ORIGINAL ARTICLE DENTISTRY

Journal of Contemporary Medicine and Dentistry
www.jcmad.com

ISSN [P-2347-4513]
ISSN [O-2349-0799]
Year: 2020
Volume: 8
Issue: 1
62-69

Efficacy of Subgingival Ozone Irrigation for Management of Chronic Periodontitis – A Clinical, Microbiological and Biochemical study

Thayyil Sivaraman Hrishi [1], Pratibha Pandurang Kundapur [2], Giliyar Subraya Bhat [3], Shashidhar Vishwanath [4], Shoba Kamath [5]

1. Assistant Professor, Department of Periodontology, Government Dental College, Calicut
2. Professor and Head, Department of Periodontology Manipal College of Dental Sciences, Manipal, Karnataka
3. Professor, Department of Periodontology Manipal College of Dental Sciences, Manipal, Karnataka
4. Associate Professor, Department of Microbiology, Kasturba Medical College, Manipal, Karnataka
5. Professor, Department of Biochemistry, Kasturba Medical College, Manipal, Karnataka

Abstract

Purpose: *Ozone, both gaseous and aqueous forms, is gaining significance among the dental fraternity as a possible alternative oral antiseptic agent. It is strongly antimicrobial and does not induce microbial resistance. The purpose of the present study was to evaluate the efficacy of subgingival irrigation of ozonated water as an adjunct to scaling and root planing (SRP) compared to SRP alone in the management of mild to moderate periodontitis patients.* ***Methods and Material****: The study was a randomized controlled trial; sixty patients were allocated to two groups, the ozone group (test group) and the scaling group (control group). All patients were subjected to scaling and root planing (SRP) in a single visit followed by irrigation with ozone water in the test group and distilled water in the control group at baseline and 14 days. The clinical parameters of Gingival Index (GI), Plaque Index (PI), percentage of sites with bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL), total microbiologic count, differential microbial count of Porphyromonas spp., Fusobacterium spp., Prevotella spp. &Veillonella spp., and biochemical parameter of total antioxidant capacity (TAOC) in gingival crevicular fluid (GCF) were recorded at baseline and after 6 weeks.* ***Results****: Statistically significant improvements in clinical, microbiological and biochemical parameters were observed in both groups following therapy. At 6 weeks it was observed that adjunctive ozone irrigation resulted in significant reductions in GI, BOP, CAL and total microbial count compared to scaling and adjunctive distilled water irrigation while the changes in PI and antioxidant levels did not reach statistical significance.* ***Conclusions****: The adjunctive use of subgingival irrigation ozonated water showed promising short-term results.*

Keywords*: Ozone, Periodontitis, antimicrobial, subgingival irrigation, non-surgical therapy*

Address for correspondence: Dr. Thayyil Sivaraman Hrishi, Assistant Professor, Department of Periodontology, Government Dental College Calicut, Kerala, India- Phone- 9846772531 Email- hrishisivram@hotmail.com

Date of Acceptance: 23/04/2020

Introduction

Periodontitis is an inflammatory disease affecting the supporting tissues of the teeth. It is caused by specific microflora leading to destruction of the periodontal ligament and alveolar bone. The pathophysiology of periodontal disease is related to microbial plaque which accumulate on teeth and host's response to these accumulations. [1] Therefore, removal and control of microbial deposits is critical for successful periodontal therapy. Clinical evidence shows that plaque control combined with scaling and root planning is effective as a therapeutic modality for arresting periodontitis, but certain factors can limit the effectiveness of scaling and root planning, for example, deep developmental grooves, furcations, operator skills, tortuous pockets,

