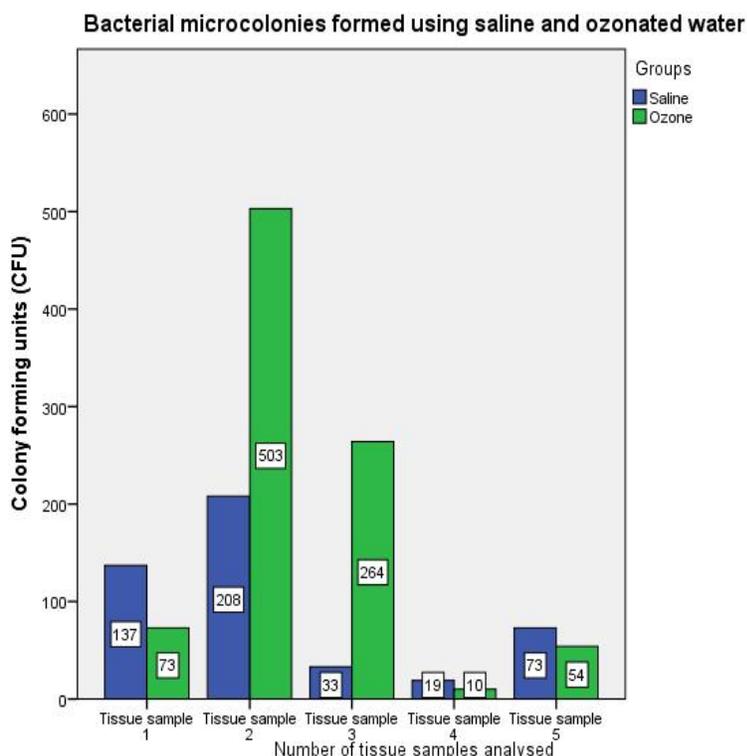


Fig. 1. Microbial colony counts from tissue samples treated with ozonated water and saline was assessed .The bar graph represents the number of colonies formed, Blue colour depicting saline and green colour depicting ozone.The x axis depicts the number of tissue samples and the Y axis represents the number of colony forming units (Group 1)

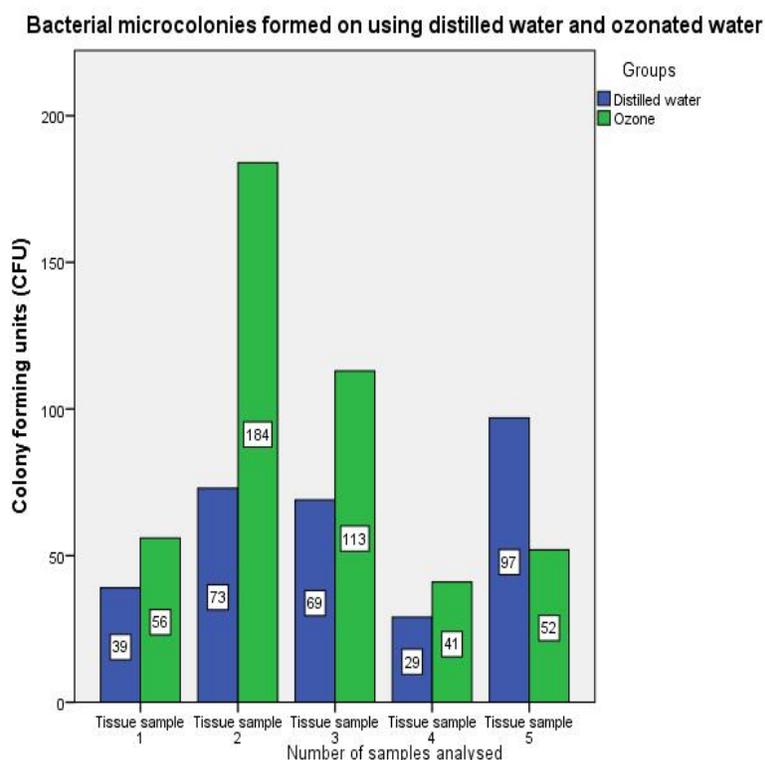


Fig. 2. Microbial colony counts from tissue samples treated with ozonated water and distilled water was assessed. The bar graph represents the number of colonies formed, blue colour depicting distilled water and green colour depicting ozone. The x axis depicts the number of tissue samples and the Y axis represents the number of colony forming units (Group 2)

