

# Ozone Enhanced Scaling as an Adjunct in the Treatment of Chronic Periodontitis: A Randomised Controlled Trial

TANYA SAHAI<sup>1</sup>, SHIVANI SHARMA<sup>2</sup>, KUMAR SAURAV SINGH<sup>3</sup>, KRITI AGARWAL<sup>4</sup>, ATUL PARASHAR<sup>5</sup>, KAVITA SINGH<sup>6</sup>

## ABSTRACT

**Introduction:** Chronic Periodontitis (CP) is an inflammatory biofilm-induced disease that damages the periodontal ligament and alveolar bone, leading to tooth loss. Ozonated water exhibits antimicrobial effects against periodontal microorganisms, like *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Ozone molecules may easily enter cells and lead to membrane lysis by release of nascent oxygen molecule. The present study was undertaken to evaluate the long-term biochemical effect of the ozone water on inflammatory cytokines.

**Aim:** To determine the clinical and biochemical effectiveness of ozonated water irrigation as an adjunct to Scaling and Root Planing (SRP) in patients with CP.

**Materials and Methods:** The present parallel-group randomised controlled trial was conducted in the Department of Periodontology, Santosh Dental College, Ghaziabad, Uttar Pradesh, India, between October 2022 and November 2024. Among 100 patients screened, a total of 72 participants with CP, aged 30-60 years were randomly allocated into two groups: Group A received SRP alone, and Group B received SRP with subgingival irrigation using ozonated water. Clinical parameters i.e., Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) were recorded at

baseline, one month, and three months, while Interleukin-1 $\beta$  (IL-1 $\beta$ ) was evaluated at baseline and three months. Wilcoxon signed-ranked and student's independent t-test were used for data analysis using Statistical Package for Social Sciences (SPSS) version 26.0, with a significance threshold of  $p \leq 0.05$ .

**Results:** The study population had a mean age of  $44.3 \pm 7.2$  years. The test group showed greater PPD reduction ( $2.14 \pm 0.49$  mm vs  $2.08 \pm 0.16$  mm) and greater CAL gain ( $3.080 \pm 0.47$  versus  $1.63 \pm 0.25$ ) in control group ( $p < 0.05$ ) three months postoperatively. At three months, the difference in mean value of PPD and CAL of control group and test group was found to be statistically significant ( $p = 0.008^*$  and  $p = 0.001^{**}$ , respectively). IL-1 $\beta$  levels reduced significantly in the test group by  $2.16 \pm 1.39$  and the control group by  $1.26 \pm 1.02$  at three months from baseline ( $p < 0.05$ ). At three months, the difference in mean value of salivary IL-1 $\beta$  level in control group and test group was statistically significant ( $p = 0.03^*$ ). Both the groups showed significant reduction in PI and GI three months postoperatively ( $p < 0.05$ ).

**Conclusion:** Subgingival irrigation with ozonated water as an adjunct to SRP provides superior improvements in clinical and biochemical parameters compared to SRP alone in the management of patients with CP.

**Keywords:** Aqueous ozone, Clinical attachment level, Inflammatory markers, Interleukin-1 $\beta$ , Ozone therapy

## INTRODUCTION

Inflammation that is host-mediated and microbiologically linked to periodontitis causes the loss of periodontal attachment. Using a standardised periodontal probe to circumferentially check the secondary dentition with respect to the Cementoenamel Junction (CEJ), helps in the identification of Clinical Attachment Loss (CAL) [1]. The intricate interplay between periodontopathogenic bacteria and the immune inflammatory retaliation is frequently linked to CP [2]. Alveolar bone loss, CAL, gingival inflammation, and periodontal pockets are clinical characteristics of CP that can lead to tooth loss [3]. Two crucial cytokines that initiate and sustain the systemic inflammation are Tumour Necrosis Factor- $\alpha$  (TNF $\alpha$ ) and IL-6 [4].