osseous defects etc.[2] Hence, use of adjunctive modalities like local and systemic chemotherapeutics,[3-7]host modulatory agents,[8,9] lasers [10,11] and photodynamic therapy[12] has been advocated to overcome these limitations. Chemotherapeutics can be delivered to the periodontal pockets in many ways such as irrigation, mouth rinses, fibers, membranes, gels, chips etc.[13] Irrigation (supra and subgingival irrigation) has the potential to be a cost effective method that can be used by the clinicians and patients to help suppress bacterial etiologic agents causing periodontitis.[14] The biologic rationale for performing supra and subgingival irrigation is to nonspecifically reduce microbial deposits and count respectively, thereby reducing the pocket microflora to prevent initiation of periodontitis or to facilitate its reduction [15] The conventional antimicrobial or antiseptic agents may allow for development of bacterial resistance following long term use.[16] Presently, ozone, O_3 is contemplated as a possible alternative oral antiseptic agent owing to its strong antimicrobial property and incidence of no microbial resistance. Evidence has shown that both aqueous and gaseous forms of ozone are detrimental to oral pathogens. [17, 18] Ozonated water (0.5-4 mg/L) was found to be highly effective in killing both gram-positive and gram-negative microorganisms. Even lower concentrations of ozone water (0.12 to 0.19 mg/L) were found to be effective against bacteria. [19] Ozone, therefore, may have a potential role in the treatment of periodontal disease. Administration of ozone into periodontal pockets would conceivably reduce the bacterial load in periodontal pockets. Ozone water could either be used as a coolant in ultrasonic scalers or as an irrigant along with scaling and root planing. Clinical evidence is however, limited in this area. Kshitish et al. [20] in a study used ozonated water in the treatment of periodontitis patients and observed that ozone water (0.1mg/L) was effective against periodonto-pathogenic bacteria. The purpose of this study is to evaluate the efficacy of ozone water irrigation as an adjunct to scaling and root planing in improving the clinical, microbiological and biochemical parameters in mild to moderate periodontitis patients.

Materials and Methods

The present study followed a randomized controlled clinical trial design. A total of one hundred twenty four patients within the age group of 18 to 60 years of both sexes reporting to the Department of Periodontology were screened for eligibility for participation in the study. Out of these, sixty patients having at least 6 sites with probing pocket depth of >4mm with Clinical Attachment Loss (CAL) of 1 - 2 mm (mild periodontitis) or CAL of 3 – 4mm (moderate periodontitis) were included in the study. [21] Patients who had undergone any surgical or non-surgical periodontal therapy in the past 6 months or patients with Diabetes mellitus, Rheumatoid arthritis, Osteoporosis and bleeding disorders, pregnant and lactating women, smokers and patients with less than 20 teeth or history of use of antibiotics, anti-inflammatory agents and any chemotherapeutics in the past 3 months were excluded from the study. The study design and purpose was explained to the patients and a written consent was obtained. Ethical approval for the study was obtained from the Hospital Ethics Committee.

Subjects were randomly divided into two groups, the test group and control group, with 30 subjects in each group. Patients in test group received scaling and root planing in a single visit using ‘EMS Piezon Master 150’ ultrasonic scaler with 0.1 mg/L ozone water (produced by “Kent ozone Dental Jet TY-820”, Kent Ro Systems Ltd., Noida, India) (figure: A) used as the coolant and hand instruments (Gracey Curettes; Hu Friedy, Chicago, IL, USA). Following these pockets were irrigated with ozone water using a sterile 5 cc syringe with a blunt 21 gauge needle for 3 min (figure B). Repeated irrigation ensured that irrigants filled up pockets for a period of 3 min. In the control group, scaling and root planing was performed and pockets were irrigated with distilled water using blunt 21 gauge needle. Patients were instructed to brush with Modified Bass method twice daily using a standard tooth paste and were recalled after 2 weeks to repeat the irrigations. During this visit personal plaque control of patients was monitored and oral hygiene instructions were reinforced. Clinical, microbiological & biochemical parameters were evaluated at baseline and the sixth week.

Clinical parameters

Gingival Index, [22] Plaque Index[23] probing pocket depth (PPD), number of teeth which bled on probing and clinical attachment level (CAL) were recorded prior to SRP and at 6 weeks recall visits. Probing depth was measured from the gingival margin to the base of the pocket only in areas where the depth was more than 3mm and CAL attachment was measured from the CEJ to base of the pocket using William's periodontal probe.

