RESEARCH PAPER

Ozonized Water Versus 0.2% Chlorhexidine Against Candida Albicans & Streptococcus Mutans in Chronic Periodontitis

Dr. Gaurav Chari[1], Dr. Ronak Kabra[2], Dr. Amit Jain[3], Dr. Rohit Mundra[4], Dr. Surbhi Porwal[5], Dr. Rohit Sharma[6]

[1]Senior Lecturer in the Department of Periodontics, Pacific Dental College and Research Centre, Bedla, Udaipur, Rajasthan

[2]Reader in the Department of Periodontics, RR Dental College and Hospital, Umarda, Udaipur, Rajasthan

[3]Professor in the Department of Oral Medicine and Radiology, Pacific Dental College and Hospital, Debari, Udaipur, Rajasthan

[4]Reader in the Department of Prosthodontics and crown and bridge and Implantology, Pacific Dental College and Research Centre, Bedla, Udaipur, Rajasthan

[5]Reader in the Department of Periodontics, Pacific Dental College and Research Centre, Bedla, Udaipur, Rajasthan

[6]Former Professor in the Department of Prosthodontics and crown and bridge and Implantology, Pacific Dental College and Research Centre, Bedla, Udaipur, Rajasthan

Corresponding Author: Dr. Rohit Mundra, Email: rohitmundra4188@gmail.com

Received: 25 Oct, 2026, Revised: 28 Oct, 2026, Accepted: 26 Oct, 2026, Published: 28 Oct, 2026

ABSTRACT

Chronic periodontitis is associated with microbial plaque accumulation and periodontal destruction. Ozonated water has been proposed as a biocompatible antimicrobial adjunct to conventional periodontal care. Therefore the aim of our study was to compare the antimicrobial effects of ozonated water and 0.2% chlorhexidine on salivary Streptococcus mutans and Candida albicans in patients with chronic periodontitis among 30 patients who were randomly allocated into three groups (n = 10): saline irrigation, ozonated-water irrigation, and 0.2% chlorhexidine irrigation. Subgingival irrigation was performed, and salivary samples were collected at baseline and on day 14. Microbial counts were assessed by culture methods, while plaque index, gingival bleeding index, probing pocket depth, and clinical attachment level were recorded. We have found that, all groups showed a significant reduction in *S. mutans* counts at day 14 ($p < 0.0001$); the greatest reduction was observed in Group I (88%), followed by Group II (44%) and Group III (9%). *C. albicans* counts decreased significantly in Groups I and II ($p < 0.05$), whereas Group III showed a non-significant reduction. Intergroup analysis demonstrated significant differences in S. mutans. Thus, we have come to conclude that, Ozonated-water irrigation demonstrated antimicrobial activity and may be used as an adjunctive measure for reducing periodontal microbial load in chronic periodontitis.

KEY WORDS: Ozonated Water, Chlorhexidine, Chronic Periodontitis, Streptococcus Mutans, Candida Albicans.

How to cite this article: Gaurav Chari1 Dr. Ronak Kabra2 Dr. Amit Jain3 Dr. Rohit Mundra4 Dr. Surbhi Porwal5 Dr. Rohit Sharma6. Ozonized Water Versus 0.2% Chlorhexidine Against Candida Albicans & Streptococcus Mutans in Chronic Periodontitis. Int J Drug Deliv Technol. 2026;16(87s):1908-1913. DOI: 10.25258/ijddt.16.87s.205.

