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Comparison of ozone therapy and antimicrobial photodynamic therapy in periodontitis: A randomized clinical trial

Shilpi Mangal [a], Shalini Kaushal [a,*], Nand Lal [a], Anjani Kumar Pathak [a], Bhaskar Agarwal [b], Suruchi Shukla [c]

[a] Department of Periodontology, Faculty of Dental Sciences, King George's Medical University, U.P., Lucknow, India
[b] Department of Prosthodontics and Crown & Bridge, Faculty of Dental Sciences, King George's Medical University, U.P., Lucknow, India
[c] Department of Microbiology, King George's Medical University, U.P., Lucknow, India

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ABSTRACT

Background: This randomized clinical trial evaluated the comparative efficacy of ozone therapy and ICG-mediated antimicrobial photodynamic therapy as adjunctive to scaling and root planing in the management of periodontitis, by assessing clinical outcomes, inflammatory biomarkers, specifically interleukin-1β (IL-1β) and Pentraxin-3 (PTX-3), and microbial load via quantitative PCR.

Methods: This is a parallel-arm, single-blinded, randomized controlled trial that included 36 patients with Stage III Grade B periodontitis, allocated randomly to three groups. Group 1 {Scaling and Root planing (SRP)}, Group 2 {SRP + Ozone Therapy (OT})}, and Group 3 {SRP + Antimicrobial Photodynamic Therapy (aPDT)}. Clinical parameters, biochemical markers, and microbial count were recorded at baseline, 1 month, and 3 months.

Result: The change in Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) from baseline to 1 month and from baseline to 3 months was significantly higher in the SRP + OT group ($p < 0.001$). Reduction in the microbial count of *Porphyromonas gingivalis* (Keystone pathogen) (Mean difference = −0.70, $p < 0.001$) and *Tannerella forsythia* (−0.51, $p < 0.001$), whereas **SRP + aPDT provided superior suppression** for *Fusobacterium nucleatum* (−0.76, $p < 0.001$) and *Prevotella intermedia* (0.40, $p < 0.001$). No meaningful difference was observed between the two adjuncts for *Treponema denticola* ($p = 0.994$).

Conclusion: All treatment modalities resulted in significant improvements in all the parameters over time. However, SRP + Ozone Therapy consistently achieved the improvement across all parameters, when compared to SRP alone or SRP with aPDT.

Clinical Relevance

Background.

This randomized clinical trial evaluated the efficacy of two adjunctive therapies, ozone therapy (OT) and antimicrobial photodynamic therapy (aPDT), in enhancing the outcomes of scaling and root planing (SRP) in patients with periodontitis. Thirty-six individuals were randomly assigned to three groups: SRP alone (control), SRP + OT, and SRP + aPDT. Clinical periodontal parameters, inflammatory biomarkers (IL-1β, PTX-3), and microbial load of key periodontopathogens were assessed at baseline, 1 month, and 3 months.

Added Value of the Study.

The results demonstrated that the SRP + OT group exhibited significantly greater improvements in plaque index, gingival index, probing pocket depth, and clinical attachment levels compared to the other groups. IL-1β levels showed the most

* Corresponding author.
E-mail addresses: shilpimngl6@gmail.com (S. Mangal), shalinikaushal@kgmcindia.edu (S. Kaushal), nandlalkgmc@yahoo.in (N. Lal), anjanipathak@kgmcindia.edu (A.K. Pathak), bhaskaragarwal@kgmcindia.edu (B. Agarwal), shuklasuruchi@yahoo.com (S. Shukla).

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pronounced reduction in the ozone group. Microbial analysis revealed superior suppression of *Porphyromonas gingivalis* and *Tannerella forsythia* with SRP + OT, while SRP + aPDT showed more selective effects against *Prevotella intermedia* and *Fusobacterium nucleatum*.

Clinical Implications.

Both adjunctive therapies provided measurable benefits beyond SRP alone, with OT showing more sustained and comprehensive effects. These findings suggest that ozone therapy may serve as an effective non-surgical adjunct to SRP in the management of periodontitis, offering enhanced periodontal health outcomes compared to SRP alone or SRP combined with aPDT.

1. Introduction

Periodontal disease is characterized by inflammation that impacts the soft and hard tissues supporting the teeth, being the primary cause of tooth loss[1] and is the sixth most common condition globally.[2] In most cases, chronic forms of periodontitis are brought on by a prolonged polymicrobial challenge. This challenge then proceeds to elicit a host immunological response, which in turn promotes a persistent inflammatory state.[3] The clinical manifestation results from the loss of periodontal attachment, culminating in subsequent tooth exfoliation. The pathogenesis of periodontitis is largely driven by bacterial biofilm and the host's inflammatory response, particularly through the action of pro-inflammatory cytokines.[4,5]

The intention in any periodontal therapy is to halt the progression of inflammation-related disease process, impede or stop disease development, and facilitate periodontal regeneration.[6] This will lead to enhanced periodontal solace and functionality. Scaling and root planing (SRP) is the gold standard non-surgical periodontal therapy, but alone cannot fully eliminate pathogens in deep or anatomically complex sites like furcations and root concavities.[7–11] Therefore, adjuvant therapies have been effectively studied at multiple levels to enhance the outcomes of SRP in the treatment of periodontal conditions.[12,13]

Among the numerous non-invasive therapies for improving plaque control, ozonated water therapy (OT) and antimicrobial photodynamic therapy (aPDT) are well recognised as two effective methods.