Microbiological Evaluation

Microbiological parameters evaluated included total anaerobic bacterial count & differential count of *Porphyromonas spp., Fusobacterium spp., Prevotella spp. &Veillonella spp.*

Plaque samples were collected from 4 deepest periodontal pocket prior to SRP. The supragingival plaque was removed from the tooth surface using a gauze piece, tooth was isolated, air dried before collection. The same sites were used for sampling during final visit. The collected samples were transported in 10 ml thioglycollate broth immediately to Department of Microbiology and culturing was done within 30 minutes. Neomycin Blood Agar which is a selective media for obligate anaerobes (*Peptostreptococcus, Treponema, Fusobacterium, Porphyromonas, Veillonella*, and *Actinomyces*species) was used for culture.

The plaque samples were vortexed and serially diluted up to 10^{-3}. The different dilutions i.e., undiluted, 1:10, 1:100 & 1:1000 were inoculated on the culture plates by means of loop. The plates were incubated in anaerobic jars with GasPak™ EZ Anaerobe Container System Sachets for 5 days. Metronidazole discs were placed on the agar plates before incubation, for identification of anaerobes. Specific species were recognized from colony morphology, gram staining and biochemical differentiation tests using kanamycin 1mg, Vancomycin 5 mg, colistin 10mg discs.

Biochemical Parameter

Gingival crevicular fluid was collected from all subjects for biochemical analysis of total antioxidant capacity. GCF samples were taken prior to plaque collection to avoid bleeding from pockets during GCF collection, from same sites where plaque samples were intended to be collected. Teeth were isolated with cotton rolls and gently air dried. Standardized Whatman's filter paper strips of dimensions 2x8 mm dimensions were used, 6 strips of filter paper were placed in the pocket and a total of 4 pockets were sampled. The filter paper were removed after 60 seconds and transferred to Eppendorf vials containing phosphate buffered saline (pH=7.4).

The TAOC in GCF was measured using Ferric Reducing Antioxidant Power Assay(FRAP). FRAP measures antioxidant reduction of ferric tripyridyltriazine complex to intense blue colored ferrous tripyridyltriazine, which is monitored by measuring the change in absorption at 593nm.[24]

Statistical Analysis

Statistical analysis was performed by SPSS 19 software. Shapiro Wilk test was utilized to determine the normality of variables. The variables were expressed in Mean ± Std. deviation. Paired t-test was used to compare the variables which showed normal distribution (GI, PI, CAL, PD) between baseline and follow up in both groups and the intergroup comparisons were made by independent t-test. Non-parametric Wilcoxon test was used to compare baseline and follow up values of parameters which didn't show normal distribution (BOP, TAOC, Total anaerobic count) and Mann-Whitney U Test was used for intergroup comparisons.

Results

The present study was a randomized controlled clinical trial. A total of 60 subjects including 23 male and 37 females were enrolled in the study. The mean age of the patients was 38.6 years.

Clinical Parameters

The mean gingival index score showed significant reduction at 6 weeks in both ozone and scaling group compared to baseline values (table 1 & 3) and intergroup comparison at 6 weeks showed significant mean GI reduction in ozone group compared to scaling group. The mean plaque index scores showed statistically significant reduction in both ozone and scaling groups from baseline to 6 weeks follow up visits (table 1 & 3), but the mean reduction between the groups were not significant. (table 5). The

reduction in percentage of bleeding sites were statistically significant in both control and test groups from baseline to 6 weeks (table 1 & 3). The reduction in ozone group was statistically significant than scaling group on intergroup comparison (table 5). At 6 weeks follow up significant reduction in mean probing depth was observed in both the ozone and the scaling group (table 1 &3), but this change was not significant between the groups (table 5). The mean attachment level gain was statistically significant in both the groups after 6 weeks (table 1 & 3) and Intergroup comparison showed significant change in ozone group (table 5).

Table 1: Changes in Clinical, Microbiological & Biochemical Parameters in Ozone Group

Parameters	Baseline	6 weeks	Mean change	P value
Gingival index†	1.73±0.14	0.84±0.18	0.89±0.14	<0.001
Plaque index†	1.62±0.20	0.97±.17	0.67±0.23	<0.001
Bleeding on probing %*	82.03±10.2	26.03±13.75	56.00±14.02	<0.001
Probing depth†	5.34±0.34	3.87±0.51	1.47±0.45	<0.001
Clincal attachment level†	3.43±0.54	2.04±0.60	1.39±0.36	<0.001
Totalantioxidant capacity* (µmol/l)	237.33±74.0	307.63±40.32	70.3±68.6	<0.001
Anaerobes*(log of cfus)	6.57±0.44	5.16±0.75	1.41±0.50	<0.001

†Evaluate by Paired t test, *Evaluated by non-parametric Wilcoxon's test.

