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Comparative evaluation of ozonized water versus normal saline irrigation with mechanical debridement in chronic periodontitis patients – A clinical study

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ABSTRACT

Background: Ozone therapy is a rather novel approach for managing many diseases, encompassing oral disorders such as tooth caries and periodontal disease. It possesses the ability to disrupt microbial cell walls, resulting in rapid cell lysis. Moreover, characteristics of ozone such as anti-inflammation, stimulation and modulation of immunity contribute to mitigating the host response aspect of periodontal disease.
Objective: To assess the adjunctive advantages of irrigation of ozonized water in conjunction with mechanical debridement for the treatment of periodontitis.
Methodology: 50 patients with chronic periodontitis and pocket probing depth ≥4 have been divided into 2 groups i.e. Test (n = 25) and control groups (n = 25). While the control group was administered normal saline irrigation and mechanical debridement, the test group was given ozonized water irrigation. Evaluations were conducted at baseline and four weeks following treatment to determine the plaque score, bleeding score, pocket probing depth, and clinical attachment level.
Results: Clinical measures such as clinical attachment level, pocket probing depth, bleeding index, and plaque index, significantly improved with adjunctive ozone water irrigation. The frequency of sites with bleeding on probing that had a pocket depth ≥4 mm was significantly reduced as a result of ozone water irrigation.
Conclusion: Ozone irrigation alongside nonsurgical periodontal therapy offers adjunctive benefits by reducing clinical parameters.

1. Introduction

Periodontitis is a chronic immuno-inflammatory disease having a multifactorial origin, primarily caused by an accumulation of pathogenic bacteria in the form of dental plaque or biofilm which gradually destroys the supporting structures surrounding the tooth such as periodontal ligaments and alveolar bone.[1] In the subgingival environment, the pathogens create communities (biofilms) adhered to the root surface, typically exhibiting resistance to both chemical antibacterial treatments and the oral cavity's natural antibacterial defences. Mechanical root debridement stands as the foundation of cause-related periodontal therapy, targeting the removal of subgingival biofilm and calculus. This traditional approach, known as scaling and root planing (SRP), is regarded as the gold standard for periodontal therapy for most patients with chronic periodontitis.[2,3] Still, this method does not fully eradicate pockets and periodontopathic bacteria, especially in areas such as furcations, root concavities, interproximal spaces, deep developmental grooves, and areas that are difficult to reach with periodontal instruments such as deep, tortuous pockets.[4] For improving treatment outcomes, adjunctive therapies such as local and systemic antibiotic administrations, host modulating agents, etc. are extensively researched.[5] Subgingival environments are mostly predominated by anaerobic microbes that cause further disease progression.[6] These environments can be altered by delivering oxygen-free radicles, hyperbaric oxygen, hydrogen peroxide etc.

Ozone is a recent treatment strategy that acts as a powerful antioxidant with antimicrobial activity through the release of reactive oxygen species in the subgingival environment when administered locally.[7,8]

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Ozone disrupts microbial cell membranes leading to their rapid inactivation. Ozone hinders and disturbs the cell's enzymatic regulatory system during this process by targeting glycoproteins, glycolipids, and other amino acids.[9,10] Ozone's immunostimulatory, immunomodulatory, and anti-inflammatory properties target the host response component of periodontal disease.[11]

The literature concerning ozone therapy in dentistry highlights its significance in terms of various properties, including its therapeutic effects as a modulator of biological reactions, through the distribution of oxygen, growth factor stimulation and cytokine production, and oxidative stress reduction.[12] The findings from de Ramon et al. validate ozone's efficacy in reducing bleeding from the gingiva, demonstrating a reduction of more than 50 % in key periodontal pathogenic bacteria: Porphyromonas gingivalis, Bacteroides forsythus, and Aggregatibacter actinomycetencomitans. Moreover, there was a notable decrease in IL-1β and TNF-α levels.[7,13]

Nevertheless, ozone irrigation's therapeutic efficacy when used in conjunction with scaling and root debridement remains a topic of controversy. The results of a recent systematic review and meta-analysis suggest that ozone irrigation as an adjuvant improves clinical parameters, however, the difference was not statistically significant.[14] Therefore, to further clarify its effectiveness, well-controlled randomized clinical trials are still needed. The current study aims to assess the effects of ozonized water irrigation as an adjuvant to scaling and root planing in the management of periodontitis. Even though scaling and root planing have been well established as a gold standard for periodontitis patients, the value of adjunctive modalities that have minimal or no adverse effects on application to patients needs to be evaluated. Ozone having its inherent properties as antibacterial and anti-inflammatory seems to be a sound strategy to be incorporated in conventional therapy to increase its effectiveness in treating periodontitis patients.