2. Ozone therapy

Ozone therapy is an excellent treatment for periodontitis due to its unique features, non-invasive nature, and versatility. Ozone (O_3), commonly referred to as triatomic oxygen or trioxygen, is the third most potent oxidising agent globally[14] and is biocompatible with oral epithelial cells, periodontal cells, and gingival fibroblasts.[15] Ozone (O_3) is acknowledged as one of the most effective bactericidal, antiviral, and antifungal agents. The antimicrobial impact of ozone arises from its action on cells, which involves disrupting the cytoplasmic membrane by ozonolysis.[16] Ozonated water also exhibits mild haemostatic properties by promoting platelet aggregation, enhancing local vasoconstriction, and accelerating fibrin clot formation. These effects are attributed to ozone's oxidative stimulation of platelet-derived mediators such as TGF-β and PDGF, which contribute to improved control of bleeding and faster healing in inflamed periodontal tissues. Moreover, ozone enhances the synthesis of various free radical scavenger enzymes, including glutathione peroxidase, superoxide dismutase, and catalase, all of which are essential in mitigating oxidative stress.[17]

3. Antimicrobial photodynamic therapy

The antibacterial efficacy of lasers can be further augmented by incorporating a photosensitizing dye, a technique also called photodynamic therapy (PDT). Photodynamic therapy relies on three key components that interact specifically within diseased tissues to produce therapeutic effects:

1) A photosensitizer is a molecule that captures light energy to initiate chemical reactions within specific tissues.
2) Light at the correct wavelength activates the photosensitizer.
3) Oxygen present in the cells interacts with it to produce reactive oxygen species that trigger therapeutic effects.[18]

The working principle involves the photosensitizer absorbing light energy and transitioning from its ground state (stable, low energy) to a triplet state (excited, higher energy). This transition triggers the creation of highly reactive molecules known as reactive oxygen species that cause significant cellular damage. These interactions occur via two distinctive ways, designated as Type I and Type II. Photocytotoxic reactions emerge exclusively within diseased tissues, in regions where the photosensitizer is distributed, facilitating selective or targeted destruction. Indocyanine green (ICG) dye constitutes a tri-carboxylated compound from the cyanine family, exhibiting peak absorption at wavelengths around 805–810 nm. ICG possesses amphiphilic characteristics. The ICG differs from other available photosensitizers; it demonstrates 20 % photodynamic impact, with its primary action occurring through photothermal therapy (PTT), triggering cellular destruction by elevating intracellular temperature.[18]

Interleukin 1β (IL-1β) serves as a crucial pro-inflammatory cytokine. It is one of the major mediators for periodontal tissue degradation. It directly influences the course of periodontitis by enhancing the expression of collagenolytic enzymes and matrix metalloproteinases (MMPs), upregulating RANKL, reducing osteoprotegerin synthesis, and inducing osteoclastogenesis.[19] Pentraxin-3 (PTX-3) appears to be both sensitive and specific in diagnosing and possibly predicting the prognosis of ongoing diseases.[20] PTX-3, typically present at low levels under normal circumstances, rises swiftly and significantly during states of inflammation or infection, correlating with the severity and progression of the disease.

Periodontopathogenic microorganisms are crucial in the initiation and development of a disease, rendering microbiological analysis essential for prognosis. Quantitative PCR (qPCR) is an advanced variant of conventional PCR that facilitates the precise quantification of specific DNA targets. This method employs distinct chemical approaches, including SYBR Green dye and the TaqMan assay. SYBR Green is highly specific and enables the detection of product accumulation in real-time during the amplification process.[21] By enabling precise quantification of bacterial DNA, qPCR with SYBR Green aids in understanding the microbial dynamics in periodontal conditions, advancing diagnostic and therapeutic approaches in dental medicine.

Few studies have evaluated the efficacy of these two therapies in conjunction with a standard plaque-control strategy. By exploring the effectiveness of OT and aPDT, this study seeks to provide insight into their potential role in enhancing the overall treatment of chronic periodontitis, offering a more comprehensive approach to periodontal care without the need for surgical intervention.

Hence, this study intends to evaluate the clinical parameters, biochemical markers, i.e., IL-1β and PTX-3, and reduction in microbial load in periodontitis patients following scaling and root planing, ozone therapy, and antimicrobial photodynamic therapy.

4. Materials and Methods

This research has been done in the Department of Periodontology, King George's Medical University (F.O.D.S), in collaboration with the Department of Microbiology at KGMU, U.P., Lucknow. Before the initiation of the investigation, ethical approval was secured from the Institutional Ethics Committee (Reference code: XIX-PGTSC IIA/P4). This clinical trial is filed at ClinicalTrials.gov under the code CTRI/2024/08/072049, and the reference number is REF/2024/04/082112.

Informed written consent was acquired from each participant after they were thoroughly briefed and comprehended the objectives and specifics of the study. De-identified data and analysis codes will be shared upon reasonable request, subject to institutional and ethical compliance.

STUDY DESIGN: This single-blind, parallel-arm RCT was conducted over a follow-up duration of three months. The CONSORT principles were adhered to; Fig. 1 illustrates the CONSORT flowchart. A total of 36 individuals were enlisted from the Periodontology OPD,12 participants in each group, with 4 females and 8 males in Group 1 (SRP), 6 females and 6 males in Group 2 (SRP + OT), and 7 females and 5 males in Group 3 (SRP + aPDT). Participants were recruited for the study based on the established criteria of inclusion and exclusion.

5. Inclusion criteria

1. Adults between 18 and 75 years of age.
2. Based on the criteria of the ***2017 Classification of Periodontal and Peri-Implant Diseases and Conditions,***[22] a Stage III Grade B Periodontitis patient.
3. Generalized involvement (≥30 % of sites affected)
4. Probing pocket depth (PPD) ≥5 mm.
5. Clinical attachment level (CAL) of 4 mm or more should be present in at least 10 % of the sites.
6. Radiographic bone loss (RBL) extending to the middle third of the root or beyond.
7. Tooth loss limited to four teeth or fewer due to periodontitis.
8. Along with complexity features such as probing depths ≥6 mm or vertical defects.
9. Participants of all genders were eligible.

6. Exclusion criteria

1. Current smokers or previously used tobacco products.
2. Any systemic disorders that may influence the progression and risk of the periodontal disease.
3. Any periodontal therapy in the last 6 months.
4. Intake of antibiotics or antioxidant therapy within the past 3 months.
5. Pregnant women or women in the lactation period.
6. Patients who were allergic to the photosensitizer dye.
7. Patients with lower Glucose-6-Phosphate Dehydrogenase (G6PD) levels.