The major goal of periodontal therapy is to remove biofilm, stop the disease's progression, and lower inflammation. Reducing aetiologic factors below the threshold level that might cause breakdown is the goal of periodontal therapy so as to enable the periodontal regeneration. By using non-surgical periodontal therapy or phase I in conjunction with a chemotherapeutic medication, some techniques might enhance the new attachment of damaged periodontium [5]. Nonetheless, in certain cases, the intricate structure of the root and the lesion's shape may make therapy difficult and prevent the bacterial load from being sufficiently reduced to render the tooth surface physiologically acceptable [6]. This may be related to the bacteria's generation of particular virulence factors that compromise the host's

defenses. Hence, chemotherapeutic agents are highly desirable and might be useful supplements to mechanical treatment [7].

These adjuvant treatments are categorised according to whether they are administered systemically (low concentration at the periodontal pocket) or locally (high concentration at the periodontal pocket) [8]. When compared to systemic treatment, Local Drug Delivery (LDD) for periodontitis reduces the systemic impact by directly delivering an antibacterial agent into subgingival locations. Among the methods are mouth rinses, gels, chips, ointments, and pocket irrigation. Antimicrobials are most often used via subgingival irrigation. Numerous antimicrobial medications, including tetracycline, hydrogen peroxide, boric acid, stannous fluoride, and chlorhexidine, have been utilised for this [9].

Theoretically, therapists utilise Chlorhexidine Gluconate (CHX), a cationic bis-biguamide salt with a wide antibacterial range, it is being regarded as the gold standard, as supra and sub-gingival irrigant to inhibit bacterial etiologic agents. However, it has been dissuaded as a long-term therapy option due to some of its adverse effects.

Schonbein C made the discovery of ozone in 1840, and it has since been employed extensively. It's a strong oxidant, very poisonous, and corrosive agent [10]. Ozone molecules may easily enter cells when the membrane is broken because it becomes more permeable [11-13]. The antibacterial effect is produced by direct interactions of molecular ozone and additional mechanisms

that are mediated by free radicals [14]. Studies have demonstrated the antibacterial capabilities of ozonised water against periodontal infections caused by *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* [15-17]. In a previous systematic review, varying findings were revealed on the impact of ozone on periodontal tissue when used in conjunction with SRP based on clinical and radiographic parameters [18]. Several authors have undertaken studies [19-21] to evaluate the biochemical effect of the ozone water which had smaller follow-ups and hence this study was undertaken to assess the long term effect of ozone water on inflammatory cytokines.

The present study aimed to evaluate the adjunctive use of ozonated water with SRP in patients with CP, combining clinical parameters and biochemical markers for comprehensive assessment. By exploring a biocompatible alternative to conventional antimicrobials, it aimed to establish ozonated water as a LDD agent.

## MATERIALS AND METHODS

The present randomised controlled trial was conducted in the Department of Periodontics and Oral Implantology at Santosh Dental College, Ghaziabad, Uttar Pradesh, India. The investigation was conducted from October 2022 to November 2024, with follow-up assessments scheduled at one and three months. The study protocol was approved by the Institution's Ethical Committee (SU/2023/2480). The present study was in accordance with the Declaration of Helsinki guidelines (2013). Each patient was informed about the limitations and benefits of treatment, and written consent was obtained before the procedure.

**Sample size calculation:** The sample size was calculated using the n Master 2.0 software, based on the formula for comparison of two independent means using the study by Habashneh AL et al., as reference [19]. Using the sample-size formula for comparing two means and assuming a standard deviation of 0.9 and an expected difference of 0.5 units in clinical periodontal outcomes, with 95% confidence and 80% power, the required sample size was 36 participants per group, a total of 72 subjects.

**Inclusion criteria:** Systemically healthy patients in the age group of 30-60 years with stage II grade B periodontitis [1] participated in the study. Out of the total individuals screened, patients with a PPD of 4-6 mm and those who did not receive any surgical or non-surgical periodontal therapy in the past six months were included.