2. Methods

The current study was a parallel-group randomized controlled clinical trial that was patient-blinded and carried out in the Department of Periodontology from March 2024 to October 2024 with institutional ethics committee approval [Ref no. 219/03/23]. The study is registered under the clinical trial registry of India [CTRI/2024/11/076547]. The study followed the guidelines outlined in the 1975 Helsinki Declaration, which has been revised in 2013. The study also followed the consolidated standards of reporting trials (CONSORT) guidelines. Statistical formula $n = 2\sigma^2 (Z\alpha + Z\beta)^2/\delta$ was used to calculate sample size for this study. As from the pilot study, Zα was kept as 1.96, Z β as 1.28, probing pocket depth (PPD) reduction of 1 mm between groups (δ) and standard deviation (σ) of 1. The calculated minimum sample size in each group was 21. Twenty-five subjects were assigned to each group to make up for study dropouts.

2.1. Inclusion criteria

The study comprised fifty systemically healthy patients between the ages of 25 and 60 who had stage III periodontitis, and PPD $\geq$4 mm with at least one site/quadrant having bleeding on probing (BOP).

2.2. Exclusion criteria

Participants were excluded if they had undergone periodontal therapy in the preceding six months, had systemic diseases that preclude periodontal surgery, history of taking any antibiotics and/or anti-inflammatory medications within the previous three months, were pregnant or nursing, were receiving orthodontic treatment, or used tobacco or tobacco products in any form.

2.3. Procedure

All participants were granted informed consent following a comprehensive description of the study's nature and the potential advantages of their involvement. The UNC-15 probe (Hu Friedy, IL, USA) was used to provide a thorough periodontal examination that included the full mouth bleeding score (FMBS), the plaque score (FMPS), and an assessment of the periodontal condition including PPD and clinical attachment loss/level (CAL) examined on six sites per tooth. Full mouth subgingival and supragingival scaling for every selected patient is done by a trained clinician. If required, local anaesthesia was used during the procedure. Approximately 2 h were spent on each patient, and the treatment was finished within 24 h.

The block randomization method was used to randomly assign the chosen subjects to the test group and control group (1:1 ratio). The participants selected a sequentially numbered opaque, sealed envelope that was prepared by the statistician with the code for the treatment procedure they were to receive. Following a random allocation, the participants in this trial were assigned to one of two treatment groups. In addition to scaling and root debridement, ozonized water irrigation was administered to the test group. Normal saline solution (0.9 %w/v) was given to the control group instead of ozonized water irrigation. Clinical parameters were evaluated by an examiner who was not present during the procedure. The examiner was trained for precision and reproducibility of the clinical parameters and indices.

Ozonized water was created by employing an ozone generator (Ganga Basil O3 Sagar) and promptly utilized for irrigation. The concentration of ozone water was about 2 parts per million (ppm). Using a 10-mL syringe with a blunt-tipped 22-gauge needle, full-mouth subgingival irrigation with ozonized water was performed, at a rate of 2 mL for each tooth over 1 min. Using high-power vacuum suction and cotton rolls, excess ozonated water was eliminated. The control group received irrigation with normal saline employing a similar procedure. Additionally, each patient received reinforcement on proper oral hygiene. Four weeks later, a re-evaluation was carried out. The study design in the form of a Consort flow chart is given in Fig. 1.

2.4. Statistical analysis

Per-subject analysis was done on the data. Each patient's mean and standard deviations for each parameter were calculated, and any differences between and within groups were examined. Primary outcome variables were clinical attachment level (CAL) and pocket probing depth (PPD). While relationships between categorical variables were assessed using the chi-square test, quantitative parameters between groups were evaluated using the independent *t*-test. Before and after the intervention, intragroup comparisons of the parameters were conducted using the paired *t*-test. The significance level for the statistical interpretations was set at $p < 0.05$. The analysis was conducted using the statistical software program SPSS, version 20.

