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KEY WORDS: Ozone, Chlorhexidine, Chronic periodontitis, subgingival irrigation.

OZONE VERSUS CHLORHEXIDINE IN THE TREATMENT OF CHRONIC PERIODONTITIS

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ABSTRACT

Background: study was to compare and evaluate the effects of Subgingival Irrigation with Ozonated water and 0.2% Chlorhexidine as an adjunct to scaling and root planing in the improvement of periodontal health in patients with moderate to severe chronic periodontitis.

Material & Method: A randomized, split-mouth design was performed in 22 subjects. Each half (right and left) of the oral cavity was randomly assigned into 2 groups (A and B) of 22 subjects. After performing scaling and root planing, subgingival Irrigation with either OZ or CHX was performed once in a week for 6 weeks. Clinical parameters were recorded and subgingival plaque samples were collected, at baseline and at 6 weeks.

Result: When comparing the indices of individual groups at baseline and 6 weeks, it was seen that there was statistically significant reduction in the mean values of **PI, GI, PPD, CAL** and in all the four periodontal pathogens which were ***Aa, Pg, Pi,*** and ***Tf*** in both the groups (A and B). The differences were statistically nonsignificant when comparisons were made between group A and group B.

Conclusion: ozonated water can be used in treating chronic periodontitis patients as it is equally effective as chlorhexidine in subgingival irrigation as an adjunct to SRP.

INTRODUCTION:

Periodontitis is a plaque induced chronic inflammatory disease, which affects the integrity of the periodontal tissues that surround and support the teeth (periodontal ligament, gingiva, and alveolar bone; collectively known as the periodontium) and may exert an adverse impact on systemic health.

Removal of plaque forms an important part of controlling and treating periodontal disease, which brings about both qualitative as well as quantitative changes in the subgingival microflora. However, the effectiveness of mechanical debridement is limited by various factors such as inaccessibility to periodontal pockets, tissue invasive micro-organisms, concave tooth surfaces, the unfavourable anatomy of roots, the overhanging margins of restorations, the bacterial invasion into dentinal tubules & intra oral microbial translocation.[1]

A number of chemical adjuncts have been used to improve the outcome of mechanical oral hygiene procedures.[2] Currently, the established oral antiseptics for periodontal treatment include chlorhexidine gluconate (CHX, 0.2–2%). Regarding side-effects, it is known that chlorhexidine may cause mucosal desquamation, impaired wound healing and fibroblast attachment to the tooth surfaces, tooth staining and altered taste sensation.[3]

Now a days, ozone gas dissolved in water or in oil such as olive oil (ozonated oil) is being discussed in dentistry for its excellent antimicrobial property without the development of drug resistance.[1] Ozone (also known as triatomic oxygen and trioxygen O3, molecular weight 47.98g/mol) is a naturally occurring compound consisting of three oxygen atoms. The use of ozone in different dental fields comes as a result of physico-chemical properties. There are various known actions of ozone on human body, such as immunostimulating and analgesic, antimicrobial, antihypoxic and detoxicating, bioenergetic and biosynthetic (activation of the metabolism of carbohydrates, proteins, lipids) etc.[4] Microbiological investigation of chronic periodontitis have been carried out in various studies support the concept that chronic periodontitis is associated with specific bacterial agents revealing high percentages of anaerobic (90%) gram-negative (75%) bacterial species. Detectable levels of P. gingivalis, P. intermedia, T. forsythia, C. rectus, and A. actinomycetemcomitans are associated with disease progression, and their elimination via periodontal therapy is associated with an improved clinical response.[5]

In proposing ozone as another potential antimicrobial for use in the oral cavity, it is important to compare its potential with that of the potential of established agents. With this background, the proposed study was conducted with a randomized, split-mouth study design. The aim of the study was to compare and evaluate the effects of Subgingival Irrigation with Ozonated water and 0.2% Chlorhexidine as an adjunct to scaling and root planing in the improvement of periodontal health in patients with moderate to severe chronic periodontitis.

MATERIALS & METHODS:

The present study was conducted in the Department of Periodontics, Govt. Dental College And Hospital, Aurangabad with the approval of the Institutional Ethical Committee.