7. Sample size estimation

Sample size was calculated based on variation in PPD level before and after the intervention of ozone therapy **(Ambill Ranjith *et al.*,2022).**[47]

The sample size calculation is as follows:

$$n = k\frac{(z_\alpha + z_\beta)^2(\sigma_1^2 + \sigma_2^2)}{d^2}$$

Where $\sigma_1 = 0.18$, the SD of the pre-intervention PPD level,$\sigma_2 = 0.16$, the

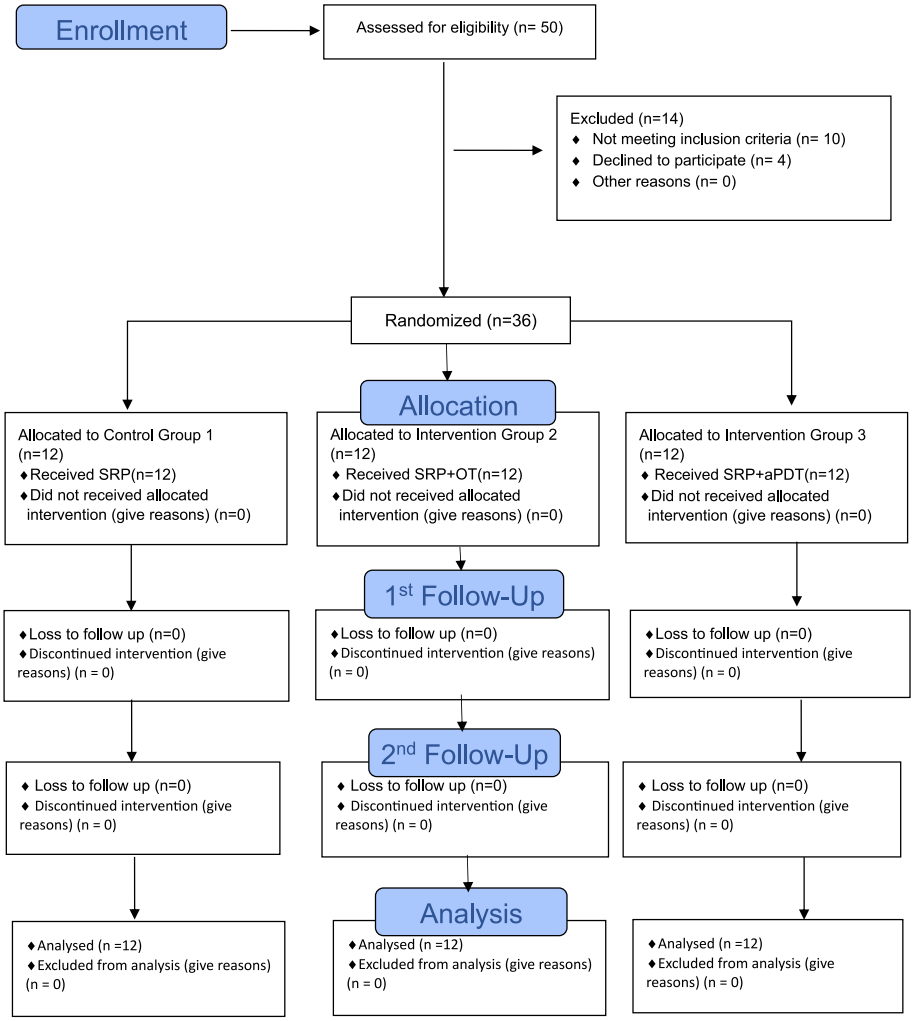

Fig. 1. CONSORT 2025 flow diagram.

SD of post-intervention PPD level, $d = 30$ % of the mean difference in PPD level, considered to be clinically significant, $k = 1.0$, the effect size, Type I error α with 5 %, (95 % confidence level), Type II error β with 10 % (90 % power of the study). So, the sample size comes out to be 12 for each group.

8. Randomization and allocation concealment

The randomization sequence was generated using a computerized method (block randomization of 4 block size) via the online tool www.sealedenvelope.com. The sequence was prepared by a postgraduate student not involved in participant recruitment, intervention delivery, or outcome assessment, thereby ensuring allocation concealment. The random allocation list was concealed in opaque sealed envelopes to prevent selection bias.

9. Study groups

Group 1: Control group: Patients underwent SRP.
Group 2: Ozone group (Test Group I): Patients received ozonated water application after SRP.
Group 3: Antimicrobial Photodynamic therapy (Test Group II): Patients received aPDT after SRP.

10. Blinding

A single-blind design was employed in this study. Participants were blinded to their group allocation to minimize performance bias. Due to the nature of the intervention, which included AMD Picasso Laser, blinding of the principal investigator was not feasible.

11. Intervention protocol

SRP was performed using an **ultrasonic scaler unit (Acteon Satelec Suprasson P5 Booster, France),** followed by **manual root planing with Universal Gracey curettes 2R/2L and 4R/4L (Hu-Friedy, Chicago, IL, USA).** To ensure consistency, all treatments were performed by a single calibrated periodontist. Calibration training was conducted over several consecutive days using 10 volunteers. Repeated assessments were performed until the examiner achieved the predefined threshold for measurement consistency, defined by an intra-class correlation coefficient (ICC) of 0.85.

Armamentarium for Ozone Therapy: Oxygen cylinder, ozone generator, ozone destructor, and diffuser, 22-gauge needle, 10 ml syringes.

Ozone water preparation:

Ozonated water was prepared 30 min before the start of the procedure. A glass bottle was kept overnight in the refrigerator at 5–7 °C. The ozone inlet was pushed through the bottle. A second outlet opening was made in the same glass bottle to which the ozone destructor was attached. Oxygen flow rate was kept at 0.5 L/ml; thereafter, the ozone generator was switched on and set to attain an ozone concentration of 60 μg/ml. After 30 min of ozone bubbling in the water, the ozonated water was ready to use.

Procedure: After scaling and root planing, 2 ml of ozonized water was applied to each tooth for 5–10 min using a 22-gauge blunt tip needle connected to a syringe (10 ml). Group 2 patients were then recalled after 1 month, and again therapy was repeated similarly.

Armamentarium for aPDT: Indocyanine green dye (Aurogreen®, Aurolabs, Madurai, India), Diode laser (AMD Picasso) with 400-μm fiber-optic tip, 22-gauge needle, 10 ml syringes.

