

Comparison of the amount of bacterial microcolonies formed on using distilled water and ozonated water as an irrigating agent

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ABSTRACT

Background: Periodontal diseases are constantly associated with anaerobic bacterial species 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. Bacteria are the prime etiological agent in periodontal disease, nearly 500 bacterial agent colonize the adult mouth, and anaerobic bacterial microorganism is known to cause destructive periodontal diseases. Mechanical removal of the biofilm and adjunctive use of antibiotics have been the conventional methods of periodontal therapy, and the emergence of ozone therapy in periodontal treatment seems to have promising future. Ozone is a triatomic molecule (O_3), it has three oxygen atoms, and it is a gas with extremely pungent odor. It is an allotrope of oxygen that is much less stable than its diatomic allotrope O_2 oxygen. Ozone gas has high oxidation potential and it is 1.5 times greater than chloride when used as an antimicrobial agent against bacteria, fungi, and protozoa; it can be used in the treatment of alveolitis as a replacement of antibiotic mouthwash for oral microflora. The objective of the study is to find the effect of ozone in the form of ozonated water used as an irrigating agent in scaling and root planning (SRP) procedure and finding the amount of bacterial microcolonies before and after SRP from the subgingival plaque samples comparing with distilled water as a control irrigating agent. **Aim:** The aim of the study is to assess the amount of microbial colonies formed on using ozonated water compared to distilled water as irrigating agent during SRP procedure and to know whether ozonated water is effective in bringing out changes in the number of microbial colonies under anaerobic environment. **Objective:** The objective of the study is to find the effect of ozone in the form of ozonated water used as an irrigating agent in SRP procedure and finding the amount of bacterial microcolonies before and after SRP from the subgingival plaque samples comparing with distilled water as a control irrigating agent. **Materials and Methods:** A randomized, double-blinded study was performed. A total of 20 patients suffering from chronic generalized periodontitis were recruited into the study; subgingival plaque samples were obtained from the patients before SRP and immediately after SRP. 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 under anaerobic conditions; 24 h after culture, the samples were collected and total colony forming units were calculated. **Results:** On counting the number of colony forming units formed, it was found out that the amount of colonies formed in the ozone group had significantly lesser colonies formed when compared with the distilled water group from which we could interpret that ozonated water has better antibacterial properties. **Conclusion:** It is observed that there is drastic reduction in the bacterial microcolonies formed. It is clear that ozonated water is more effective in reduction of anaerobic organisms which are difficult to eradicate from the subgingival bacterial ecosystem henceforth ozonated water can be used as an alternative for water which is used commonly in daily practice.

KEY WORDS: Anaerobic microorganism, Bacterial colony count, Distilled water, Ozone, Plaque samples

INTRODUCTION

The word ozone originated from the Greek word OZIEN which means odorant Ozone is a triatomic molecule

(O_3), it has three oxygen atoms, and it is a gas with extremely pungent. It is an allotrope of oxygen that is much less stable than its diatomic allotrope O_2 oxygen. It occurs naturally on earth atmosphere. It surrounds the earth at an altitude of 50,000 to 100,000 feet. It has the capacity to absorb the harmful ultraviolet rays which present in the light. It is a pale blue gas that condenses to a deep blue liquid at very low temperatures.

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Ozone is a gaseous triatomic molecule with three oxygen atoms.^[1] The gas has a pungent odor and it is less stable^[2] it is a powerful oxidizer.^[3] It has the ability to disperse light and absorb harmful UV rays.^[4,5]

Ozone is an unstable gas; it quickly gives up nascent oxygen molecule to form oxygen gas. Due to the property of releasing nascent oxygen, it has been used in medicine to kill bacteria and fungi and to control hemorrhages;^[6] commercially, it is produced in ozone generators, which involves sending an electrical discharge through a condenser containing oxygen.^[7]

Ozone was first discovered in the year 1840 by a German chemist Christian Friedrich Schonbein at the University of Basil in Switzerland and was used in medicine by Lander in the year 1932. Ozonated water was used as disinfectant by Dr. E.A. Fisch a Swiss dentist.^[8] German physicist Joachim Hansler and Hans Wolff developed the first ozone generator for medical use.