Table 2: Reduction in Count of Different Bacterial Species (in Log Cfus) in Ozone Group

Species	Prevalence	Baseline	6 weeks	Mean change
Porphyromonas	7(23%)	5.16±.65	4.5±0.77	0.66±0.34
Fusobacterium	10(33%)	5.49±0.1	4.14±1.22	1.34±1.25
Veillonella	12(40%)	4.88±0.68	4.37±0.59	0.51±0.41
Prevotella	9(27%)	5.02±0.61	4.11±0.70	0.91±0.29

Table 3: **Comparison of Parameters at Baseline & 6 Weeks in Scaling Group**

Parameter	Base line	6 weeks	Mean change	P value
Gingival index†	1.63±.19	1.06±0.22	0.57±0.03	<0.001
Plaque index†	1.65±0.19	1.09±0.20	0.55±0.03	<0.001
Bleeding on probing %*	78.87±10.64	40.17±16.81	38.70±14.92	<0.001
Probing depth†	5.32±0.35	4.03±0.51	1.29±0.06	<0.001
Clinical attachment level†	3.50±0.69	2.39±0.75	0.80±0.18	<0.001
Totalantioxidant capacity (µmol/l)*	239.00±33.7	292.50±40.3	53.50±33.12	<0.001
Anaerobs* (log of CFUs)	6.36±0.50	5.37±0.48	0.99±0.48	0.003

†Evaluated by paired t test, *Evaluated by non-parametric wilcoxon's test

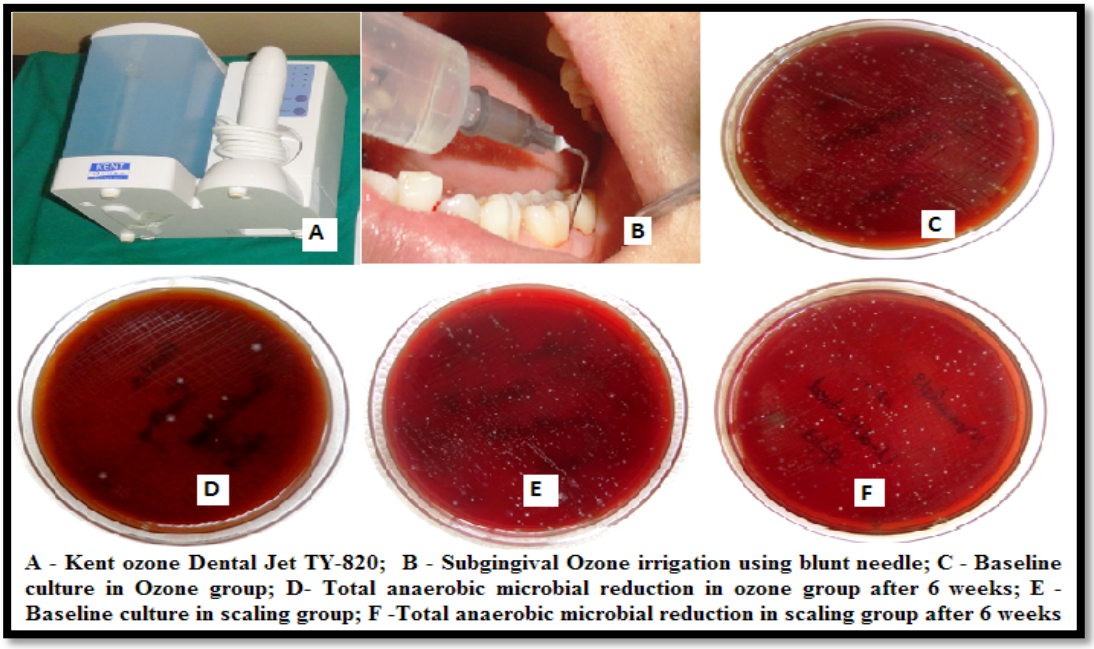
Table 4: Comparison of CFUs of Different Microorganisms at Baseline and 6 Weeks in Scaling Group