3. Results

Out of 62 patients who were screened for the study, 50 patients were selected after matching inclusion and exclusion criteria and they were randomly allocated into test and control groups having 25 subjects in each group. Both groups are age and gender-matched with no adverse effects reported during the study period. Table 1 represents the baseline clinical data and demographics of the two therapy groups. At the baseline, no statistically significant difference was found between the test and control groups in terms of PPD and CAL ($p > 0.05$). Fig. 2 represents the clinical photographs of the comparison between both groups.

Table 2 and Fig. 3 illustrate changes in the clinical parameters. From baseline to re-evaluation at four weeks, all clinical measures significantly decreased in both the test and control groups. The percentage of

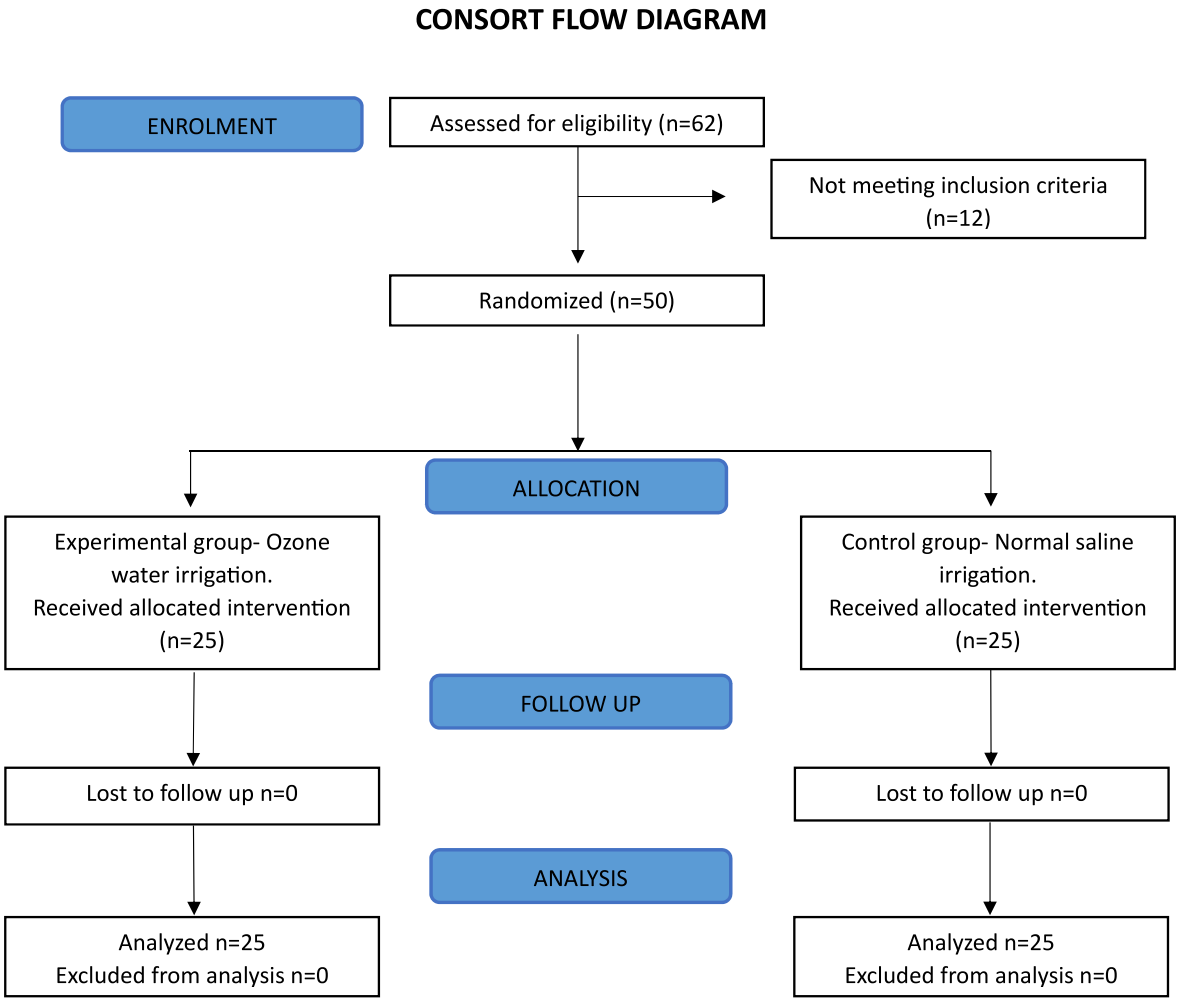

Fig. 1. CONSORT flow diagram.

Table 1
Demographic and clinical characteristics of participants at baseline.