INTRODUCTION

Ozone (O3) is a triatomic molecule, thermodynamically highly instable consisting of three oxygen atoms. Its molecular weight is 47,98 g/mol.. It has many different applications in various fields; one of them is usage of ozone in medicine.[1] Ozone is an elemental form of oxygen occurring naturally in the Earth's atmosphere, it surrounds the Earth at an altitude of between 50,000 and 100,000 feet.[2] In nature, ozone is formed in the Earth's stratosphere, where high energy radiation acts as a catalyst for its formation from molecular oxygen. Ozone can be generated artificially via the same reaction. Ozone is found in ambient air and humans are continually exposed to ozone during their daily lives. Corona Discharge system: produces high concentrations of ozone. It is the most common system used in the medical / dental field.[3] Ozone forms a protective layer that absorbs much of the sun's ultraviolet radiation, which can cause mutation, cancer, sunburn, immunosuppression and other problems. The depletion of the ozone layer by the use of chloroflourocarbons (CFC's), mostly released into the atmosphere by refrigerators, air conditioner and aerosol containers is of grave concern to scientists and physicians the world over.[4]

A German dentist, Dr. E.A. Fisch, in 1950, used ozonated water for dental procedures and pioneered its use in medicine.[5] Ozone therapy applications are determined by its anti-inflammatory, antiseptic, oxidative stress modulation, peripheral circulation improvement and tissular oxygenation properties. This specifies the number of pathologies in which it is useful, either by itself or most commonly as a complementary treatment. Thus, pathologies of inflammatory, infectious, ischemic and oxidative-stress-related origins can benefit from ozone therapy.[6] Dentistry is changing as we are now using modern science to practice dentistry. In dentistry, ozone has got its role in various dental treatment modalities. The ozone therapy has been more beneficial than present conventional therapeutic modalities. This state of the art technology allows us to take a minimally invasive and conservative approach to dental treatment. The main use of ozone in dentistry is relays on its anti-microbial properties. The application of ozone therapy in periodontics showed promising results. Both gaseous and aqueous ozone are used as a substitute

to mechanical debridement. Ozonated water (4 mg/l) strongly inhibited the formation of dental plaque and reduced the number of sub gingival pathogens. Gram negative bacteria, such as P. endodontalis and P. gingivalis were substantially more sensitive to ozonated water than gram positive oral streptococci and C. albicans in pure culture. Ozone gas found to be toxic to the human oral epithelial and gingival fibroblast cells and aqueous ozone is more biocompatible than gaseous ozone. The application of ozone therapy in chronic gingival and periodontal diseases, showed subjective and objective improvement of their status, as well as patients with periodontal abscess, with no exudation was observed.[7]

AIM

To compare antimicrobial presence by effects of ozonized water and chlorhexidine 0.2% on micro organisms Candida albicans and streptococcus mutans in patients with Chronic Periodontitis.

MATERIAL AND METHODS

This randomized, controlled, double-blind clinical study included 30 patients with chronic periodontitis who were recruited by convenience sampling and randomly allocated into three groups (n = 10 each): Group I received saline irrigation (control), Group II received ozonated-water irrigation as shown in figure 1



FIGURE 1 : OZONE IRRIGATION and Group III received chlorhexidine (CHX) irrigation as shown in figure 2.



FIGURE 2 : 0.2% CHLORHEXIDINE IRRIGATION

The study duration was 14 days, comprising baseline to day 7 and day 7 to day 14 intervals; patients and the clinical examiner were blinded to the allocated irrigant. Participants refrained from all oral-hygiene measures for 24 hours before sampling. Unstimulated saliva was collected at baseline and 10 minutes after irrigation; after a water rinse and 10-minute waiting period, participants expectorated approximately 2 mL saliva into sterile labelled containers, which were immediately maintained on ice. Ozonated-water irrigation was delivered using the Kent Ozone Dental Jet TY-820 was used as shown in figure 3,



FIGURE 3 : KENT OZONE DENTAL JET TY-820

through a bent 20-gauge blunt needle inserted approximately 3 mm subgingivally for 5–10 minutes as shown in figure 1, whereas saline and CHX were delivered with a 2-mL syringe and a 24-gauge needle bent to approximately 110 degrees, using 10 mL irrigant over 20 seconds as shown in figure 2. For microbiological assessment, saliva was serially diluted and cultured for Streptococcus mutans on Mitis Salivarius agar supplemented with bacitracin and potassium tellurite under microaerophilic conditions at 37 degrees Celsius for 48 hours as hsown in figure 4 & 5,