**Exclusion criteria:** Patients who were on antimicrobial medications or antibiotic mouth rinses taken in the past six months prior to the study. Those on medications that would induce gingival enlargement or immunosuppressants (like tacrolimus, cyclosporin). Pregnant women and lactating mothers and those who were smokers and tobacco chewers were excluded in this study.

Patients were randomly divided into two groups using the coin toss method: Group A and Group B.

Group A (control group)- SRP alone

Group B (test group)- Ozone water irrigation with SRP

## Study Procedure

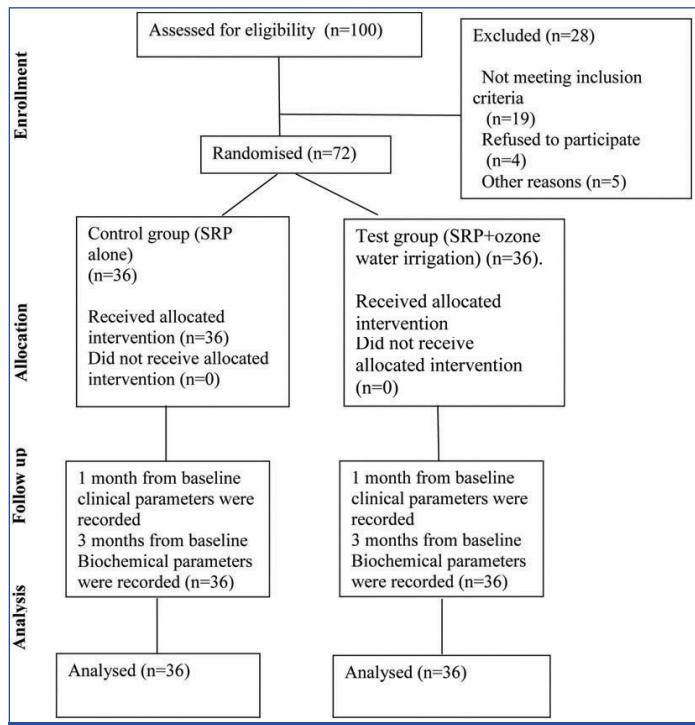
**Clinical parameters:** Following clinical parameters were recorded using UNC-15 (Hu-Friedy, USA) periodontal probe: 1 Periodontal Probing Depth (PPD); 2 Clinical attachment level (CAL); 3 Plaque index (PI); and 4 Gingival index (GI). All measurements were recorded at baseline, and subsequently at one and three-month intervals [Table/Fig-1,2]. Standards of Reporting Trials (CONSORT) guidelines has been presented in [Table/Fig-3]. GI and PI were assessed using standard criteria published by Löe H and Silness J (1963) and Silness J and Löe H (1964), respectively [14,15].

In the present randomised controlled trial, a total of 72 patients were selected based on the inclusion and exclusion criteria. On the day of treatment, a detailed case history was recorded and a complete



[Table/Fig-1]: Test group measurements at baseline: Probing Pocket Depth (PPD).

[Table/Fig-2]: Control group measurements at baseline: Probing Pocket Depth (PPD). (Images from left to right)



[Table/Fig-3]: CONSORT flow diagram.

patient examination was done. SRP was performed for all patients [Table/Fig-4], irrespective of the group, using an ultrasonic scaler (Woodpecker, Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, Guangxi, China). In the test group, SRP was followed by subgingival irrigation with 2 mL of ozonated water using a 3 mL syringe [Table/Fig-5].



[Table/Fig-4]: Scaling and Root Planing (SRP).

[Table/Fig-5]: Subgingival irrigation with freshly prepared ozonated water (test Group). (Images from left to right)

Fresh ozonated water was generated for every patient on the day of treatment using an ambient air-based ozone generator (ADC Inc. Dentozoneindia) in the department, using cold distilled water that was active for 20 minutes. For generating 50 mL of ozone water, the time of preparation was five minutes [Table/Fig-6].