The use of ozone in dentistry: The use of ozone is increasing day by day in dentistry; due to safety considerations, the gas is not directly used for intraoral use. Only dissolved ozone in water and ozonated oils is used. The various routes of administration of ozone are gaseous ozone, ozonated oil, and ozonated water.

Gaseous ozone: Administration of gaseous form of is through open system or sealing suction system as a prerequisite to avoid inhalation of ozone.^[5]

Ozonated oil: Ozone can be infused into oil, and also, ozonized sunflower oil is available as Oleozone and Bioperoxoil; they are shown to be effective against staphylococci, *Streptococcus*, enterococci, *Pseudomonas*, *Escherichia coli*, and mycobacteria.^[9,10]

Ozonated water: Ozonated water is effective against bacteria,^[11] ozone gas is proven to have toxic effects if inhaled,^[12,13] and ozonated water is a safe alternative agent which is cheap when compared to other disinfectants available ozonated water may be useful into control oral infections and various pathogens.

According to German dentist Fritz Kramer, ozone in the form of ozonated water can be used in the following ways – as a powerful disinfectant, in controlling bleeding, and cleanse wounds from bones and wounds. Ozone aids in healing. Ozonated water can be used as mouth rinse, to cleanse, disinfect oral mucosa, cavities, and in general dental surgery. Ozone water jet can be used in root canal therapy and in treating gingivitis.^[14]

Some of the actions of ozone in human body are immunostimulating, antihypoxic, detoxicating, and

antimicrobial actions. Ozone works distinctively against bacteria, fungi, and protozoa. The antimicrobial effect of ozone is a result of its action on cells by damaging its cytoplasmic membrane due to ozonolysis of dual bonds and also ozone-induced modification of intracellular contents like oxidation of proteins because of its secondary oxidant effect. This action is non-specific and selective to microbial cells. It does not damage the human body cells due to their antioxidative capacity. Ozone is more effective in against antibiotic resistant strain. Since ozone is highly antioxidant, it joins with biomolecules containing cysteine, methionine, and histidine which are the part of bacterial cell membrane. Few seconds of application of ozone stops all the vital functions of bacteria. Gram-positive bacteria are more sensitive to destruction than Gram-negative bacteria. Anaerobic bacteria also react well to ozone therapy.^[15]

The ozone therapy aims in achieving the following therapeutic outcomes.

Pathogen elimination, immune activation, restoration of proper oxygen metabolism, and in periodontology its use extend in the treatment of gingivitis, periodontitis, and periimplantitis.^[16] The main use of ozone in periodontal therapy is that it is effective against microorganisms.

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. Ebersberger *et al.*^[17] evaluated the effect of irrigation with ozonated water on the proliferation of cells in the periodontal ligament adhering to the root surfaces. He used twenty three freshly extracted fully erupted third molar, the teeth, and these teeth were irrigated with ozonated water it was observed that the the ozonated water cleansed and decontaminated the root surface and it had no negative effect on the periodontal cells remaining on the root surface.

The objectives of this study is to find the amount of subgingival anaerobic microorganisms before and immediately after scaling and root planing (SRP) procedures using ozonated water as irrigating agent in the test group and distilled water as irrigating agent in the control group and assess the amount of reduction in anaerobic microbial count right before and after scaling from the subgingival plaque samples obtained from the patients with periodontal diseases since distilled water is water that has been heated to the boiling point so that impurities are separated from the water, which itself becomes vapor or steam. Steam is then condensed back into pure liquid form. The impurities remain as residue and are removed. Pure Water Distillation Systems remove waterborne

biological contaminants such as bacteria, viruses, organic and inorganic chemicals, heavy metals, volatile gases, and other contaminants. Distilled water contains virtually no solids, minerals, or trace elements. It is clean, natural, and healthy. Steam distilled water is the standard by which all other waters are measured and compared.