Procedure: The indocyanine green dye was administered at a concentration of 0.05 mg/ml via a 22-gauge needle, through continuous horizontal movements to ensure thorough flushing of the pocket for 30 s at each site. After that, irradiation was performed inside the pockets with an AMD Picasso diode laser with an 810 nm wavelength and 0.5W power for 1 min in noncontact continuous wave mode via fiber-optic application using a tip of 10 mm with a default angle and a fiber core diameter of 60° and 400 μm, respectively. After that, sterile water was used to completely remove any remaining dye. Group 3 patients were then recalled after 1 month, and again therapy was repeated similarly.

12. Outcome variables and follow-up timepoints

The primary outcomes were plaque index (PI), gingival index (GI), PPD, and CAL, using the UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA). All clinical measurements (PI, GI, PPD, CAL) were performed by a calibrated, independent examiner blinded to the group allocation.

The secondary outcomes were IL-1β and PTX-3 levels using the commercially available *Fine test*™ ELISA kits and a reduction in the microbial count through qPCR.

The clinical parameters of each participant were recorded at baseline using a graduated periodontal probe (UNC-15, Hu-Friedy, Chicago, IL, USA), and customized stents with vertical grooves were prepared for each participant to standardize the location and angulation of the periodontal probes and to provide a reproducible insertion axis at all subsequent appointments. Number of sites assessed per tooth and per patient (six sites per tooth: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) at different follow-up visits.

The patients were asked to report at 1 and 3 months. Clinical, biochemical parameters, and microbiological assessment were done at baseline, 1 month, and 3 months.

12.1. Gingival crevicular fluid (GCF) sampling

To avoid saliva and/or plaque contamination, isolate the targeted locations with cotton rolls, and effectively remove any supragingival biofilm with a curette. GCF was collected for 30 s using Meta absorbent paper points, size #30 with a 4 % taper, and gently inserted in the deepest pocket until minimal resistance was felt.[23] Samples were consolidated into Eppendorf tubes, which contained 500 μl of phosphate buffer solution (PBS), and kept in the laboratory freezer at −80 °C until processed, to minimize the loss of biomarker reactivity. On the trial day, Eppendorf tubes with the sample solution were taken from −80 °C, paper points were discarded, and centrifuged at 1000×*g* for 15 min at 2–8 °C.

13. Microbiological monitoring by qPCR

13.1. Methodology

The GCF sample collected was immediately delivered to the Department of Microbiology for qPCR. Genomic DNA from bacteria was extracted from 200 μl of normal saline after processing for further laboratory investigations employing a Qiagen DNA extraction kit. Initial real-time PCR was carried out for the human housekeeping gene to verify the extraction process and quality of DNA. Furthermore, Real-time PCR was conducted using universal primers, followed by the detection of *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia*, and *Fusobacterium nucleatum* with SYBR Green real-time PCR.[24]

The SYBR Green primers were designed by Integrated DNA Technology, USA. The amplifications were carried out using Real Time thermal cycler system using SYBR™ Green Universal Master Mix (Thermo Fisher Scientific, USA) with the conditions: 95 °C for 10 min, followed by 40–45 cycles at 95 °C for 15 s, annealing at 52–64 °C for 30 s, and elongation at 72°C for 1 min, followed by a melting stage. The fluorescence was detected in real time during PCR thermal cycling.

13.2. Quantitative PCR for bacterial load calculation

The **target PCR product** was used to prepare **quantitative standards,** with copy number expressed as **copies/ml**. A **10-fold serial dilution** was performed, ranging from **10^7 to 10^3 copies/ml,** to calibrate a standard curve and determine the copy number of the target sample, which allowed for precise quantification.

14. Data and statistical analysis

Data analysis was generated with SPSS version 25 software. Two-way repeated-measure ANOVA was employed to compare PI, GI, PPD, CAL, IL-1β, PTX-3 levels, and microbial counts of pathogens across three groups, followed by Tukey's Post Hoc test for multiple comparisons. A significance threshold of $p < 0.05$ was used. All analyses followed the Intention-to-Treat (ITT) approach.

15. Result

15.1. Participant characteristics

A total of 36 participants were enrolled after meeting eligibility criteria and providing informed consent. Recruitment occurred in Aug 2024, with follow-up assessments continuing through December 2024. Baseline demographic analysis confirmed that the three study groups were comparable and free from selection bias. Group 1 exhibited a mean age of 43.0 ± 8.62 years with 33.3 % female participants, Group 2 had a mean age of 43.75 ± 8.80 years with 50 % females, and Group 3 demonstrated a mean age of 43.5 ± 10.40 years with 58.3 % females. Male participants were 66.7 %, 50 % and 41.7 %, respectively. Statistical analysis revealed no significant differences in either age ($p = 0.980$) or gender distribution ($p = 0.458$) among the groups, indicating that the study population was well balanced and comparable at baseline.

15.2. Primary outcome

15.2.1. Clinical parameters

At 1 month, PI decreased to 0.93 ± 0.37 in the SRP + OT group, with the most notable reduction at 3 months (0.20 ± 0.14). Repeated measure ANOVA showed significant effects of time ($F = 22.1$, $p < 0.001$), group ($F = 293.0$, $p < 0.001$), and interaction ($F = 15.7$, $p < 0.001$) (Table 1).

Significant reductions in GI were observed at 1 month and 3 months across all groups, with the SRP + OT group showing the maximum improvement, reaching 0.13 ± 0.08 at 3 months. Repeated measure ANOVA revealed significant time ($F = 11.18$, $p < 0.001$, $\eta^2 = 0.253$), group ($F = 279.5$, $p < 0.001$, $\eta^2 = 0.944$), and group × time interaction effects ($F = 23.6$, $p < 0.001$, $\eta^2 = 0.588$) (Table 1). The post hoc intergroup comparisons revealed significant differences between the groups (SRP + OT vs SRP + aPDT, mean difference-0.43, $p < 0.001$), indicating both progressive improvement over time and superior performance of the SRP + OT group (mean difference-1.31, $p < 0.001$) (Table 1).