Saliva samples were collected twice (at baseline and after three month). Saliva sample collection was performed prior to SRP in Eppendorf tubes and were placed in ice box and transferred to lab at baseline. Level of IL-1 $\beta$  were assessed at baseline and at three months because it is an acute phase marker of periodontal

inflammation and usually after periodontal therapy its level reduces after 3-4 weeks and only minimal changes are observed after one month, using Enzyme Linked Immunosorbent Assay (ELISA {Elabscience}) [Table/Fig-7]. For this, saliva samples were collected using the Eppendorf tubes. Saliva samples were then placed in an ice box with a temperature ranging between 4-6°C and transported to the laboratory for assessment.



[Table/Fig-6]: Ozonolysis of cold distilled water in ambient based ozone generator (Dentozoneindia).

[Table/Fig-7]: ELISA Kit (Elabscience™). (Images from left to right)

**Postoperative period:** Patients were recalled after one and three months for assessment of clinical parameters i.e PPD, CAL, PI and GI and salivary level of IL-1 $\beta$  were assessed at three months from baseline [Table/Fig 8,9].



[Table/Fig-8]: Probing Pocket Depth (PPD) at 3 months (test group).

[Table/Fig-9]: Probing Pocket Depth (PPD) at 3 months (control group). (Images from left to right)

## STATISTICAL ANALYSIS

Descriptive statistics were performed by calculating the mean and standard deviation for the continuous variables. Categorical variables are presented as absolute numbers and percentage. The data was entered into Microsoft Excel and analysed using the SPSS 26.0 for relevant statistical comparisons. Results were presented in the form of tables and graphs. Wilcoxon signed-rank test was used to check whether the continuous variables were following normal distribution or not. Student independent sample t-test was used for the comparison of values between two groups while intragroup comparison was done using paired t-test between 2-time intervals. Level of statistical significance was set at p-value less than or equal to 0.05.

## RESULTS

The present study included a total of 72 patients with a mean age of 30-45 years with a mean age of 44.3 $\pm$ 7.2 years.

**Intergroup comparison:** The differences in mean value of PPD of control group (4.20 $\pm$ 0.401) and test group (4.19 $\pm$ 0.624) at baseline was found to be statistically insignificant (p>0.05). Post one month, the difference in mean PPD of control (2.14 $\pm$ 0.351) and test group (2.11 $\pm$ 0.319) was found to be statistically insignificant (p>0.05). Furthermore, at three months, the difference in mean value of PPD of control group (2.12 $\pm$ 0.447) and test group (2.05 $\pm$ 0.609) was found to be significant statistically (p=0.008\*). Difference in mean of CAL in control group (4.19 $\pm$ 0.632) and test group (4.44 $\pm$ 0.689) at baseline was found to be statistically non-significant (p>0.05). At one month, the difference in mean value of clinical attachment level of control group (3.99 $\pm$ 0.451) and test group (3.69 $\pm$ 0.657)

was statistically insignificant (p>0.05). Post three months, mean of CAL in control group (2.56 $\pm$ 0.348) and test group (1.36 $\pm$ 0.289) was found to be significant statistically (p<0.001\*\*). The differences in mean value of salivary IL-1 $\beta$  level in control group (13.76 $\pm$ 1.65) and test group (14.05 $\pm$ 1.13) at baseline was insignificant statistically (p>0.05). At three months, the difference in mean value of salivary IL-1 $\beta$  level in control group (12.50 $\pm$ 1.28) and test group (11.89 $\pm$ 1.18) was statistically significant (p=0.03\*).

The differences in mean value of PI of control and test group at baseline was statistically insignificant (p>0.05). However, the three months difference in PI (mean) of control group (1.70 $\pm$ 0.591) and test group (1.08 $\pm$ 0.280) was significant statistically (p=0.002\*). Differences in mean GI of control group and test group at baseline was statistically insignificant (p>0.05). Furthermore the three months difference in mean GI in control group (1.64 $\pm$ 0.683) and test group (1.53 $\pm$ 0.654) was statistically insignificant (p>0.05) [Table/Fig-10].