Species	prevalence	Baseline	6 weeks	Mean change
Porphyromonas	6 (20%)	3.97±0.62	3.39±0.34	0.58±0.32
Fusobacterium	9 (30%)	5.44±0.59	4.54±0.59	0.90±0.19
Veillonella	13 (43.33%)	4.76±0.69	4.25±0.70	0.50±0.38
Prevotella	12(40 %)	5.14±0.55	4.37±0.70	0.76±0.40

Table 5: Comparison of mean changes at 6 Weeks

Parameters	Ozone	Scaling	Ozone v/s scaling
Gingival index†	0.89±0.14	0.57±0.03	P<0.001
Plaque index †	0.67±0.23	0.31±0.23	NS
Bleeding on probing %*	56.00±14.0	38.70±14.92	P<0.001
Pocket depth†	1.47±0.45	1.29±0.06	NS
Clinical attachment level†	1.39±0.03	0.80±0.04	P=0.006
Total antioxidant capacity *	70.3±68.6	53.50±33.12	NS
Anaerobes *	1.41±0.50	0.99±0.48	P=0.05
Porphyromonas	0.66±0.34	0.58±0.32	-
Fusobacterium	1.34±1.25	0.90±0.19	-
Veillonella	0.51±0.41	0.50±0.38	-
Prevotella	0.91±0.29	0.76±0.40	-

†Evaluated by independent t test, *Evaluated by Mann-Whitney U Test



A - Kent ozone Dental Jet TY-820; B - Subgingival Ozone irrigation using blunt needle; C - Baseline culture in Ozone group; D- Total anaerobic microbial reduction in ozone group after 6 weeks; E - Baseline culture in scaling group; F -Total anaerobic microbial reduction in scaling group after 6 weeks

Microbiologic Parameters

The total anaerobic count and differential count of anaerobic bacteria was expressed in log of colony forming units (Log CFUs). At 6 weeks follow up the mean log CFUs of total anaerobic bacteria showed significant reduction in both the ozone and the scaling groups (figure 3 & 4, table 1 & 3). Comparison between groups showed statistically significant reduction in the ozone group compared to the scaling group (table 5). The differential bacterial count in both the groups was expressed in log of CFUs (table 2 & 4). The changes in differential count at 6 weeks between the various counts was represented in mean differences ± Standard deviations, p values were not calculated because the size of samples of different bacteria was not sufficient. At follow up, the ozone group showed increased reduction in CFUs of *Porphyromonasspp*, *Fusobacterium spp* & *Prevotellaspp* compared to scaling group

Biochemical Parameter

The total antioxidant capacity (TAOC) was evaluated at baseline & 6 weeks in both the groups. At 6 weeks, TAOC showed significant increase in both the groups (table 1 & 3). Even though the mean increase in Ozone group was more compared to the Scaling group, intergroup comparison at 6 weeks showed no statistically significant differences (table 5).

Discussion

Adjunctive use of local agents with SRP has met with moderate success. [4] Considering possible development of resistant bacteria and adverse host reactions, the use of a broad-spectrum antiseptic agent with low potential for adverse reactions seems a desirable choice than topical antibiotics. [25, 26] Ozone is an interesting prospect because of its strong antimicrobial activity and reduced risk of bacterial resistance. In the present study, the efficacy of adjunctive subgingival irrigation of 0.1 mg/L ozone water with SRP was compared to SRP and adjunctive distilled water irrigation. Clinical, microbiological and biochemical parameters were recorded at baseline and 6 weeks. Significant improvements in clinical, microbiological and biochemical parameters were observed in both groups following therapy. At 6 weeks it was observed that adjunctive ozone irrigation resulted in significant reductions in GI, BOP, CAL and Total Microbial count compared to scaling and adjunctive distilled water irrigation while the changes in PI and antioxidant levels did not reach statistical significance. The improvements at 6 weeks in all groups can be attributed primarily to removal of local factors and diseased cementum by SRP and thereby reduction of microbial load from periodontal pockets. Various authors have reported improvements in clinical [27-31]and microbiologic parameters following SRP.[32-34]