PPD and CAL also improved significantly across all groups. At 3 months, reductions in PPD were greatest in the SRP + OT group (2.93 ± 0.18), followed by SRP (4.04 ± 0.33) and SRP + aPDT (4.22 ± 0.42). Time ($F = 46.9$, $p < 0.001$), group ($F = 44.2$, $p < 0.001$), and interaction effects ($F = 13.1$, $p < 0.001$) were significant. For **CAL**, the greatest gain was at 3 months in the SRP + OT group (3.22 ± 0.43), SRP and SRP + aPDT showed modest changes (4.18 ± 0.43 and 4.28 ± 0.41). Time ($F = 10.2$, $p = 0.003$), group ($F = 29.81$, $p < 0.001$), and interaction ($F = 9.89$, $p < 0.001$) effects were significant (Table 1).

Overall, all groups demonstrated significant clinical improvement over time, with **SRP + OT consistently achieving the greatest reductions in PI, GI, and PPD, and the highest CAL gain**, supported by significant group and interaction effects and intergroup post hoc analysis, showing its enhanced therapeutic efficacy (SRP vs SRP + OT, $p < 0.001$) (Table 1).

Table 1
Changes in Clinical Parameters and Intergroup Comparisons among different groups at different time intervals.

Clinical Parameters	Time Point/Duration	Group 1 (SRP)		Group 2 (SRP + OT)		Group 3 (SRP + aPDT)		Intergroup Differences (Tukey's Post Hoc)	
		Mean	SD	Mean	SD	Mean	SD	Mean Difference	*p*- value
Plaque Index (PI)	Baseline	2.25	0.34	2.45	0.33	2.42	0.2	1.21	Group 1 vs Group 2*: $p < 0.001$
	1 month	1.42	0.38	0.93	0.37	1.14	0.28	1.76	Group 1 vs Group 3*: $p < 0.001$
	3 months	1.23	0.35	0.2	0.14	0.41	0.17	0.55	Group 2 vs Group 3*: $p < 0.001$
	time effect	$F = 22.1$, $p < 0.001$, $\eta2 = 0.401$							
	group effect	$F = 293.0$, $p < 0.001$, $\eta2 = 0.947$							
	group*time	$F = 15.7$, $p < 0.001$, $\eta2 = 0.489$							
Gingival Index (GI)	Baseline	1.94	0.45	2.47	0.18	2.32	0.32	1.31	Group 1 vs Group 2*: $p < 0.001$
	1 month	1.17	0.39	0.63	0.27	1	0.3	1.74	Group 1 vs Group 3*: $p < 0.001$
	3 months	1.06	0.36	0.13	0.08	0.31	0.13	0.43	Group 2 vs Group 3*: $p < 0.001$
	time effect	$F = 11.18$, $p < 0.001$, $\eta2 = 0.253$							
	group effect	$F = 279.5$, $p < 0.001$, $\eta2 = 0.944$							
	group*time	$F = 23.6$, $p < 0.001$, $\eta2 = 0.588$							
Probing Pocket Depth (PPD)	Baseline	4.35	0.24	4.57	0.33	4.73	0.47	0.55	Group 1 vs Group 2*: $p < 0.001$
	1 month	4.11	0.33	3.49	0.35	4.39	0.46	0.82	Group 1 vs Group 3*: $p < 0.001$
	3 months	4.04	0.33	2.93	0.18	4.22	0.42	0.27	Group 2 vs Group 3*: $p = 0.014$
	time effect	$F = 46.9$, $p < 0.001$, $\eta2 = 0.587$							
	group effect	$F = 44.2$, $p < 0.001$, $\eta2 = 0.728$							
	group*time	$F = 13.1$, $p < 0.001$, $\eta2 = 0.443$							
Clinical Attachment Level (CAL)	Baseline	4.49	0.32	5.04	0.61	4.79	0.46	0.61	Group 1 vs Group 2*: $p < 0.001$
	1 month	4.25	0.43	3.79	0.64	4.45	0.45	0.88	Group 1 vs Group 3*: $p < 0.001$
	3 months	4.18	0.43	3.22	0.43	4.28	0.41	0.27	Group 2 vs Group 3: $p = 0.073$ (NS)
	time effect	$F = 10.2$, $p = 0.003$, $\eta2 = 0.237$							
	group effect	$F = 29.81$, $p < 0.001$, $\eta2 = 0.644$							
	group*time	$F = 9.89$, $p < 0.001$, $\eta2 = 0.375$							

Legends: Values are expressed as Mean ± Standard Deviation (SD), Repeated Measures ANOVA was used to assess time effect, group effect, and time × group interaction, Tukey's post hoc test was applied for between-group comparisons at each individual time point (baseline, 1 month, and 3 months), *$p < 0.05$ considered statistically significant, η^2 = partial eta squared (effect size), NS = Not significant.
Abbreviations: SRP: Scaling and Root Planing; OT: Ozone Therapy; aPDT: Antimicrobial Photodynamic Therapy.

15.2.2. Intergroup comparison

Post hoc analysis showed that both SRP + OT and SRP + aPDT produced significantly greater improvements than SRP alone across all clinical parameters (PI, GI, PPD, CAL; all $p < 0.001$). SRP + OT showed a modest advantage for PI, GI, and PPD, while CAL gains were comparable between the SRP + OT and SRP + aPDT groups ($p = 0.073$) (Table 1).

15.2.3. Inflammatory biomarkers

IL-1β levels decreased across all groups from baseline to 3 months. SRP, SRP + OT, and SRP + aPDT showed progressive reductions (53.32 → 31.14 pg/ml; 67.98 → 22.34 pg/ml; 52.16 → 22.26 pg/ml). Repeated measure ANOVA showed a significant time effect ($F = 66.3$, $p < 0.001$, $\eta^2 = 0.668$) and a significant group × time interaction ($F = 2.96$, $p = 0.026$), indicating a marked reduction in IL-1β levels over the study period, while the group effect was non-significant ($F = 0.74$, $p = 0.484$, $\eta^2 = 0.043$). The significant group × time interaction indicates that the pace of reduction differed between treatments. Notably, the SRP + OT and SRP + aPDT groups achieved greater reductions by the 3-month follow-up compared with SRP alone, suggesting that adjunctive therapies may enhance or accelerate the resolution of inflammatory burden, even though their cumulative mean values remained comparable across the study period (Table 2).