Parameters	Intergroup Comparison					
	Baseline	p-value	1 month	p-value	3 months	p-value
PPD-control (mm)	4.20 $\pm$ 0.401	1.000	2.14 $\pm$ 0.351	0.726	2.12 $\pm$ 0.447	0.008*
PPD-test (mm)	4.19 $\pm$ 0.624		2.11 $\pm$ 0.319		2.05 $\pm$ 0.609	
CAL-control (mm)	4.19 $\pm$ 0.632	0.443	3.99 $\pm$ 0.451	0.696	2.56 $\pm$ 0.348	0.001**
CAL-test (mm)	4.44 $\pm$ 0.689		3.69 $\pm$ 0.657		1.36 $\pm$ 0.289	
IL-1 $\beta$ -control (ng/L)	13.76 $\pm$ 1.65	0.398	-	-	12.50 $\pm$ 1.28	0.03*
IL-1 BETA-test (ng/L)	14.05 $\pm$ 1.13		-		11.89 $\pm$ 1.18	
PI-control (mm)	2.83 $\pm$ 0.507	.802	1.72 $\pm$ 0.566	0.076	1.70 $\pm$ 0.591	0.002*
PI-test (mm)	2.86 $\pm$ 0.424		1.47 $\pm$ 0.609		1.08 $\pm$ 0.280	
GI-control (mm)	2.99 $\pm$ 0.399	1.000	1.81 $\pm$ 0.467	0.147	1.64 $\pm$ 0.683	0.483
GI-test (mm)	2.89 $\pm$ 0.398		1.61 $\pm$ 0.645		1.53 $\pm$ 0.654	

[Table/Fig-10]: Intergroup comparison of clinical and biochemical parameters at various study intervals using student independent sample t-test

**Intragroup comparison:** For the control group, the 4.20 $\pm$ 0.401 at baseline showed significant reduction of 2.06 $\pm$ 0.249 at one month (p<0.05), which further decreased by 2.08 $\pm$ 0.169 at three months, (p<0.05). For the test group, mean PPD of 4.19 $\pm$ 0.624 at baseline showed significant reduction of 2.00 $\pm$ 0.539 at one month (p<0.05), which further decreased by 2.14 $\pm$ 0.497 at three months (p<0.05), with a significant difference from baseline. For the control group, the mean CAL at baseline of 4.19 $\pm$ 0.632 decreased by 0.2 $\pm$ 0.602 at one month (p>0.05) and by 0.63 $\pm$ 0.253 at three months (p<0.05), being statistically significant from baseline. Similarly, the baseline CAL of 4.44 $\pm$ 0.689 decreased by 0.75 $\pm$ 0.572 at one month (p=0.05) and by 3.08 $\pm$ 0.478 (p<0.05) at three months, for the test group.

For the control group and test group, the baseline IL-1 $\beta$  decreased by 1.26 $\pm$ 1.02 & 2.16 $\pm$ 1.39 at three months (p=0.02\* and 0.001\*\*), respectively, demonstrating a significant decrease in inflammatory cytokine level following treatment. Mean PI of the control group decreased by 1.11 $\pm$ 0.471 at one month (p<0.05), which further decreased by 1.13 $\pm$ 0.381 at three months (p<0.05) with a significant difference from baseline. Similarly for the test group, the baseline mean PI score significantly decreased by 1.39 $\pm$ 0.389 at one month (p<0.05), further decreasing by 1.78 $\pm$ 0.284 at three months (p<0.05) from baseline. The mean GI score of 2.99 $\pm$ 0.399 & 2.89 $\pm$ 0.398 at baseline significantly reduced by 1.18 $\pm$ 0.117 and 1.28 $\pm$ 0.285 at one month (p<0.05), further decreasing statistically by 1.35 $\pm$ 0.049 and 1.36 $\pm$ 0.169 at three months, from baseline (p<0.05) for the control and test group, respectively [Table/Fig-11].