The greater reduction in GI & percentage of bleeding sites in the ozone group can be attributed to the antimicrobial properties of ozone water, which would result in resolution of inflammation in periodontal tissues by reducing the pathogenic bacterial count. Nagayoshi et al.,[18]observed that ozone is highly effective against oral microorganisms, the viability of S. *mutans, P. gingivalis, P. endodontalis, and A. actinomycetemcomitans* was found to be significantly decreased when treated with ozonated water of concentration 0.5mg/L. Hence it can be implied that Ozone may have antibacterial properties against gram negative periodontopathogens like *P gingivalis, Tannerella forsythia and Treponemadenticola* which are associated with bleeding on probing. The change in plaque index (0.67±0.23 in the ozone group and 0.31±0.23 in the scaling group) was not statistically significant, this may be due to strict oral hygiene maintained by the patients during the study period, which was evaluated and reinforced every 2 weeks by the examiners. The ozone concentration used in the present study was 0.1mg/m. Kshitish & Laxman in a short term comparative study of 21 days used the same concentration of ozone water as an irrigant in the treatment of periodontitis patients. It was observed that irrigation with ozone water resulted in higher percentage reduction of Plaque Index (12%), Gingival Index (29%) and bleeding sites (26%) compared to chlorhexidine. [20] It has also been observed that ozonated water at 0.1 ppm concentration is found to be effective in reducing the load of 24-hour plaque bacteria but it did not eliminate them completely. [35]

The total anaerobic counts (expressed in log of CFUs) were significantly reduced from baseline to 6 weeks in both the groups and the comparison of total anaerobic count among the groups showed a statistically significant reduction in the ozone group (1.41±0.50) compared to the scaling group (0.99±0.48). These results are consistent with a study by Ramzy et al., 2005, who noticed significant improvements regarding bacterial count in aggressive periodontitis patients following SRP& irrigation with ozone water compared to SRP alone.[36] The mechanism by which ozone kills bacteria is by oxidation of cell walls and cytoplasmic membranes of microorganisms. Microorganisms will have different susceptibility to ozonated water due to differences in the structure of the cell walls of microorganisms. After the membrane is damaged by oxidation, the permeability of the membrane increases, and ozone molecules can readily enter the cells and cause lysis. This action is non-specific and selective to microbial cells; it does not damage human body cells because of their major antioxidative ability.[37]

In the present study total antioxidant status was evaluated in order to determine the oxidative stress in periodontal tissue and to evaluate the effect of various treatment modalities on oxidative stress. Oxidative stress has been implicated in the pathobiology of many human diseases including periodontitis. In periodontal disease, the exaggerated inflammatory and immune response to the presence of specific

periodontal pathogens results in elevated levels of local neutrophil-derived degradative enzymes, circulating cytokines and C-reactive protein (CRP). Binding of bacteria directly or indirectly by the surface receptors, such as Toll-like receptors [TLR] and Fcγ-receptors of neutrophils, triggers phagocytosis and superoxide radical formation which may subsequently be converted to hydrogen peroxide and highly reactive hydroxyl radicals.[38]Hence in periodontitis the balance between pro-oxidants and antioxidants is disturbed with more production of reactive oxygen species resulting in destruction of host tissue.[39] The antioxidant defense system scavenges the increased ROS and in this process the antioxidant levels are depleted. Similarly a reduction in oxidative stress restores the antioxidant levels representing the healthy state of tissue. Based on this concept many authors have suggested that antioxidant levels and pro-oxidant levels in GCF can be utilized as biomarkers of periodontal disease.[40]