MATERIALS AND METHODS

The participants of the study were selected from the outpatient department of periodontics, Saveetha Dental College and Hospitals, Chennai. A randomized, double-blinded study was performed. 20 patients who were suffering from chronic generalized periodontitis were selected for the study; subgingival plaque samples were obtained from the patients before SRP and immediately after SRP. Both the patient and the clinician were 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. Subgingival plaque was collected before SRP using a sharp-tipped instrument without inducing bleeding. The collected plaque 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 13W for 15–30 min. The ozone generator consists of tubes, bubble stones, and wires. The tube is connected to the ozonator machine, and bubble stone was connected to the other end of tube and the other end is placed into booster bottle containing plain water. A 15 min timer is set in the ozonator and the power is set on. The water gets ozonized, and the booster bottle with ozonized water is connected to the chair, using this ozonated water as irrigating agent scaling is done with the aid of ultrasonic dental scaler by woodpecker UDS-J. Post-scaling the residual subgingival plaque is scraped out and transferred into Eppendorf tube containing brain–heart infusion broth. 10 µl of the collected samples are then cultured under anaerobic condition. Under aseptic condition, the samples are cultured into sterilized brain–heart infusion agar plates (BHI agar plates) by lawn culture method and incubated at 37°C in an anaerobic jar for 24 h using anaerobic gas pack by Himedia. After 24 h, the colonies formed were counted manually. The same procedure was done for both groups.

RESULTS

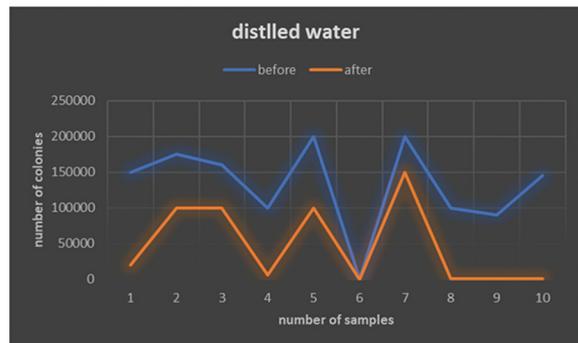
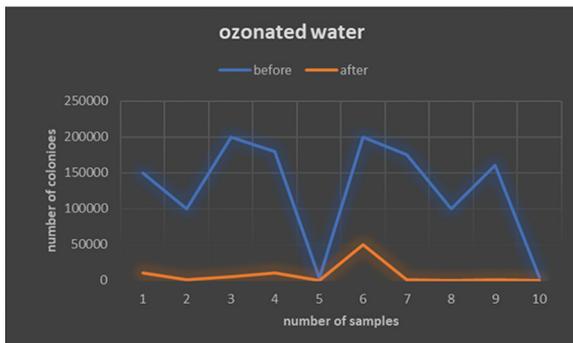
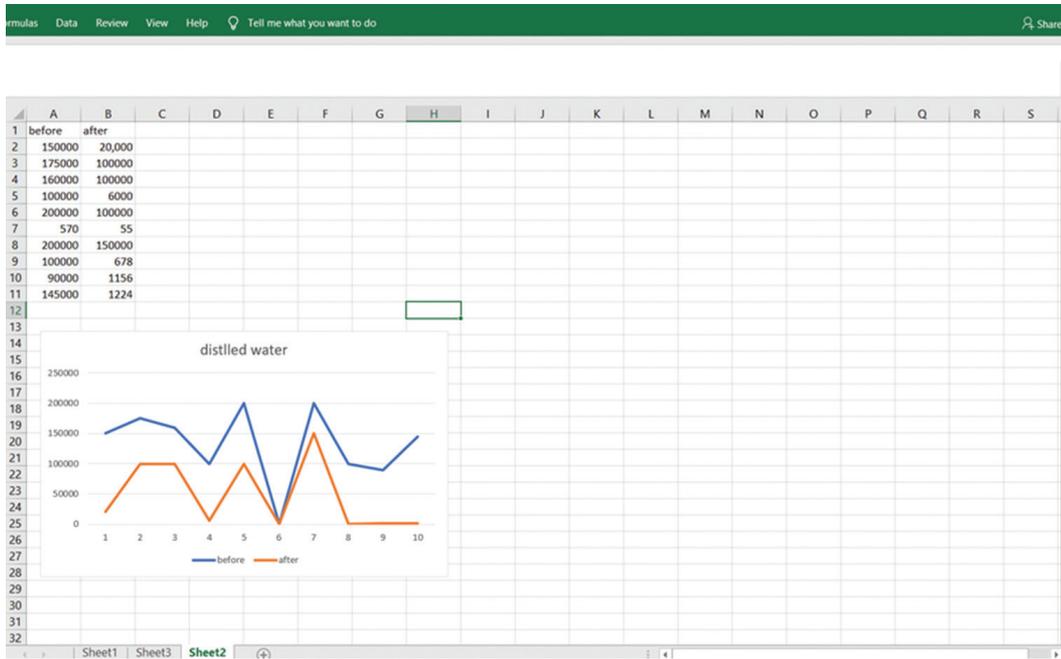
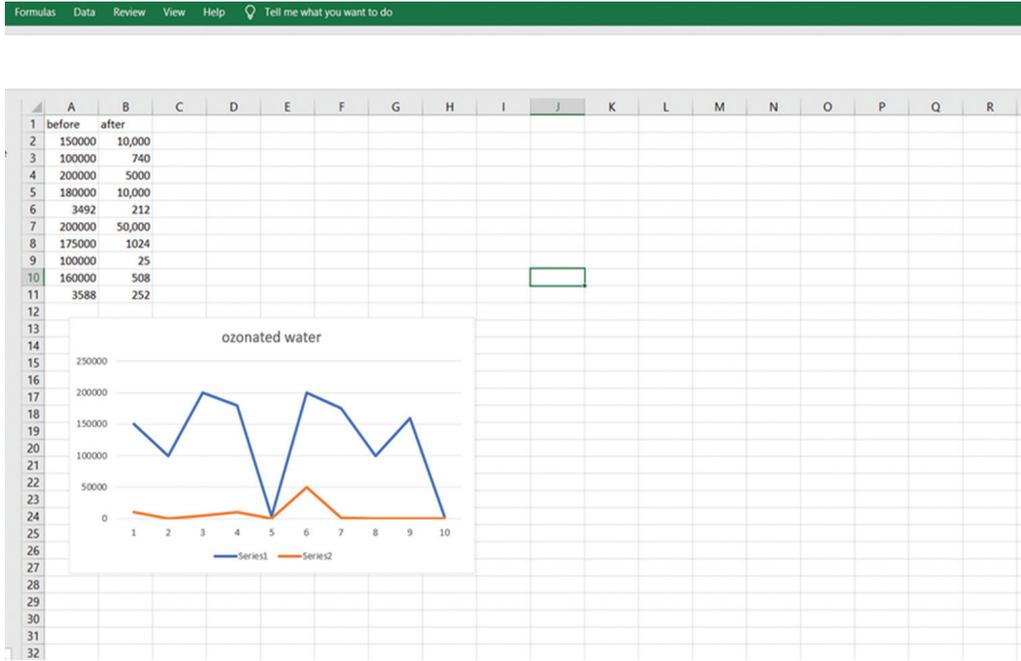
On counting the number of colony forming units formed, it was found out that the amount of colonies