PTX-3 levels declined progressively across all groups from baseline to 3 months, confirming a significant time effect ($F = 11.3$, $p < 0.001$). Neither the group effect ($F = 1.63$, $p = 0.212$, $\eta^2 = 0.090$) nor the group × time interaction ($F = 0.16$, $p = 0.956$, $\eta^2 = 0.010$) was significant, indicating that the magnitude and pattern of PTX-3 reduction were comparable among SRP, SRP + OT, and SRP + aPDT (Table 2).

Overall, PTX-3 showed a uniform downward trend across interventions, in contrast to IL-1β, which demonstrated differential temporal responses among groups.

15.2.4. Secondary outcome

Reduction in the microbial load of *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Prevotella intermedia*.

All target periodontal pathogens showed **significant longitudinal reductions** in microbial load across the three treatment groups (all time effects $p < 0.001$).

For ***P. gingivalis***, the SRP + OT group demonstrated the greatest early reduction at 1 month (2.78 ± 0.40), with all groups reaching comparably low levels by 3 months. This was supported by significant group differences ($p < 0.001$) and a strong group × time interaction ($p < 0.001$) (Table 3).

T. forsythia followed a similar pattern, with SRP + OT showing more pronounced reductions at 1 month (2.59 ± 0.04), confirmed by significant group ($p < 0.001$) and interaction effects ($p < 0.001$) (Table 3).

For ***T. denticola***, the largest 1-month suppression occurred in the SRP + aPDT group (2.60 ± 0.66), consistent with significant group differences ($p < 0.001$) and a modest interaction effect ($p = 0.031$) (Table 3).

F. nucleatum *and* ***P. intermedia*** exhibited steady, progressive reductions in all groups, with the lowest 3-month values generally observed in the SRP + OT group; both species showed significant group effects ($p < 0.001$) and interaction effects ($p = 0.001$) (Table 3).

Collectively, the data indicate that while SRP alone produced substantial microbial suppression, **adjunctive ozone therapy and aPDT enhanced early bacterial reduction**, with SRP + OT demonstrating the most consistent reductions across pathogens.

15.2.5. Intergroup comparison

Tukey's post hoc comparison of the two adjunctive therapies, SRP + OT vs SRP + aPDT showed that **SRP + OT achieved greater reductions** for ***P. gingivalis*** (mean diff = −0.70, $p < 0.001$) and ***T. forsythia*** (−0.51, $p < 0.001$), whereas **SRP + aPDT provided superior suppression** for ***F. nucleatum*** (−0.76, $p < 0.001$) and ***P. intermedia*** (0.40, $p < 0.001$). No meaningful difference was observed between the two adjuncts for ***T. denticola*** (0.02, $p = 0.994$) (Table 3).

Overall, these findings confirm that **both adjunctive therapies enhance microbial reduction beyond SRP**, with ozone therapy showing broader effects across ***P. gingivalis*** and ***T. forsythia*** and aPDT offering stronger inhibition for selective species such as ***F. nucleatum*** and ***P. intermedia***.

16. Discussion

Dysbiosis of the oral microbiome is closely connected to periodontal inflammation, playing a driving force in the initiation and advancement of periodontal disease.[25] Persistence of inflammation and the anaerobic condition within periodontal pockets creates a favorable environment for pathogenic bacteria, potentially leading to excessive inflammation and tissue damage.[26] Modifying the subgingival environment could be an effective approach to suppress those obligate anaerobic subgingival microorganisms. Oxygen-releasing and redox mediators, such as molecular oxygen and hyperbaric oxygen therapy, aid in accomplishing this process.

The core purpose of the treatment is the elimination of biofilms that harbor pathogenic microorganisms. It is also important to recognize that biofilm-associated dysbiosis is not solely driven by classical pathogens but may also involve commensal or opportunistic species (pathobionts) that acquire virulent characteristics in response to environmental stressors or host-related alterations within the biofilm exposome, thereby contributing to disease onset and progression. Non-surgical mechanical therapy serves as the cornerstone of periodontal

Table 2
Changes in Inflammatory Markers in different groups at different time intervals.

Inflammatory Markers	Time Point/Duration	Group 1 (SRP)		Group 2 (SRP + OT)		Group 3 (SRP + aPDT)	
		Mean	SD	Mean	SD	Mean	SD
IL-1β (pg/ml)	Baseline	53.32	31.25	67.98	13.27	52.16	16.52
	1 month	36.81	19.29	41.39	19.26	34.12	9.32
	3 months	31.14	21.53	22.34	15.51	22.26	9.95
	time effect	$F = 66.3$, $p < 0.001$, $\eta2 = 0.668$					
	group effect	$F = 0.74$, $p = 0.484$, $\eta2 = 0.043$					
	group*time	$F = 2.96$, $p = 0.026$, $\eta2 = 0.152$					
PTX-3 (ng/ml)	Baseline	6.68	4.86	7.31	6.81	9.24	6.73
	1 month	4.68	4.92	3.64	3.85	6.19	4.17
	3 months	2.9	2.88	2.53	2.08	5.25	4.29
	time effect	$F = 11.3$, $p < 0.001$, $\eta2 = 0.255$					
	group effect	$F = 1.63$, $p = 0.212$, $\eta2 = 0.090$					
	group*time	$F = 0.16$, $p = 0.956$, $\eta2 = 0.010$					

Legends: *$p < 0.05$ is statistically significant, Repeated Measure ANOVA applied.
Abbreviations: SRP: Scaling and Root Planing; OT: Ozone Therapy; aPDT: Antimicrobial Photodynamic Therapy.

Table 3
Reduction in Microbial Count and Intergroup Comparisons among different groups at different intervals through qPCR.