Parameter	Baseline vs 1 Month	p-value	Baseline vs 3 Months	p-value
PPD-control	2.06±0.249	0.03*	2.08±0.169	0.002**
PPD-test	2.00±0.539	0.02*	2.14±0.497	0.001**
CAL-control	0.2±0.602	0.06	1.63±0.253	0.001**
CAL-test	0.75±0.572	0.05	3.08±0.478	0.002*
IL1 BETA-control	-	-	1.26±1.02	0.02*
IL1 BETA-test	-	-	2.16±1.39	0.001**
PI-control	1.11±0.471	0.04*	1.13±0.381	0.02*
PI-test	1.39±0.389	0.03*	1.78±0.284	0.01*
GI-control	1.18±0.117	0.03*	1.35±0.049	0.01*
GI-test	1.28±0.285	0.02*	1.36±0.169	0.004*

**[Table/FIG-11]:** Intragroup comparison of clinical and biochemical parameters at various study intervals using paired t-test.

## DISCUSSION

The present study showed the effects of subgingival irrigation using ozone water irrigation at one-month and three-month post-scaling. PPD, a key clinical metric for evaluating periodontal disease and the efficacy of treatment at baseline and at one month did not differ significantly between groups, according to intergroup comparisons, indicating that both groups had similar baseline circumstances and early healing responses. Post 3-month observation period, a statistically significant difference ( $p<0.05$ ) had been seen between the test and the control group. Oxidative activity makes ozone capable of breaking down bacterial biofilms, which may be responsible for the test group's further improvement in PPD. It also increases tissue response and stabilise pocket reduction by its stimulatory effects on local circulation and oxygenation. These findings are in line with those of Musalaiah S et al., Ramzy MI et al., and Nagayoshi M et al., which quoted, employing ozone water as a supplement to oral prophylaxis in the treatment of CP significantly decreases PPD both within and across groups [12,16,22]. These results are in contrast with the results of Habashneh AL et al., who showed a non-significant difference in PPD between control and test group as according to them the use of distilled water in control group could have attributed to the non-significant results between the two groups [19]. Intragroup comparison revealed progressive improvement in PPD within both the groups as shown is a study by Kshitish D and Laxman VK [23].

CAL as an index of disease severity and treatment effectiveness is an important measure for assessing the efficacy of periodontal therapy. The intergroup differences were not statistically significant ( $p>0.05$ ) at baseline, and one month after treatment initiation, indicating the comparable initial situation and early therapeutic effect of both groups. However, the CAL at three months for test group and control group was statistically detectable, indicating that using ozonated water as an adjuvant had a higher long-term advantage in clinical attachment gain. Ozone has improved antibacterial, local oxygenation, and healing-promoting qualities which may be responsible for the test group's greater CAL improvement. In a study to determine the clinical effectiveness of ozone water, Nagayoshi M et al., and Huth KC et al., also found similar findings, reporting a substantial decrease in clinical attachment level both within and across groups [22,24]. Contrary results have been shown by Habashneh AL et al., which demonstrated a non-significant reduction in CAL between the groups [19]. The above observations are also in accordance with prospective split-mouth research of Musalaiah S et al., and Alsakr A et al., who evaluated the effectiveness of ozone therapy in treating stage II and stage III periodontitis and they found that all clinical variables significantly decreased both within and between groups from baseline to six weeks [12,25].