formed in the ozone group had significantly lesser colonies formed when compared with the distilled water group from which we could interpret that ozonated water has better antibacterial properties. The number of colonies formed drastically reduced from more than a lack to few hundred microcolonies in the group which received ozonated water as irrigating agent from the results obtained; it is clear that ozonated water is more effective in reduction of anaerobic organisms which are difficult to eradicate from the subgingival bacterial ecosystem henceforth ozonated water can be used as an alternative for water which is used commonly in daily practice.

DISCUSSION

Plaque accumulation in the gingival crevice area causes changes in the oral environment causing gingivitis and periodontitis. It is difficult for antibiotics to target the pathogenic microorganism and requires higher concentration of antimicrobial agents to inactivate or to destroy the microorganisms. Ozone can be used to treat periodontal diseases.

Researches have been done previously on ozone. Nagayoshi *et al.* tested the efficacy of different concentration of ozone water with time-dependent inactivation of cariogenic, periodontopathogenic, and endodontopathogenic microorganism, and they confirmed that ozonated water is highly effective in killing Gram-positive and Gram-negative microorganism. Huth *et al.*^[18] declared that aqueous form of ozone has a potential like that of antiseptic agent, and it showed less cytotoxicity when compared to that of gaseous ozone. Kshitish and Laxman^[19] conducted a randomized, double-blind, crossover split-mouth study on 16 patients suffering from generalized chronic periodontitis. They observed the percentile reduction of Aa (25%) using ozone was appreciable as compared to no change in Aa occurrence using chlorhexidine. Using chlorhexidine, there was no antibacterial effect on *Porphyromonas gingivalis* and *Tannerella forsythensis*. They concluded that despite the substantivity of chlorhexidine, the single irrigation of ozone is quite effective to inactivate microorganisms. In concordance with other studies, in this study, the microbial colonies formed in the group whom received ozonated water for subgingival scaling procedure as an irrigating agent showed lesser number of colonies post-treatment when compared to samples obtained from the group receiving distilled water as an irrigating agent. The colony counts drastically reduced from confluent number of colonies to few thousands and hundreds in groups who received ozonated water, whereas there was reduction in the number of microbial colonies who received distilled water; the number of colonies developed post-therapy using distilled water was comparatively higher when



compared to the ozone group. Even though ozone has excellent antimicrobial property, it has poor stability and it emits unpleasant odor.

CONCLUSION

Ozone can be considered as a promising antimicrobial agent in antimicrobial therapy and serves as an effective tool in supportive periodontal therapy; ozonated water is more effective in reduction of anaerobic organisms which are difficult to eradicate from the subgingival bacterial ecosystem henceforth ozonated water can be used as an alternative for water which is used commonly in daily practice.

REFERENCES

1. Burns DT. Early problems in the analysis and the determination of ozone. *Fresenius J Anal Chem* 1997;357:178-83.
2. Saini R. Ozone therapy in dentistry: A strategic review. *J Nat Sci Biol Med* 2011;2:151-3.
3. Srikanth A, Sathish M, Sri Harsha AV. Application of ozone in the treatment of periodontal disease. *J Pharm Bioallied Sci* 2013;5:S89-94.
4. Holmes J. Clinical reversal of root caries using ozone, double-blind, randomised, controlled 18-month trial. *Gerodontology* 2003;20:106-14.
5. Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. *Arch Med Res* 2006;37:425-35.
6. Garg R, Tandon S. Ozone: A new face of dentistry. *Int J Dent Sci* 2009;7:2.
7. Gupta G, Mansi B. Ozone therapy in periodontics. *J Med life* 2012;5:59.
8. Edmondo F. Apparatus for the Production and Use of Ozone in Therapeutics. New York, US: United States Patent No. 2,054,367; 1936.
9. Sechi LA, Lezcano I, Nunez N, Espim M, Duprè I, Pinna A, *et al.* Antibacterial activity of ozonized sunflower oil (Oleozone). *J Appl Microbiol* 2001;90:279-84.
10. Rodrigues KL, Cardoso CC, Caputo LR, Carvalho JC, Fiorini JE, Schneedorf JM. Cicatrizing and antimicrobial properties of an ozonised oil from sunflower seeds. *Inflammopharmacology* 2004;12:261-70.
11. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. *J Endod* 2004;30:778-81.
12. Maiya A. Applications of ozone in dentistry. *Int J Clin Dent Sci* 2011;2:23-7.
13. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, *et al.* Effect of ozone on oral cells compared with established antimicrobials. *Eur J Oral Sci* 2006;114:435-40.
14. Krammer F. Ozone in the Dental Practice. Medical Applications of Ozone. Norwalk, CT: International Ozone Association, Pan American Committee; 1983. p. 258-65.
15. Seidler V, Linetskiy I, Hubálková H, Stankova H, Smucler R, Mazánek J. Ozone and its usage in general medicine and dentistry. A review article. *Prague Med Rep* 2008;109:5-13.
16. Mollica P, Harris R. Integrating Oxygen/ozone Therapy into Your Practice. Available from: <http://www.Toxin free smile. Dom/ images/ozone integrating % 20 oxygen ozone 20% therapy your practice.Pdf>. [Last accessed on 2010 Jan 13].
17. Ebensberger U, Pohl Y, Filippi A. PCNA-expression of cementoblasts and fibroblasts on the root surface after extraoral rinsing for decontamination. *Dent Traumatol* 2002;18:262-6.
18. Huth KC, Saugel B, Jakob FM, Cappello C, Quirling M, Paschos E, *et al.* Effect of aqueous ozone on the NF-κB system. *J Dent Res* 2007;86:451-6.
19. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. *Indian J Dent Res* 2010;21:341-8.

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