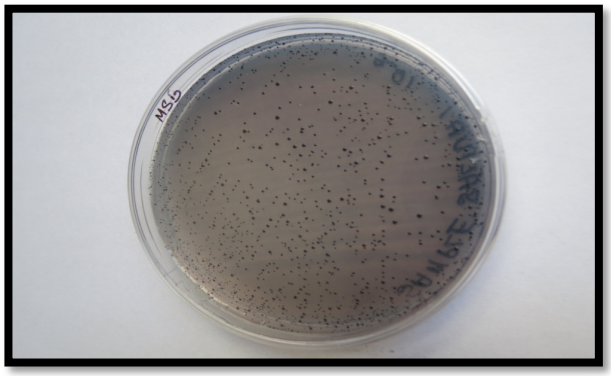


FIGURE 4 : COLONIES OF S.MUTANS (PRE IRRIGATION)

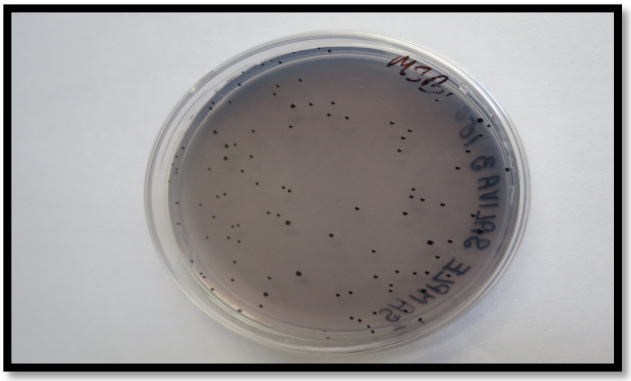


FIGURE 5: COLONIES OF S.MUTANS (POST IRRIGATION)

while Candida albicans was cultured on Sabouraud Dextrose Agar after dilution in Sabouraud broth and incubated at 30 degrees Celsius for 48 hours to 7 days; colonies were identified morphologically and quantified as colony-forming units per millilitre as shown in figure 6 & 7.

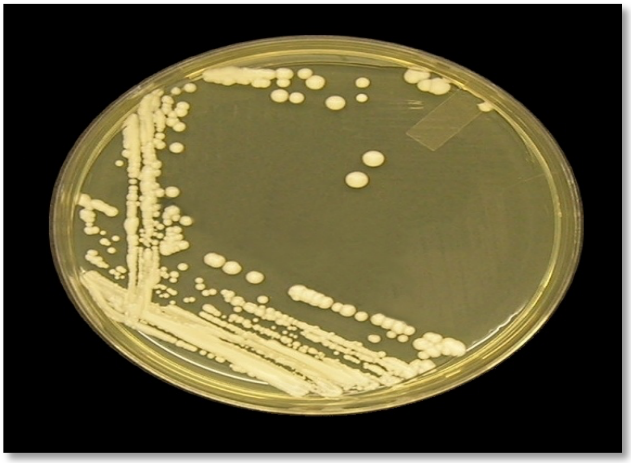


FIGURE 6 : COLONIES OF C.ALBICAN (PRE IRRIGATION)



FIGURE 7: COLONIES OF C.ALBICAN (POST IRRIGATION)

INCLUSION CRITERIA

1. Patient's age between 30-65 years.
2. Clinical diagnosis of Chronic generalized periodontitis with evident bone loss on radiograph and PD ≥4mm or more at 30% of proximal sites, with Clinical attachment loss.
3. No history of periodontal therapy in last 6 months

EXCLUSION CRITERIA

1. Patients who are pregnant or lactating
2. Patients who are suffering from any known systemic diseases which could effect the course of assessment.
3. Patients who have received any surgical or non-surgical therapy 6 months prior to the start of the study
4. Patients who have received any antibiotic therapy in the last 6 months,
5. Patients who have received any chemotherapeutic mouth rinses and oral irrigation during the past 6 months
6. Patients who are smokers.
7. Radiation therapy