Inclusion criteria

1. Subjects to be aged between 25 and 65 years with minimum of 3 teeth in each quadrant and diagnosed with chronic moderate to severe periodontitis.
2. Patients with at least one site in each quadrant with Probing Pocket Depth≥5mm.
3. Patients willing to give and sign written informed consent for study

Exclusion criteria

1. Patient with any known Systemic disease.
2. Pregnant or lactating Females.
3. Tobacco chewing in any forms, smokers and/or alcoholics
4. Treatment with chlorhexidine 6 weeks before inclusion.
5. Treatment with antibiotics, any periodontal therapy within last 6 months.

Clinical parameter

1. Plaque Index (Turesky, Gilmore, Glickman modification of Quigely Hein Index, 1970)
2. Gingival Index (Loe and Silness, 1963)
3. Probing pocket depth (PPD) measurement using University of North Carolina probe (UNC-15 Probe).
4. Clinical attachment level (CAL)

Study Method:

- Clinical parameters were recorded and subgingival plaque samples were collected, at baseline and at 6 weeks.
- A randomized, split-mouth design was performed in 22 subjects. Each half (right and left) of the oral cavity was randomly assigned into two groups of 22 subjects by using lottery method:

Group A- Subgingival Irrigation with Ozonated Water (OZ) as an adjunct to scaling and root planing.(Figure 1)

Group B- Subgingival Irrigation with 0.2% Chlorhexidine (CHX) as an adjunct to scaling and root planing.(Figure 2)

- At baseline, the clinical parameters were assessed and subgingival plaque samples were collected.
- Scaling and root planing was performed by using ultrasonic scaler and area specific gracey curettes respectively.
- After isolation with cotton rolls, each half of the mouth was subjected to Subgingival Irrigation with either OZ or with CHX simultaneously.
- Excess irrigant solution was continuously aspirated.
- The Subgingival Irrigation of each half of the mouth was carried out for an approximate period of 5 min with a 2 ml syringe, once weekly for 6 weeks.

Figure 1: Subgingival Irrigation With Ozonated Water

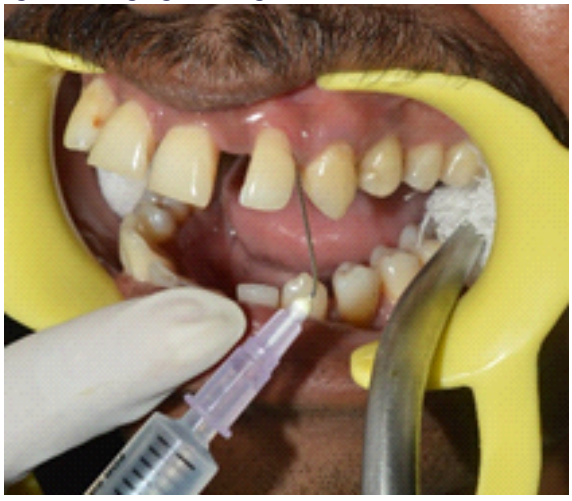
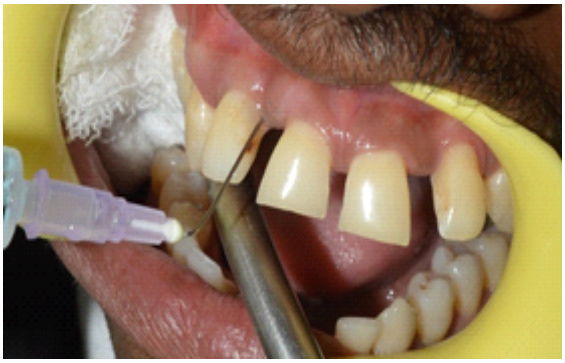


Figure 2: Subgingival Irrigation With Chlorhexidine



Ozonated water preparation:

This aqueous ozone (4 mg/L) was freshly prepared. Aqueous ozone was prepared by using Amozonics medical generator **(Figure 3)**. For input gas medical grade oxygen cylinder was attached. 230V current was supplied to ozone generator. It produced ozone in gaseous form, which was added to one liter of distilled water. Concentration was measured by using ozone aqua test colour comparator. The freshly prepared aqueous ozone was carried to the periodontal pocket by sterile syringe with needle.