Microbial Count {log_{10}(DNA Copies/ml)}	Time Point/Duration	Group 1 (SRP)		Group 2 (SRP + OT)		Group 3 (SRP + aPDT)		Intergroup Differences (Tukey's Post Hoc)	
		Mean	SD	Mean	SD	Mean	SD	Mean Difference	*p*- value
Porphyromonas gingivalis	Baseline	5.13	0.16	5.65	0.96	5.9	1.08	0.40	Group 1 vs Group 2*: $p = 0.015$
	1 month	4.07	0.85	2.78	0.4	4.28	0.23	−0.29	Group 1 vs Group 3: $p = 0.079$ (NS)
	3 months	2.59	0.22	2.17	0.09	2.51	0	−0.70	Group 2 vs Group 3*: $p < 0.001$
	time effect	$F = 251.4$, $p < 0.001$, $\eta2 = 0.884$							
	group effect	$F = 13.70$, $p < 0.001$, $\eta2 = 0.454$							
	group*time	$F = 8.36$, $p < 0.001$, $\eta2 = 0.336$							
Tannerella forsythia	Baseline	4.63	0.12	3.22	0.12	4.71	1.23	1.01	Group 1 vs Group 2*: $p < 0.001$
	1 month	4.02	0.03	2.59	0.04	2.47	0.65	0.50	Group 1 vs Group 3*: $p < 0.001$
	3 months	2.45	0.21	2.25	0.12	2.42	0.42	−0.51	Group 2 vs Group 3*: $p < 0.001$
	time effect	$F = 109.2$, $p < 0.001$, $\eta2 = 0.768$							
	group effect	$F = 51.2$, $p < 0.001$, $\eta2 = 0.756$							
	group*time	$F = 14.9$, $p < 0.001$, $\eta2 = 0.474$							
Treponema denticola	Baseline	6.57	0.58	5.85	0.4	6.08	0.58	0.76	Group 1 vs Group 2*: $p < 0.001$
	1 month	4.01	1.01	3.24	0.09	2.6	0.66	0.78	Group 1 vs Group 3*: $p < 0.001$
	3 months	3.06	0.47	2.26	0.34	2.63	0.73	0.02	Group 2 vs Group 3: $p = 0.994$ (NS)
	time effect	$F = 408.8$, $p < 0.001$, $\eta2 = 0.925$							
	group effect	$F = 16.5$, $p < 0.001$, $\eta2 = 0.500$							
	group*time	$F = 3.9$, $p = 0.031$, $\eta2 = 0.189$							
Fusobacterium nucleatum	Baseline	4.71	0.93	4.19	0.51	5.67	0.42	0.04	Group 1 vs Group 2: $p = 0.966$ (NS)
	1 month	3.22	0.87	3.92	0.03	4.53	0.66	−0.72	Group 1 vs Group 3*: $p < 0.001$
	3 months	2.8	0.74	2.48	0.61	2.68	0.42	−0.76	Group 2 vs Group 3*: $p < 0.001$
	time effect	$F = 136.6$, $p < 0.001$, $\eta2 = 0.805$							
	group effect	$F = 12.1$, $p < 0.001$, $\eta2 = 0.423$							
	group*time	$F = 8.7$, $p = 0.001$, $\eta2 = 0.345$							
Prevotella intermedia	Baseline	5.13	0.03	5.85	0.4	4.78	0	0.10	Group 1 vs Group 2: $p = 0.501$ (NS)
	1 month	4.34	0.43	3.58	0.28	3.08	0.86	0.49	Group 1 vs Group 3*: $p < 0.001$
	3 months	2.6	0.18	2.34	0.16	2.73	0	0.40	Group 2 vs Group 3*: $p < 0.001$
	time effect	$F = 482.2$, $p < 0.001$, $\eta2 = 0.936$							
	group effect	$F = 17.9$, $p < 0.001$, $\eta2 = 0.521$							
	group*time	$F = 23.5$, $p = 0.001$, $\eta2 = 0.588$							

Legends: Values are expressed as Mean ± Standard Deviation (SD), Repeated Measures ANOVA was used to assess time effect, group effect, and time × group interaction, Tukey's post hoc test was applied for between-group comparisons at each individual time point (baseline, 1 month, and 3 months), *$p < 0.05$ considered statistically significant, η^2 = partial eta squared (effect size), NS = Not significant.
Abbreviations: SRP: Scaling and Root Planing; OT: Ozone Therapy; aPDT: Antimicrobial Photodynamic Therapy.

management. However, in certain cases, conventional approaches may not effectively control the progression of periodontal disease, highlighting the need for innovative treatment methods. Ozone therapy, a relatively recent approach, causes instant lysis of the microbial cell wall.[27] Additionally, ozone possesses antibacterial, immunostimulatory, and immunomodulatory properties, which help regulate the host immunoinflammatory response in periodontal disease.[28,29]

Due to demographic changes, dentistry is now encountering a growing number of geriatric patients who experience systemic side effects from antibiotics and complications from drug interactions caused by polypharmacy. Developing innovative and effective therapeutic strategies is essential to tackle the pressing issue of antibiotic resistance directly. One emerging approach being explored as a supplementary treatment for periodontitis management is antimicrobial photodynamic therapy (aPDT).[30]

As probing depths increase in periodontal pockets, the environment becomes more anaerobic. Elemental composition analysis revealed a notable rise in calcium levels in an anaerobic environment. Bivalent cations hinder the methylene blue uptake in microbial cells; however, they facilitate increased ICG absorption.[30] Therefore, this study monitors the impact of ICG dye, a photosensitizer used in aPDT. Additionally, a quantitative analysis of microorganisms was conducted to enhance understanding and demonstrate the efficacy of ICG-mediated aPDT and OT.

IL-1β plays a pivotal part in triggering inflammation and participating in the advancement of periodontal disease. It also promotes the secretion of PTX-3 in neutrophils. IL-1β levels are elevated in individuals with periodontitis compared to those with a healthy periodontium.[31,32] PTX-3 functions as both a diagnostic and prognostic marker for periodontal disease. It serves as a dependable indicator of periodontal tissue damage, with its levels aligning with clinical parameters of periodontal disease/severity. PTX-3 is produced locally in inflamed gingival tissues, influencing its concentration in the GCF, a potentially more accurate reflection of disease severity.[33,34]

The present study findings showed that both OT and ICG mediated-aPDT improve periodontal clinical parameters compared to the baseline. Ozonated water shows a promising outcome in treating mild to moderate periodontal pockets, reducing the IL-1β and PTX-3 levels and promoting resolution of inflammation, thereby reducing the load of the five key Gram-negative anaerobic pathogens (***P.g, T.f, T.d, P.i,*** and ***F.n)*** associated with the progression of periodontitis microbial communities related to the progression of periodontitis.