STATISTICAL ANALYSES

Statistical Analyses of the entire data were performed using SPSS 16 software program. Data were analysed using one-way analysis of variance (ANOVA) to assess statistical difference between various groups (Group I, II, II). Turkey-karmer multiple comparisons test was done for comparison between groups. Mean values and SD values for each parameter included were calculated. P values from all statistical tests were presented, but were considered statistically significant at $p \leq 0.05$ and highly significant at $p \leq 0.001$.

RESULTS

TABLE 1 : MEAN VALUE OF AGE DIFFERENCE BETWEEN THE STUDY GROUPS

GROUPS	AGE	SEX	
	Mean±SD	Male	Female
GROUP I	37.90 ± 5.04	1	9
GROUP II	39.80 ± 10.18	6	4
GROUP III	41 ± 6.65	8	2

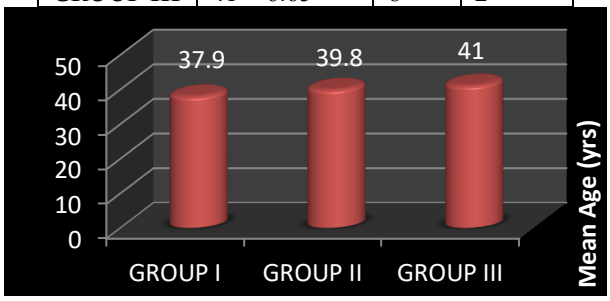


FIGURE 8 : MEAN DIFFERENCE IN AGE BETWEEN GROUP I, II, III

Table 1 & figure 8 showed that , a total of thirty patients with chronic periodontitis were allocated equally among the three study groups (n = 10 each). The mean age was 37.90 ± 5.04 years in Group I, 39.80 ± 10.18 years in Group II, and 41.00 ± 6.65 years in Group III, indicating broadly comparable age distribution. Group I consisted predominantly of females (9 females, 1 male), whereas Groups II and III had a higher proportion of males (6 and 8, respectively). Thus, the groups showed variation in sex distribution, while age characteristics were generally similar before assessing the antimicrobial effects of saline, ozonated water, and 0.2% chlorhexidine on Candida albicans and Streptococcus mutans.

TABLE 2 : COMPARISON OF MEAN VALUES OF S.MUTANS COUNT OF DIFFERENT TREATMENT GROUPS (AT BASELINE AND 14TH DAY)

GROUPS	0 DAY	14 DAY	0-14 DAY	% REDUCTION	P-VALUE
	Mean± SD	Mean ±SD	Mean ±SD		
GROUP I	564.50 ±161.18	67.50 ±49.17	497±132.08	88	<0.0001
GROUP II	614±137.53	343±144.15	271±109.79	44	<0.0001
GROUP III	682±146.27	619±139.40	63±18.29	9	<0.0001