Figure 3: Ozone Generator

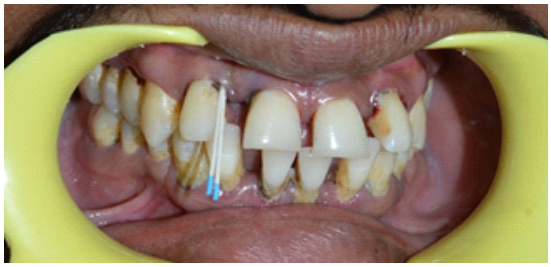
Collection of subgingival plaque sample (Ercole et al.)[6]

- Sub-gingival plaque was collected at the preliminary visit and after 6 weeks from the two predetermined sites for microbiologic evaluation.
- Supra gingival plaque was removed by using a sterile curette and the gingival surface was dried with cotton roll.
- The plaque samples **(Figure 4)** were obtained by insertion of two standardized #30 sterile paper points into the deepest periodontal pocket of each half of the mouth, which were left in situ for 30 seconds.
- The plaque samples were collected in Eppendorf vials containing transport media, viz. TE buffer (10 mMTris-HCl, 1 mM EDTA, pH 8) were send to the microbiological laboratory, (Department of Molecular Biology and Immunology, MMNGH Institute of Dental Sciences and Research Centre, Belgaum) where they were subjected to multiplex polymerase chain reaction (PCR) analysis.

The following periodontal pathogens were analyzed by Multiplex PCR analysis:

Aggregatibacteractinomycetemcomitans
Porphyromonasgingivalis
Tannerella forsythia
Prevotella Intermedia

Figure 4: Collection Of Plaque Sample



RESULTS:

Based on the comparison of two means and standard deviation with 95 % of Confidence Interval and power 80, a sample size of n=9 per group was required.

Data analysis was done for ***PI, GI, PPD*** and ***CAL*** and concentrations of ***Aa, Pg, Tf****and* ***Pi*** in subgingival plaque samples by using **SPSS, version 20**.

- Quantitative data were recorded as the **mean** value and **standard deviation (SD)** for all investigated parameters.
- **Paired t test** was used to evaluate **intra group**

comparisons of the clinical and microbiological parameters in pairs for group A and group B, and statistical significance was set at P < 0.005.

- **Unpaired Student t test** was used to evaluate the **intergroup comparisons** of the mean differences of various clinical and microbiological parameters between group A and group B, and statistical significance was set at P < 0.05.

Table 1- Intra-group comparison of mean values of various clinical parameters of Group A at baseline and 6 weeks

S. No.	Clinical Parameter	Baseline	6 Weeks	't' test value#	P - value
1	PI	2.70±0.51	1.20±0.57	14.31	<0.0001*
2	GI	2.27±0.46	0.34±0.40	19.19	<0.0001*
3	PPD	4.51±0.88	2.90±0.71	10.98	< 0.0001*
4	CAL	4.77±0.96	3.18±0.73	11.06	< 0.0001*

**highly statistical significant (p<0.0001)*
#paired t test

Table 2 - Intra-group comparison of mean levels of different bacteria of Group A at baseline and 6 weeks

S. No.	Microbiological Parameter	**Baseline** (Mean±SD) (CFU/ml)	**6 Weeks** (Mean±SD) (CFU/ml)	't' test value#	Significance (2-tailed) P value
1	Aa	$1.31x10^5 \pm 1.86x10^6$	$2.28x10^4 \pm 6.94x10^4$	3.3	0.0034*
2	Pg	$7.01x10^4 \pm 9.90x10^4$	$1.57x10^3 \pm 1.31x10^3$	3.242	0.0039*
3	Pi	$8.35x10^5 \pm 1.75x10^6$	$2.00x10^3 \pm 1.01x10^3$	2.239	0.0361*
4	Tf	$5.00x10^4 \pm 6.80x10^4$	$3.46x10^3 \pm 2.66x10^3$	3.154	0.0048*

**statistically significant*
#paired t test

Table 3 - Intra-group comparison of mean values of various clinical parameters of Group B at baseline and 6 weeks

S. No.	Clinical Parameter	Baseline	6 Weeks	't' test value#	P - value
1	PI	2.71±0.58	1.35±0.65	10.98	< 0.0001*
2	GI	2.26±0.47	0.37±0.40	19.75	< 0.0001*
3	PPD	4.38±0.75	3.00±0.64	10.81	< 0.0001*
4	CAL	4.72±0.95	3.29±0.74	11.10	< 0.0001*

**highly statistical significant (p<0.0001)*
#paired t test

Table 4 - Intra-group comparison of mean levels of different bacteria of Group B at baseline and 6 weeks