Saliva with elevated IL-1 $\beta$  levels is a useful biomarker for assessing the host's reaction to periodontal treatment since it indicates active inflammation and tissue degradation. This conclusion is further supported by the intergroup comparison. At three months,

a significant difference appeared favouring the test group, even though the groups' baseline IL-1 $\beta$  levels were statistically identical. This implies that, in comparison to SRP alone, supplementary ozonated water treatment may provide improved control of the inflammatory response. The above findings are in accordance with those of Katti SS and Chava VK who found that, as compared to oral prophylaxis alone (control group), there was a substantial intergroup and intragroup decrease in the IL-1 $\beta$  level with ozone water (test group) [26]. Intragroup comparison revealed a progressive improvement in IL-1 $\beta$  level within both the groups under the study. These, observations are similar with those of Uraz A et al., who demonstrated a significant decrease in IL-1 $\beta$  both within and between groups following the additional use of ozone water [27].

For PI, the difference in mean PI scores between groups was not statistically significant at baseline and at one month. By three months, however, the test group's PI score was statistically lower as compared to the control group. This implies that when combined with traditional SRP, the supplementary use of ozonated water irrigation produced a better plaque-reducing impact over time. The above results were in accordance with Aggarwal P and Ranjith A et al., who conducted a clinic-microbiological study and showed a significant reduction both within and across groups [28,29]. However, the results of our study are in contrast with the study conducted by Habashneh AL et al., which showed a non-significant reduction in PI on intergroup comparison [19]. Intragroup comparison of both Group A and Group B showed significantly relevant decline in PI from baseline to one month. These findings signify SRP's ability in reduction of plaque and enhancement of oral hygiene over time when used with ozone water. These results are in accordance with the results of Musalaiah S et al., who compared the effectiveness of ozonated water with NSPT alone in treating CP [12].

For GI, intergroup results were insignificant at one and after three months. The short follow-up period, sample size restrictions, or the ozone dosage and administration mechanism may be responsible for the lack of significant intergroup differences. Thus, SRP by itself considerably lowers gingival inflammation but in this study ozone water irrigation as an adjuvant did not produce statistically better results. Similar observations are demonstrated by Habashneh AL et al., who found that using ozone water as a supplement to NSPT in CP resulted in non-significant intergroup difference [19]. Both control and test group showed significant decline in GI from baseline to one month in accordance with the study of Nagayoshi M et al., [22]. These results support SRP's well-established use as a primary periodontal treatment by showing how well it reduces gingival inflammation.

## Limitation(s)

Limitations of the study include small number of participants, short duration follow-up. Hence, studies with larger sample size and long follow up are necessary to authenticate these findings and investigate underlying processes along with advantages of ozone therapy in managing periodontal conditions. A key limitation of the present study is that it was a single centre study, which may have restricted the findings involving a broader population.

## CONCLUSION(S)

Within the limitations of this randomised controlled trial, it can be concluded that ozone water irrigation used as an adjunct to SRP provides significant additional clinical benefits in reducing PPD and improving clinical attachment level in patients with CP compared to SRP alone. Furthermore, adjunctive ozone therapy significantly reduces salivary levels of the pro-inflammatory biomarker IL-1 $\beta$ .

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#### PARTICULARS OF CONTRIBUTORS:

- Postgraduate Student, Department of Periodontics, Santosh Dental College, Ghaziabad, Uttar Pradesh, India.
- Professor and Head, Department of Periodontics, Santosh Dental College, Ghaziabad, Uttar Pradesh, India.
- Professor, Department of Periodontics, Santosh Dental College, Ghaziabad, Uttar Pradesh, India.
- Reader, Department of Periodontics, Santosh Dental College, Ghaziabad, Uttar Pradesh, India.
- Senior Lecturer, Department of Periodontics, Santosh Dental College, Ghaziabad, Uttar Pradesh, India.
- Postgraduate Student, Department of Periodontics, Santosh Dental College, Ghaziabad, Uttar Pradesh, India.

#### NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Shivani Sharma,  
Professor and Head, Department of Periodontics, Santosh Dental College,  
Ghaziabad, Uttar Pradesh, India.  
E-mail: docshivaniperio@gmail.com

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