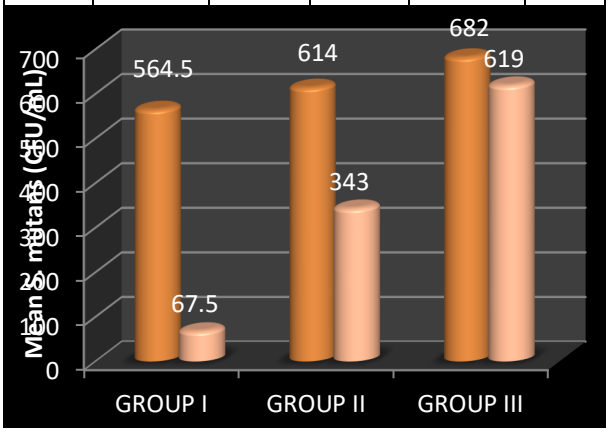


FIGURE 9 : MEAN DIFFERENCE IN S.MUTANS BETWEEN GROUP I, II, III

Table 2 & figure 9 showed that, The mean Streptococcus mutans count decreased significantly from baseline to day 14 in all treatment groups ($p < 0.0001$). Group I showed the greatest reduction, from 564.50 ± 161.18 to 67.50 ± 49.17, corresponding to an 88% reduction. Group II demonstrated a 44% reduction, with counts declining from 614.00 ± 137.53 to 343.00 ± 144.15. Group III showed the least reduction (9%), from 682.00 ± 146.27 to 619.00 ± 139.40. Overall, the findings indicate varying antimicrobial effectiveness among the tested interventions against salivary *S. mutans* in chronic periodontitis patients.

TABLE 3: COMPARISON OF MEAN VALUES OF C. ALBICANS COUNT OF DIFFERENT TREATMENT GROUPS (AT BASELINE AND 14TH DAY)

GROUPS	0 DAY	14 DAY	0-14 DAY	% REDUCTION	P-VALUE
	Mean ±SD	Mean ±SD	Mean ±SD		
GROUP I	156.25 ±79.09	88.63± 20.98	67.63 ±64.51	43	<0.05
GROUP II	157.50 ±41.93	115.75 ±37.31	41.75 ±25.14	26	<0.05
GROUP III	80±21.21	77.50± 17.68	2.50± 3.54	3	>0.05

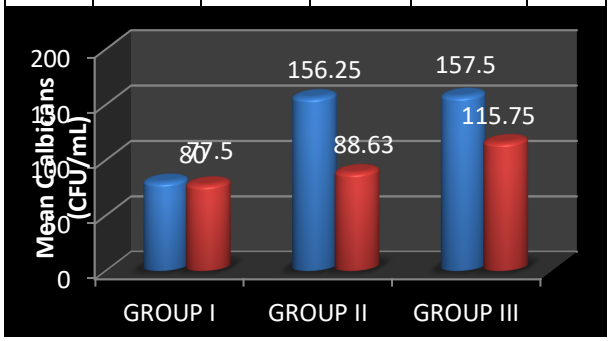


FIGURE 10 : MEAN DIFFERENCE IN C.ALBICANS BETWEEN GROUP I, II, III

Table 3 & figure 10 showed that, the mean Candida albicans count decreased from baseline to day 14 in all groups. Group I demonstrated the maximum reduction, from 156.25 ± 79.09 to 88.63 ± 20.98, representing a 43% reduction that was statistically significant ($p < 0.05$). Group II showed a significant 26% reduction, declining from 157.50 ± 41.93 to 115.75 ± 37.31 ($p < 0.05$). Group III exhibited only a 3% reduction, from 80.00 ± 21.21 to 77.50 ± 17.68, which was not statistically significant ($p > 0.05$).

TABLE 4 : ONE WAY ANALYSIS OF VARIANCE (ANOVA) FOR GROUPS I, II, III

GROUPS	P-VALUE OF S. MUTANS (14th DAY)	P- VALUE OF C. ALBICANS (14th DAY)
I VS II	<0.001	>0.05
I VS III	<0.001	>0.05
II VS III	<0.001	<0.05

Table 4 showed that At the 14th day, intergroup comparison showed a highly significant difference in Streptococcus mutans counts among all treatment groups (Groups I vs II, I vs III, and II vs III; $p < 0.001$), indicating different antimicrobial effects of the interventions. For Candida albicans, no statistically significant difference was observed between Groups I and II or between Groups I and III ($p > 0.05$). However, a significant difference was found between Groups II and III ($p < 0.05$), suggesting that these two interventions differed in their effect on C. albicans counts.