	TEST	CONTROL	p-value
	MEAN ± SD	MEAN ± SD	
Age (years)	41.56 ± 11.17	39.68 ± 10.17	0.53#
Gender, n (%)			
Male	7(28 %)	9(36 %)	0.54#
Female	18(72 %)	16(64 %)	
PPD	5.16 ± 0.31	5.03 ± 0.25	0.11#
CAL(mm)	5.89 ± 0.38	5.8 ± 0.33	0.37#
FMPS%	83.83 ± 7.04	78.08 ± 6.48	<0.05*
FMBS%	83.69 ± 5.22	74.7 ± 4.43	<0.001*

*Statistically significant, #statistically not significant, PPD probing pocket depth, CAL clinical attachment loss, FMPS full mouth plaque score, FMBS full mouth bleeding score, SD-Standard Deviation.

bleeding scores in both the groups exhibited significant differences but the mean difference was found to be more in ozone groups compared to the saline group (27.84 versus 17.05). While comparing intergroup plaque scores, the test group exhibited more mean reduction compared to the control group (27.93 versus 17.89) even though both groups exhibited statistically significant results ($p < 0.001$). Statistically significant differences were evident in the reduction of pockets from baseline to 4 weeks in ozone and saline groups i.e. 1.06 mm and 0.59 mm respectively. The ozone group exhibited more CAL gain compared to saline groups numerically (0.93 mm in the ozone group versus 0.37 mm in the saline group) but from baseline to 4 weeks both groups exhibited statistically significant values ($p < 0.001$).

4. Discussion

Periodontitis is an immunoinflammatory disease caused by the dysbiosis of polymicrobial communities and destructive and dysregulated host response which leads to the destruction of the attachment apparatus surrounding the teeth. The persistence of inflammation and the anaerobic conditions within pockets promote the proliferation of pathogens, potentially leading to uncontrolled inflammation and tissue degradation.[15] The mechanical debridement is considered a gold standard for periodontal treatment but has limitations that require adjunctive therapies for further prevention of disease. Ozone as a powerful antioxidant can disrupt bacterial cell walls and efficiently act as an adjunct to mechanical debridement.[3,13]

The results of this randomized controlled clinical trial show that incorporating irrigation with either normal saline or ozonized water to mechanical debridement significantly reduced clinical parameters. The ozone group experienced a much greater decrease in all parameters than the control group. The results of this study are in harmony with those studies done by Ambili et al.,[16] Dengizek et al.,[17] and Hayakumo et al.[18] A systematic review conducted by German et al. has proven ozone has many biological properties and may be an effective treatment.[7] Nevertheless, contradictory data have also been released, which may be related to variations in ozone concentration, duration, frequency, application method, comparator agent selection, and/or limited sample sizes.[19–21]

Fig. 2. a-application of ozone in the test group, b-application of saline in the control group, c-Pocket depth after 4 weeks in the test group, d-pocket depth after 4 weeks in the control group.

Table 2
A significant reduction in clinical parameters has been observed on re-evaluation after 4 weeks.

Intra-group comparison of clinical parameters								
		Stage	Mean	SD	N	Mean difference	Paired t	P
FMPS (%)	Test	Baseline	83.83	7.04	25	27.93	17.29	<0.001*
		After 4 weeks	55.9	4.24	25			
	Control	Baseline	78.08	6.48	25	17.89	12.28	<0.001*
		After 4 weeks	60.19	3.97	25			
FMBS(%)	Test	Baseline	83.69	5.22	25	27.84	16.79	<0.001*
		After 4 weeks	55.85	5.97	25			
	Control	Baseline	74.7	4.43	25	17.05	13.58	<0.001*
		After 4 weeks	57.65	4.02	25			
PPD (mm)	Test	Baseline	5.16	0.31	25	1.06	15.7	<0.001*
		After 4 weeks	4.1	0.43	25			
	Control	Baseline	5.03	0.25	25	0.59	15.5	<0.001*
		After 4 weeks	4.44	0.23	25			
CAL (mm)	Test	Baseline	5.89	0.38	25	0.93	16.66	<0.001*
		After 4 weeks	4.96	0.48	25			
	Control	Baseline	5.8	0.33	25	0.37	20.17	<0.001*
		After 4 weeks	5.43	0.34	25			