They also stated that ozonated water had strong bactericidal activity against bacteria in plaque biofilm [32]. Kshitish and Laxman et al in their study observed that the reduction of Aa (25%) using ozone was appreciable when compared to chlorhexidine. They concluded that despite the substantivity of chlorhexidine, the single irrigation of ozone is quite effective to inactivate microorganisms [33].



Fig. 3. Bacterial micro colonies formed after using saline as an irrigating agent



Fig. 4. Bacterial micro colonies formed after using ozonised water as irrigating agent

The results of the above mentioned studies do indicate that ozonated water can reduce bacterial load; it has to be considered that the above mentioned studies were done using plaque sample, the effects of ozonated water on tissues has to be explored in detail, hence in this pilot study we have used tissue samples and assessed the number micro colonies from the tissue samples. A smaller sample size and all the

tissue samples were obtained at single time point rather than multiple time points were the limitations of the study.



Fig. 5. Bacterial micro colonies formed after using distilled water as irrigating agent



Fig. 6. Bacterial micro colonies formed after using ozonised water as irrigating agent

4. CONCLUSION

Within the limitations of the study it was observed that, Bacterial micro colonies formed in gingival tissues after irrigating with ozonated water and when it was compared with distilled water and saline water which are the commonly used irrigating agent, it was found that there was a mixed results in both the groups. Samples treated with ozonated water has shown both increase and decrease in the microbial colony count.

CONSENT AND ETHICAL APPROVAL

The study was conducted after obtaining approval by the Institutional Ethical and Review Board, Saveetha Dental College and Hospitals, Chennai. All the procedures were done after obtaining consent from the patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cappuyns I, Gugerli P, Mombelli A. Viruses in periodontal disease - A review. *Oral Dis.* 2005;11:219–229.
2. Saini R, Marawar PP, Shete S, et al. Periodontitis, A true infection. *J Glob Infect Dis.* 2009;1:149–150.
3. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: Current concepts. *Journal of Periodontology.* 1992;63:322–331.
4. Moore WEC, Moore LVH. The bacteria of periodontal diseases. *Periodontology* 2000. 1994;5:66–77.
5. Lynch E. Ozone: The revolution in dentistry; 2004. Available: <https://www.worldcat.org/title/ozone-the-revolution-in-dentistry/oclc/57637672>
6. Burns DT, Thorburn Burns D. Early problems in the analysis and the determination of ozone. *Fresenius' Journal of Analytical Chemistry.* 1997;357:178–183.
7. Nogales CG, Ferrari PH, Kantorovich EO, et al. Ozone therapy in medicine and dentistry. *J Contemp Dent Pract.* 2008;9: 75–84.
8. Holmes J. Clinical reversal of root caries using ozone, double-blind, randomised, controlled 18-month trial. *Gerodontology.* 2003;20:106–114.
9. Edmondo F. Apparatus for the production and use of ozone in therapeutics. 1936;2054367. Available: <https://patentimages.storage.googleapis.com/ec/a2/a3/f96a4cbb58bf64/US2054367.pdf> Accessed 4 July 2020.
10. Makkar S, Makkar M. Ozone - treating dental infections. 2011;2:256–259.
11. Srikanth A, Sathish M, Sri Harsha AV. Application of ozone in the treatment of periodontal disease. *J Pharm Bioallied Sci.* 2013;5:S89–94.
12. Seidler V, Linetskiy I, Hubálková H, et al. Ozone and its usage in general medicine and dentistry. A review article. *Prague Med Rep.* 2008;109:5–13.