DISCUSSION

Gingivitis and periodontitis are characterized by a local hypoxia of tissues and also by various microbic florae that may contain over 250 species. The initiation and progression of periodontitis is caused by different bacterial accumulations in the subgingival pockets. The principle objective of periodontal therapy is to eliminate the subgingival bacterial presence and, by subsequent supragingival plaque control measures, prevent or minimize recolonization of the subgingival area by pathogenic microflora. Mechanical root debridement to remove dental calculus is important in periodontal

therapy but is frequently inadequate in curing severe periodontal infections. The mechanical methods of plaque control can also be supplemented by antimicrobial agents.[8] The most common method for controlling the growth of dental plaque is mechanical. However, the effectiveness of this method is limited by such factors as individual motivation, inaccessibility to periodontal pockets, concave tooth surfaces and the margins of restorations. Alternatives to mechanical plaque control are therefore being sought. Chlorhexidine has emerged as an important oral antibacterial agent and adjunct to periodontal therapy. It is a broadspectrum antiseptic with pronounced antimicrobial effects on Gram-positive as well as Gram-negative bacteria, some viruses and fungi.[9] Apart from direct oxygen irrigation, a few oxygenating agents such as sodium monohydrate, sodium monoxychlorosene, carbamide peroxide and sodium borate peroxyhydrate were applied to areas of wounded rat oral tissue. All of these showed complete healing in a shorter time than normally required. Recently, an allotropic form of oxygen, ozone, is being used in dentistry for the treatment of dental caries. Ozone gas application for a period of 10 sec was capable of reducing the numbers of microorganisms associated with primary root carious lesions in vitro. Four in vitro studies have reported the antimicrobial effect of ozonated water. Ozonated water has been shown to be effective against Candida albicans, adhering to acrylic denture plates, on Enterococcus faecalis and Streptococcus mutans of dentinal tubules and periodontopathic bacteria such as Aa and Pg in vitro. Ozonated water has also been shown to be effective on root surfaces after extraoral rinsing for decontamination of avulsed teeth in vitro.[9] This pilot study was performed to determine the effect of 0.1 ppm ozonated water on *in situ* plaque formation and to identify any immediate discomfort or adverse reaction an individual may have to the gas. The commonly employed method of evaluating the effectiveness of oral antiseptics antiseptics by cleaning the teeth, asking the volunteers to follow a prescribed regimen of mouth rinsing, and then testing the effectiveness after a period of time was not used in this study because ozone machines could not be given to all of the volunteers to prepare fresh ozonated water for rinsing. Taking into consideration the safety of the subjects, this study did not include concentrations of ozonated water higher than 0.1 ppm. The bacteria grown on selective media from all of the plaque samples were fastidious and required a 24- to 48-hour incubation period. The microbiological tests performed in this study suggested that the ozonated water exhibited some antimicrobial activity on the bacterial population of the 24-hour plaque.

A study conducted by Nagayoshi et al who showed that ozonated water (0.5-4mg/l) was highly effective in killing both gram positive and gram negative oral microorganisms such as Porphyromonas endodontalis and Porphyromonas gingivalis. Ozonated water has also been shown to be effective against Candida or Enterococcus fecalis and periodontopathic bacteria such as A. actinomycetemcomitans and P.gingivalis in vitro.[10] But no literature exists till date on the in vivo effect of ozonated water on organisms such as spirochetes. The oral spirochetes are often the dominant bacteria observed in subgingival plaque, and yet they are one of the least-studied and understood by the periodontal investigators. Thus this study evaluates and compares the antimicrobial effects of subgingival irrigation with ozonated water, 0.2% chlorhexidine and saline water in chronic periodontitis patients. Ramzy et al found a significant improvement in bacterial count to quadrants treated by scaling and root planing along with ozone application in patients with aggressive periodontitis.[11] Huth et al found significant reduction in periodontal pathogens namely P.gingivalis, Parvimonas micra, Tannerella forsythia on irrigation with gaseous/aqueoue ozone as compared to 0.2%CHX.[12] None of the agents could substantially reduce *A.* actinomycetem comitans (A.a.) count in biofilm cultures.[8] In the present study, the antimicrobial efficacy of ozone demonstrated the higher percentage of reduction in S.mutans count with ozonated water irrigation (88%) as compared to 0.2%Chlorhexidine (44%) and Normal saline (9%). Recently, it has been shown that ozonated water was highly effective in killing both Gram-positive and Gram-negative oral microorganisms like Streptococci, Pg, P. endodontalis and Candida albicans in vitro. Hence, it was suggested that ozonated water should be useful in reducing the infections caused by oral microorganisms in dental plaque. Morever, the disadvantages of CHX such as taste alteration and staining are potential drawbacks of chlorhexidine. Considering the limitation of this study in terms of short-term duration, ozone can be considered as a promising antimicrobial agent in the periodontal therapy. Further long-term studies are required to adequately assess the efficacy of ozone in vivo and to evaluate the frequency and duration of application of ozone. It is required to determine the specific ozone concentration that is effective against anaerobic periodontopathogens. It can be concluded that local ozone application can serve as a potential agent to treat periodontal disease non-surgically both for home care and for professional practice. It may serve as a good tool during supportive periodontal therapy.