In the present study, the total antioxidant levels increased significantly in all groups from baseline to 6weeks follow up which is in accordance to various studies that have demonstrated improvement in total antioxidant levels in GCF following non-surgical periodontal therapy.[40, 41] Intergroup comparison among the groups demonstrated that the ozone group showed slight improvement over the scaling group but these changes were not statistically significant. The improvement in TAOC levels is likely due to the reduction in oxidative stress in periodontal tissues following non-surgical therapy which indirectly results from reduction in the microbial load. The additional improvement in the ozone can be due to the additional antimicrobial effect when used as an adjunct to SRP. The present study was carried out as a short term clinical trial to evaluate the effect of adjunctive ozone irrigation in treating mild to moderate periodontitis and our results should be confirmed by long term follow up studies. Although, microbial culture is considered as the gold standard, it cannot detect non-viable organisms. Therefore, newer diagnostic methods like PCR would have been a better choice for consideration of the non-viable organisms killed during sampling. Oxidative stress was determined indirectly, in terms of antioxidant concentrations. Supplemental estimation of the pro-oxidant levels would have been more ideal.

Conclusion

In the present study, it was observed that the adjunctive use of subgingival irrigation of 0.1 ppm ozone produced significant improvement in clinical and microbiological parameters in periodontitis patients. Within the limits of the study, we can conclude that subgingival ozone irrigation can be effectively used as an adjunctive therapy for management of periodontal disease. It may be likely that the use of ozonated water as a coolant along with ultrasonic scalers, instead of distilled water, would provide substantial therapeutic benefits, especially in those patients for whom surgical therapy may not be a feasible option.

Conflict of Interest: *None declared*
Source of Support: *Nil*
Ethical Permission: *Obtained*

References

1. Hinrichs J, Kotsakis G. “Classification of Diseases and Conditions Affecting the Periodontium” In: Newman M, Takei H, Klokkevold P, Carranza F. Clinical Periodontology, 2014, Chapter 3, 12th Edition published by Elsevier, pp 45-67
2. Lowenguth RA, Greenstein G. Clinical and microbiological response to nonsurgical mechanical periodontal therapy. Periodontol 2000; 1995; 9:14-22.
3. Goodson JM, Offenbacher S, Farr DH, Hogan PE. Periodontal disease treatment by local drug delivery. J Periodontol 1985;56:265-72
4. Greenstein G, Polson A. The role of local drug delivery in the management of periodontal diseases: a comprehensive review. J Periodontol 1998;69:507-20
5. Greenstein G. Local drug delivery in the treatment of periodontal diseases: assessing the clinical significance of the results. J Periodontol. 2006;77:565-78
6. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. Ann Periodontol 2003;8;115–181
7. Slot J, Rams T E. Antibiotics in periodontal therapy: advantages and disadvantages. J. Clin. Periodontol 1990; 17:479–493.
8. Preshaw PM. Host response modulation in periodontics. Periodontol 2000 2008;48:92–110
9. Giannobile WV. Host-Response Therapeutics for Periodontal Diseases. J Periodontol 2008; 79:1592–1600.
10. Walsh L. The current status of laser applications in dentistry. Aust Dent J 2003; 48:146–155.
11. Ishikawa I, Aoki A, Takasaki AA, Mizutani K, Sasaki KM, Izumi Y. Application of lasers in