13. Deepa D, Gupta S. Applications of ozone therapy in dentistry. *Journal of Oral Research and Review*. 2016;8:86.
14. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. *Syzygium cumini* extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. *J Oral Pathol Med*. 2019; 48:115–121.
15. Kaarthikeyan G, Jayakumar ND, Sivakumar D. Comparative evaluation of bone formation between PRF and blood clot alone as the sole sinus-filling material in maxillary sinus augmentation with the implant as a tent pole: A Randomized Split-Mouth Study. *J Long Term Eff Med Implants*. 2019;29:105–111.
16. Arjunkumar R. Nanomaterials for the Management of Periodontal Diseases. In: Chaughule RS (ed). *Dental Applications of Nanotechnology*. Cham: Springer International Publishing. 2018;203–215.
17. Ravi S, Malaiappan S, Varghese S, et al. Additive effect of plasma rich in growth factors with guided tissue regeneration in treatment of intrabony defects in patients with chronic periodontitis: A split-mouth randomized controlled clinical trial. *J Periodontol*. 2017;88:839–845.
18. Kavarthapu A, Malaiappan S. Comparative evaluation of demineralized bone matrix and type II collagen membrane versus eggshell powder as a graft material and membrane in rat model. *Indian J Dent Res*. 2019;30:877–880.
19. Murthykumar K, Arjunkumar R, Jayaseelan VP. Association of vitamin D receptor gene polymorphism (rs10735810) and chronic periodontitis. *J Invest Clin Dent*. 2019; 10:e12440.
20. Ramesh A, Vellayappan R, Ravi S, et al. Esthetic lip repositioning: A cosmetic approach for correction of gummy smile - A case series. *J Indian Soc Periodontol*. 2019;23:290–294.
21. Ramesh A, Varghese S, Jayakumar ND, et al. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. *J Periodontol*. 2018;89:1241–1248.
22. Kavarthapu A, Thamaraiselvan M. Assessing the variation in course and position of inferior alveolar nerve among south Indian population: A cone beam computed tomographic study. *Indian J Dent Res*. 2018;29:405–409.
23. Ramesh A, Ravi S, Kaarthikeyan G. Comprehensive rehabilitation using dental implants in generalized aggressive periodontitis. *J Indian Soc Periodontol* 2017;21:160–163.
24. Jain M, Nazari N. Comparative evaluation of the efficacy of intraligamentary and supraperiosteal injections in the extraction of maxillary teeth: A randomized controlled clinical trial. *J Contemp Dent Pract*. 2018; 19:1117–1121.
25. Vijayashree Priyadharsini J. *In silico* validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol*. 2019;90:1441–1448.
26. Ramamurthy J, Mg V. Comparison of effect of hiora mouthwash versus chlorhexidine mouthwash in gingivitis patients: a clinical trial. *Asian J Pharm Clin Res*. 2018;11:84–88.
27. Skurska A, Pietruska MD, Paniczko-Dręzek A, et al. Evaluation of the influence of ozonotherapy on the clinical parameters and MMP levels in patients with chronic and aggressive periodontitis. *Advances in Medical Sciences*. 2010;55:297–307.
28. Vasthavi C, Babu HM, Rangaraju VM, et al. Evaluation of ozone as an adjunct to scaling and root planing in the treatment of chronic periodontitis: A randomized clinico-microbial study. *J Indian Soc Periodontol* 2020;24:42–46.
29. Habashneh RA, Al Habashneh R, Alsalmán W, et al. Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: A randomized controlled clinical trial. *Journal of Periodontal Research*. 2015;50:37–43.
30. Niveda R, Malaiappan S. Comparison of the amount of bacterial microcolonies formed on using distilled water and ozonated water as an irrigating agent; 2019.
Available:<https://www.semanticscholar.org/paper/859c41125d23f00484465a1d0cc910cafddc1411>
Accessed 6 July 2020.
31. Ramzy MI, Gomaa HE, Mostafa MI, et al. Management of aggressive periodontitis using ozonized water. 2005;6.
Available:<http://dx.doi.org/>
Accessed 4 July 2020.

32. Nagayoshi M, Fukuizumi T, Kitamura C, et al. Efficacy of ozone on survival and permeability of oral microorganisms. *Oral Microbiology and Immunology*. 2004;19: 240–246.
33. Laxman V, Kshitish D. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. *Indian Journal of Dental Research*. 2010;21:341.

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