CONCLUSION

Within the limitations of this study, ozonated water at a concentration of 0.1 ppm demonstrated antimicrobial potential by reducing plaque-associated microbial counts in patients with chronic periodontitis. Although it did not completely eliminate the microbial population, ozone may serve as a useful adjunct to conventional mechanical plaque-control measures, including tooth brushing, flossing, and professional periodontal therapy. Its ease of use, cost-effectiveness, broad antimicrobial activity, and reported biocompatibility make it a promising supportive modality in periodontal care. However, further well-designed studies with larger samples and longer follow-up periods are necessary to establish its clinical effectiveness and long-term role in controlling periodontal pathogens.

REFERENCE

1. Seidler V, Linetskiy I, Hubálková H, Stanková H, Smucler R, Mazánek J. Ozone and its usage in general medicine and dentistry. A review article. Prague Med Rep. 2008 Jan 1;109(1):5-13.
2. Rothchild J, Harris R, Mollica P. Current concepts of oxygen ozone therapy for dentistry in the United States. Int J Ozone Ther. 2010;9:105-8.
3. Gupta M. Ozone: An Emerging Prospect In Dentistry. Indian Journal of Dental Sciences. 2012 Mar 1;4(1):47.
4. Maslennikov OV, Kontorshchikova CN, GRIBKOVA IA. Ozone therapy in Practice. Health Manual, Ministry Health Service of The Russian Federation The State Medical Academy Of Nizhny Novgorod, Russia. 2008.
5. Arigbede AO, Dosumu OO, Shaba OP. Ozone therapy and restorative dentistry: a literature review. Nigerian Dental Journal. 2010;18(1).
6. Brazzelli M, McKenzie L, Fielding S, Fraser C, Clarkson J, Kilonzo M, Waugh N. Systematic review of the effectiveness and cost-effectiveness of HealOzone® for the treatment of occlusal pit/fissure caries and root caries. Health Technology Assessment. 2006;10(16).
7. Makkar S, Makkar M. Ozone-treating dental infections. Indian Journal of Stomatology. 2011 Oct 1;2(4):256.
8. Dodwad V, Gupta S, Kumar K, Sethi M, Masamatti S. Changing paradigm in pocket therapy-ozone vs conventional irrigation. International Journal of Public Health Dentistry. 2011 Apr 1;2(2):7-12.
9. 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 Journal of Dental Research. 2010 Jul 1;21(3):341-8.
10. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. Oral microbiology and immunology. 2004 Aug;19(4):240-6.
11. MI R, Gomaa H, MI M, Zaki B. Management of aggressive periodontitis using ozonized water. Egypt Med JNR C. 2005;6(1):229-45.
12. Huth KC, Quirling M, Lenzke S, Paschos E, Kamereck K, Brand K, Hickel R, Ilie N. Effectiveness of ozone against periodontal pathogenic microorganisms. European journal of oral sciences. 2011 Jun;119(3):204-10.