The present study has conducted full-mouth periodontal evaluation in contrast with the studies showing disparities wherein periodontal evaluation is done only in selected and localized sites. According to a recent systematic review and meta-analysis, ozone therapy has no additional benefits over scaling and root planning (SRP) in terms of microbiological or clinical aspects.[14]

This study has demonstrated a significant reduction in plaque and bleeding which could be attributed to the antiinflammatory, antihypoxic and antiplaque action of ozone.[11] Major periodontal pathogen inactivation was noted in the in vitro and in vivo investigations that were reviewed. The study by De Ramon et al. demonstrates that ozone effectively reduces gingival bleeding by reducing the prevalence of important periodontal pathogens like Porphyromonas gingivalis, Bacteroides forsythus, and Aggregatibacter actinomycetencomitans by more than 50 %.[13] Significant improvement in plaque score has been found in the ozone group in comparison with the normal saline group. According to Huth et al., this might be because ozonated water has a stronger antibacterial effect.[22] Similar results have been observed in the study conducted by Ramzy et al.[23] Nagayoshi et al. discovered that ozonated water exhibited potent bactericidal effects within a biofilm and also hindered plaque accumulation in vitro.[24]

However, Kshitish & Laxman found that ozone was highly effective in eradicating gram-negative bacteria, lowering periodontal infections, and minimizing bleeding on probing.[25] Ozone decreased the plaque index by more than 25 % in the de Ramon et al. study.[13] Conversely, in a different investigation by Kshitish & Laxman, the results indicated a 12 % reduction in dental plaque.[25]

The reduction of the bleeding score was higher in the ozone group in comparison with the normal saline group. Gingival tissue irrigation induces changes in plaque composition, leading to decreased inflammation and healthier gingival tissue, aligning with the observations made by Kshitish and Laxman.[25] Additionally, Ramzy et al.[23] reported significant enhancements in probing pocket depth (PPD), gingival score, plaque score, and also a reduction in bacterial count following ozone therapy. Additionally, Huth et al.[22] discovered that ozone in aqueous form reduced the activity of NF-kB in teeth with periodontitis, indicating