S. No.	Microbiological Parameter	**Baseline** (Mean±SD) (CFU/ml)	**6 Weeks** (Mean±SD) (CFU/ml)	't' test value#	Significance (2-tailed) P value
1	Aa	$5.20x10^4 \pm 1.19x10^5$	$5.11x10^3 \pm 1.56x10^4$	2.112	0.0469*
2	Pg	$3.12x10^4 \pm 5.37x10^4$	$5.74x10^3 \pm 1.44x10^4$	2.258	0.0347*
3	Pi	$6.25x10^6 \pm 1.34x10^7$	$3.20x10^3 \pm 3.77x10^3$	2.19	0.0400*
4	Tf	$5.04x10^4 \pm 1.03x10^5$	$1.21x10^3 \pm 2.88x10^1$	2.21	0.0383*

**statistically significant*
#paired t test

Table 5 - Intergroup comparison of Group A and Group B for clinical parameters

S No.	Clinical Parameter	Group A Difference (mean +/- SD)	Group B (Difference mean+/-SD)	't' test value#	Unpaired t test P-value (p<0.05)
1	PI	1.50 ± 0.49	1.35 ± 0.58	0.79	0.45 (NS)
2	GI	1.92 ± 0.47	1.90 ± 0.45	0.23	0.82 (NS)
3	PPD	1.61 ± 0.69	1.36 ± 0.60	0.47	0.64 (NS)
4	CAL	1.60 ± 0.68	1.43 ± 0.61	0.51	0.61 (NS)

Table 6- Intergroup comparison of Group A and Group B for microbiological parameters

S No.	Microbiological Parameter	Group A (Difference mean +/- SD)	Group B (Difference mean +/- SD)	't' test value#	Unpaired t test P-value(p<0.05)
1	Aa	$1.08x10^5 \pm 1.54x10^5$	$4.69x10^4 \pm 1.04x10^5$	1.84	0.0719
2	Pg	$6.85x10^4 \pm 9.91x10^4$	$2.54x10^4 \pm 5.28x10^4$	1.70	0.0968
3	Pi	$8.33x10^5 \pm 1.75x10^6$	$6.25x10^6 \pm 1.34x10^7$	2.21	0.0619
4	Tf	$4.65x10^4 \pm 6.92x10^4$	$4.92x10^4 \pm 1.04x10^5$	0.002	0.9984

 # unpaired t test

DISCUSSION:

Ozonated water has been shown to be effective against *Candida albicans*, adhering to acrylic denture plates,[6] on *Enterococcus faecalis*[7] and *Streptococcus mutans* of dentinal tubules[8] and periodontopathic bacteria such as Aa and Pg in vitro[9]. Ozone is a selective oxidant and effects only certain compounds, but when it dissolves in water, it becomes highly unstable and rapidly decomposes through a complex series of chain reactions. As a result, hydroxyl (OH) radicals are generated, which are among the most reactive oxidizing species. Ozone reacts with various chemical compounds in aqueous systems in two different and coexisting modes, one involving direct reactions of molecular ozone and the other a free radical-mediated reaction.[17] Both these mechanisms may be involved in the destruction of bacteria by ozone.

In this study, the primary focus was to evaluate the efficacy of subgingival irrigation once a week for six weeks with freshly

prepared 4 mg/l ozonated water (OZ) and 0.2 % chlorhexidine (CHX) on clinical parameters and on specific microorganisms present in subgingival plaque as an adjunct to scaling and root planing. There are several ways of delivering chemical agents. One of the ways such as the subgingival irrigation which interferes with the complex ecosystem required for the initiation and continued destruction of the compromised periodontium in the susceptible host. The effects of irrigation on gingival bleeding and plaque include change in plaque composition, flushing out of inflammation-inducing factors and physical change in tissue integrity. There are several ways of delivering chemical agents.[11]

In the present study, the mean and SD values of PI and GI for group A at baseline and six weeks showed statistically significant reduction which was in accordance with the findings of **Patel et al., Isaac et al., Dodwad et al., Katti et al.,** and **Habashneh et al.** whom reported a reduction in the plaque scores and gingival inflammation as compared to the baseline value.1, 12, 13, 14, 15)Also, the mean and SD values of PI and GI for group B at baseline and at six weeks were in accordance with the findings of **Southard et al., Reynolds et al., Jolkovsky et al. and Faveri et al.** whom reported a reduction in the plaque scores and gingival inflammation as compared to the baseline.(16, 17, 18, 19) Although the intergroup comparison of Group A and B showed nonsignificant results.