12. periodontics: true innovation or myth? Periodontol 2000 2009; 50: 90–126.
13. Braham P, Herron C, Street C, Darveau R. Antimicrobial photodynamic therapy may promote periodontal healing through multiple mechanisms. J Periodontol 2009; 80:1790-1798.
14. Rams TE, Slots J. Local delivery of antimicrobial agents in the periodontal pocket. Periodontol 2000 1996; 10:139–159.
15. Hoang T, Jorgensen MG, Keim RG, Pattison AM, Slots J. Povidone-iodine as a periodontal pocket disinfectant. J Periodontal Res 2003; 38:311–317.
16. Greenstein G. The role of supra- and subgingival irrigation in the treatment of periodontal diseases. J Periodontol. 2005; 76:2015-2027.
17. Rodrigues RMJ, Gonçalves C, Souto R, Feres-Filho EJ, Uzeda M, Colombo APV. Antibiotic resistance profile of the subgingivalmicrobiota following systemic or local tetracycline therapy. Journal of Clinical Periodontology 2004; 31:420–427.
18. Baysan A, Whiley RA, Lynch E. Antimicrobial effect of a novel ozone-generating device on microorganisms associated with primary root carious lesions in vitro. Caries Res 2000; 34:498–501
19. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. Oral Microbiol Immunol 2004; 19: 240–246.
20. Broadwater WT, Hoehn RC, King PH. Sensitivity of three selected bacterial species to ozone. Appl Microbiol. , 1973; 26:391-393.
21. 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;21:341-348
22. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4: 1–6.
23. Löe H, Silness, J Periodontal disease in pregnancy. Acta Odontol Scand. 1963 21:533-551
24. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22:121-135.
25. Benzie Iris FF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power"—the FRAP assay. Anal Biochem. 1996;239:70–76
26. Hoang T, Jorgensen MG, Keim, RG, Pattison AM, Slots J. Povidone-iodine as a periodontal pocket disinfectant. J Periodontal Res 2003;38:311–317
27. Slots J, Ting M. Systemic antibiotics in the treatment of periodontal disease. Periodontol 2000 2002; 28:106–176.
28. Badersten A, Nilvéus R, Egelberg J. Effect of nonsurgical periodontal therapy. I. Moderately advanced periodontitis. J ClinPeriodontol. 1981; 8:57-72.
29. Kaidahl WB, Kalkwarf KL, Patii, KD, Dyer JK, Bates RE. Jr. Evaluation of four modalities of periodontal therapy: Mean probing depth probing attachment levels and recession changes. J. Periodontol1988;59:783-793
30. Haffajee AD, Cugini, MA, Dibart S, Smith C, Kent RL, Socransky SS. The effect of SRP on the clinical and microbiological parameters of periodontal diseases. J ClinPeriodontol 1997; 24: 324–334.
31. Cobb CM Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. J ClinPeriodontol. 2002;29:6-16
32. Goodson JM, Haffajee AD, Socransky SS, Kent R, Teles R, Hasturk, H et al. Control of periodontal infections: A randomized controlled trial I. The primary outcome attachment gain and pocket depth reduction at treated sites. J ClinPeriodontol2012;39:526–536
33. Mousquéegs T, Listgarten MA, Phillips RW. Effect of scaling and root planing on the composition of the human subgingival microbial flora. J Periodontal Res 1980; 15: 144–151.
34. Haffajee AD, Cugini MA, Dibart S, Smith C, Kent RL, Socransky, SS. The effect of SRP on the clinical and microbiological parameters of periodontal diseases. J ClinPeriodontol 1997; 24:324–334.
35. Haffajee AD, Teles RP, Socransky SS. The effect of periodontal therapy on the composition of the subgingivalmicrobiota. Periodontol 2000 2006; 42:219-58.
36. Mohamed Syed Sadatullah N, Razak FA. The antimicrobial effect of 0.1 ppm ozonated water on 24-hour plaque microorganisms in situ. Braz Oral Res 2012; 26:126-131.
37. Ramzy MI, Gomaa HE, Mostafa MI, Management of Aggressive Periodontitis Using Ozonized Water. Egypt. Med. J. N R C. 2005; 6:229–245.
38. Rojas-Valencia MN, Research on ozone application as disinfectant and action mechanisms on wastewater microorganisms. In: Science against microbial pathogens: communicating current research and technological advances. Méndez-Vilas A edFormatex, p. 263, 2011
39. Allen EM, Matthews JB, O'Connor R, O'Halloran D, Chapple IL. Periodontitis and type 2 diabetes: is oxidative stress the mechanistic link? Scott Med J. 2009;54: 41-47
40. Chapple IL, Matthews JB. The role of reactive oxygen and anti-oxidant species in periodontal tissue destruction. Periodontol 2000 2007;43;160-232
41. Chapple IL, Brock GR, Milward MR Ling N, Matthews JB. Compromised GCF total antioxidant capacity in periodontitis: cause or effect? J ClinPeriodontol. 2007; 34:103-10.
42. Akpinar A, Toker H, Ozdemir H, Bostanci V, Aydin, H. The effects of non-surgical periodontal therapy on oxidant and anti-oxidant status in smokers with chronic periodontitis. Arch Oral Biol2013;58:717-723