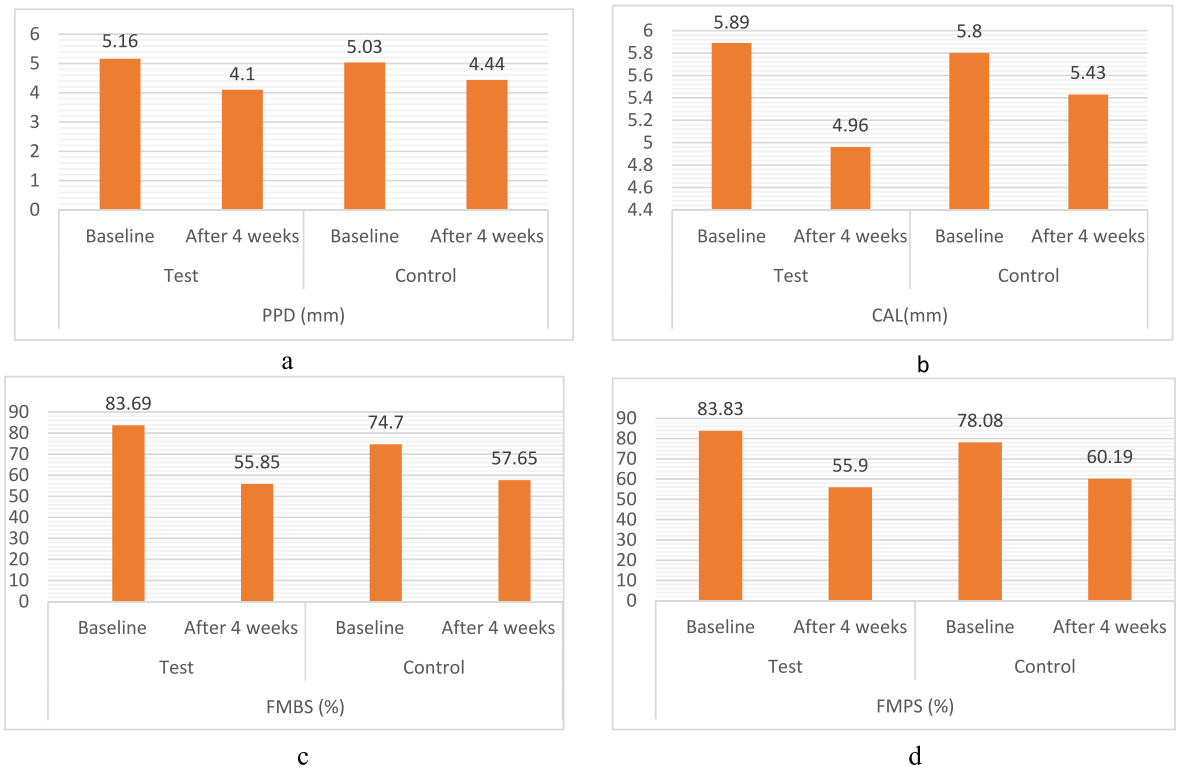

Fig. 3. a-Changes in PPD (mm), b-CAL (mm), c-FMBS (%), d-FMPS (%).

possible anti-inflammatory benefits.

Substantial clinical attachment gain might suggest superior tissue regeneration when employing ozone treatment in contrast to saline. However, Al Habashneh et al. didn't found any notable distinction amongst the experimental and control groups regarding attachment gain. They noted a significant baseline discrepancy in clinical attachment level (CAL) between the two groups, potentially contributing to this conflicting outcome.

Many of recent researchers have evaluated the use of ozone water irrigation with other comparative agents. In the study done by Habashneh et al.[19] they compared ozonated water versus distilled water and no significant results were found in the clinical parameters. Kaur et al.[20] and also Kshitish and Laxman[25] in their study compared ozone water with 0.2 % chlorhexidine gluconate and concluded that ozonated water has better results when compared to chlorhexidine irrigation. A study done by Dodwad et al.[21] also reported that the percentile reduction of spirochetes using ozonated water was comparable to 0.2 % chlorhexidine and 10 % povidone iodine. To check the practicability of ozone as a substitute for commonly used irrigants such as normal saline precluded this study from using a negative control i.e. distilled water.

In both groups, the percentage of sites with a probing pocket depth (PPD) of ≥4 mm and having bleeding on probing (BOP) was measured at baseline and follow-up. The results showed a notable decrease in diseased sites with ozone irrigation in contrast to normal saline. This suggests that irrigation with ozone water could potentially aid in decreasing the proportion of sites necessitating additional periodontal treatment. Huth et al. observed that irrigation with ozone, compared to chlorhexidine, demonstrated superior antimicrobial effectiveness against Porphyromonas gingivalis, Parvimonas micra, and Tannerella forsythia.[20,22]

Ozone therapy's anti-inflammatory effects and modification of the subgingival environment may be more effective in reducing periodontal pockets. The effectiveness of ozone in reducing the deeper pockets needs further evaluation. In the present study, the mean pocket probing depth was in the range of 5–6 mm; deeper pockets >7 mm can harbor residual subgingival calculus and microbial flora even after scaling and root planing.[4] Due to its unstable nature and lack of substantivity, ozone limits its use in deeper pockets in which an access flap surgery will be more effective. However, due to the lack of microbiological and biochemical evaluation, and the limitation of sample size in the subgroup analysis, further studies to overcome these limitations are needed for validation of this observation.

This study has been designed for the evaluation of the efficacy of a novel subgingival irrigation agent, i.e., ozone as an adjunct to mechanical debridement in chronic periodontitis patients. Utilizing a randomized, patient-blinded design and a standardized protocol for mechanical debridement and subgingival irrigation for all study participants, this study minimized potential bias and provided a reliable comparison between the study groups. By evaluating the clinical parameters, the data contributed to the growing evidence of ozone's antimicrobial and anti-inflammatory properties that have a potential for clinical application, ensuring safety and efficacy.

5. Conclusion

Mechanical debridement is considered as the gold standard of periodontal therapy but due to its limitations in inaccessible areas, newer adjunctive therapeutic options have been proposed. Ozone is a novel adjunctive therapeutic modality to mechanical debridement. Due to its anti-inflammatory, antioxidant, antimicrobial and antiplaque action, it is highly recommended to use both professional as well as in home treatment option for periodontal diseases. Future well controlled clinical trials with better methodologies to efficiently deliver ozone has to be conducted for better understanding of the efficacy of ozone as an adjunctive therapy.

Patient's/Guardian's consent

All participants provided written informed consent before enrolment, agreeing to participate in the study and allowing data usage for

publication.

Ethical clearance

Ethics approval has been obtained from the Institutional ethical committee -EC/NEW/INST/2021/1896 with the Ref no. 219/03/23 dated February 22, 2024.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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