Patel et al.(1) and **Dodwad et al.**[15]showed more reduction in PPD and gain in CAL with ozonated water subgingival irrigation as than 0.2% Chlorhexidine subgingival irrigation, whereas, in the present study inter group comparison in PPD and CAL was found to be statistically nonsignificant which could be due to the different study designs, patient populations and carrier systems. Results of our study shows that OZ and CHX are comparable and both can be used for subgingival irrigation as an adjunct to SRP.

A study conducted by **Nagayoshi et al**.(20)showed that the ozonated water (0.5-4mg/l) was highly effective in disrupting the plaque biofilm and subsequent killing of both gram positive and gram negative oral microorganisms such as *Porphyromonasendodontalis* and *Porphyromonasgingivalis*. Ozonated water has also been shown to be effective against *Enterococcus fecalis* and periodontopathic bacteria such as *A. actinomycetemcomitans*. Invitro Anti-hypoxic effect is another mechanism that can explain the improved clinical and microbiological outcomes after the ozone treatment.

Mosques et al.(21), concluded that after SRP, bacteria recolonize and reach the baseline approximately within 42 days but in the present study all the four periodontal pathogens reduced from day 0 to day 42. This could be due to the adjunctive antimicrobial action effect of the OZ and CHX along with SRP.

Kshitish et al.(3)reported reduction of *A. actinomycetemcomitans* in ozone treated group which was in accordance to the present study. Moreover, they demonstrated that there was no antibacterial effect of ozone on *Porphyromonasgingivalis* and *Tannerella forsythia* but in our study there was significant reduction in A. *actinomycetemcomitans, Prevotella Intermedia, Porphyromonasgingivalis and Tannerella forsythia* in both the groups from baseline to six weeks. This could be due to the repeated sub gingival irrigation with ozonized water and chlorhexidine which might have interfered with the recolonization of subgingival micro flora. **Huth et al.(22)**reported a significant reduction in periodontal pathogens namely *P.gingivalis, Parvimonasmicra, Tannerella forsythia* on irrigation with gaseous/aqueous ozone as compared to 0.2% CHX. In the present study, reduction in periodontal pathogens were comparable between OZ and CHX groups which suggests that OZ and CHX are equally effective and can be used for the subgingival irrigation along with SRP. **Huth et al.**(22)reported that neither gaseous/aqueous ozone nor 0.2% chlorhexidine could substantially reduce *A. actinomycetemcomitans*count in biofilm cultures but in our study there was significant reduction in A. *actinomycetemcomitans* in both the groups from baseline to six weeks. This could be due to the repeated sub gingival irrigation with ozonized water and chlorhexidine which might have interfered with the recolonization of subgingival micro flora.

Riggioet al.(23)reported that PCR is a powerful diagnostic tool that can detect low numbers of periodontal pathogens with a high degree of accuracy in subgingival plaque samples. It is rapid, with results being available within hours of sample acquisition, cheaper and less labour-intensive than conventional culture methods and permits many more samples to be easily screened at one time. With these advantages in mind, in the present study, PCR technique was used for detecting, quantifying and differentiating microorganisms before and after subgingival irrigation with ozone and chlorhexidine in treating chronic moderate to severe periodontitis patients. According to **Loesche et al.,(24)**culture method demonstrated an inability or difficulty in growing several bacterial species, e.g. *T. forsythia*. Because *T. forsythia* was one of the microbiological parameters of our study, we opted for PCR technique for the identification and quantification of microorganisms.

Limitations and Future Perspectives of the present study

1. The groups were not compared to a 'negative control' such as Placebo + SRP.
2. The stability of ozone in water was low and ozone dissipated very quickly at room temperature over 5 minutes, so every time, the preparation of fresh ozonated water was required before subgingival irrigation.
3. Future studies with longer follow up periods are warranted to confirm the effectiveness.
4. A clinical trial with a larger study population will yield a better understanding of the therapeutic effect of *Ozone* in periodontal therapy.
5. Further studies should be conducted using ozonated water at different concentrations so that optimum concentration of ozonated water can be acquired for the treatment of chronic periodontitis.

CONCLUSION

Within the limitations of this study, we can say that subgingival irrigation with OZ is equally effective as CHX in improving periodontal health, when used as an adjunct to SRP in treating chronic moderate to severe periodontitis patients. Further studies utilizing larger sample sizes and longer follow-up periods are recommended for supporting the finding of this study.

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