Since the primary etiology for periodontitis is plaque, and the plaque index assessed the oral hygiene status of the patients, and the successful periodontal treatment depends on good patient compliance, the current study found at baseline, the mean PI scores were 2.25 ± 0.34 in the SRP group, 2.45 ± 0.33 in the SRP + OT group, and 2.42 ± 0.20 in the SRP + aPDT group. By 1 month, all groups showed a reduction in PI, with the SRP + OT group achieving the greatest decrease to 0.93 ± 0.37, followed by SRP + aPDT (1.14 ± 0.28) and SRP alone (1.42 ± 0.38). At 3 months, further improvement was observed, particularly in the SRP + OT group (0.20 ± 0.14), indicating superior plaque control, validating the long-term effectiveness of the ozone water therapy (Table 1). Results of our study were in conjunction with the previous research done by **Kshitish and Laxman (2010),**[35] **and Ramzy *et al.* (2005)**[36] who observed that subgingival irrigation with ozonated water was the most effective in reducing the plaque accumulation. In agreement, we have a study by **Oliveira *et al.* (2009)**[37], where there was a marked reduction in plaque after months of antimicrobial photodynamic therapy.

Statistically significant difference was observed in PI between Group

2 and Group 3 (Mean difference = 0.05, $p < 0.001$) (Table 1). This observation from our study is in contrast with the study by **R. Mehrotra *et al.* (2023),**[38] who found no significant difference ($p > 0.05$).

Mean difference in GI was 1.31 ($p < 0.001$) between SRP and SRP + OT, 1.74 ($p < 0.001$) for SRP and SRP + aPDT, showing significant improvement in gingival inflammation with combination therapies compared to SRP alone (Table 1). This could be attributed to the accelerated healing of the oral mucosa associated with haemostatic action and bactericidal effect of ozonized water. This finding is in conjunction with the study by **Katti and Chava (2013)**[39]. Similarly, **Betsy *et al.* (2014)**[40] also showed a significant reduction in 2 weeks–3 months, and **Bundidpun *et al.* (2018)**[41] observed improvement in the gingival index at 3 and 6 months among the aPDT, followed by thorough SRP. In intergroup comparison, a significant difference in the mean gingival index was observed between Group 2 and Group (mean difference = 0.43, $p < 0.001$) (Table 1). This finding is in conjunction with the study by **Ameyaroy *et al.* (2020)**[42] who found a significant improvement in GI from baseline to 2 and 6 months in the ozone therapy group as well as in the aPDT group, but intercomparison of the gingival index change between the two groups did not reveal much change. This finding is in contrast to the present study findings.

Baseline PPD values were 4.35 ± 0.24, 4.57 ± 0.33, and 4.73 ± 0.47 for the SRP, SRP + OT, and SRP + aPDT groups, respectively. At 3 months, the greatest reduction in pocket depth was observed in the SRP + OT group (2.93 ± 0.18), followed by SRP + aPDT (4.22 ± 0.42) and SRP alone (4.04 ± 0.33) (Table 1). This can be explained by the fact that oral irrigation disrupts and detoxifies subgingival plaque. This finding is in conjunction with the study by **Kaur *et al.* (2019)**[43] who observed that ozonated water showed better reduction in PPD after 1 month (14.5 %) and 3 months (34.5 %) from baseline. Further study by **Pandya *et al.* (2012)**[44] is in accordance with the results of the present study, which evaluated a significant reduction in PPD in the ozonated water therapy group after 30 days. Although the mean PPD reduction observed in the ozone therapy group was approximately 1 mm, this magnitude of improvement is considered clinically meaningful, particularly in sites presenting with moderate baseline pocket depths. Periodontal literature indicates that even a 1 mm reduction is associated with reduced periodontal inflammation, improved access for oral hygiene, and a lower likelihood of disease progression. Therefore, the improvements observed in our study, while modest in absolute numerical terms, reflect a beneficial therapeutic response with potential implications for long-term periodontal stability.

Clinical Attachment Gain is known to be the gold standard for evaluating the success of periodontal therapy. In the present study, at 3 months, the maximum gain in clinical attachment was observed in the SRP + OT group (3.22 ± 0.43), while SRP alone and SRP + aPDT showed modest improvement (4.18 ± 0.43 and 4.28 ± 0.41, respectively) (Table 1). The findings are consistent with the study done by **Hrishi *et al.* (2020),**[45] who observed statistically significant improvement in CAL at 6 weeks from baseline with adjunctive ozone irrigation. Another study by **Habashneh *et al.* (2014)**[46] was also in agreement with our study.

IL-1β and PTX-3 levels decreased across all groups from baseline to 3 months ($p < 0.001$) (Table 2). The result of our findings is in conjunction with the study by **Ranjith *et al.* (2021)**[47] who showed a significant reduction after 4 weeks in IL-1β after ozonated water therapy, pointing to the superior long-term anti-inflammatory effect of the ozone. However, the group effect ($F = 1.63$, $p = 0.212$, $\eta^2 = 0.090$) and the group × time interaction ($F = 0.16$, $p = 0.956$, $\eta^2 = 0.010$) were not statistically significant in PTX-3, suggesting that reductions in PTX-3 were comparable across all treatment groups. This finding is in alignment with the study by **Tasdemir *et al.* (2019)**[48] who found a statistically significant decrease in PTX-3 level in the ozonated water group at baseline to 3 months follow-up. Although the result of our intergroup comparison of IL-1β and PTX-3 levels between the OT group and the aPDT group cannot be directly compared, as no study to the best of our knowledge has been done so far to compare the efficacy of irrigation between OT and ICG-aPDT. This study represented an initial attempt to compare cytokine levels between ozone therapy and ICG-aPDT. Given the lack of prior studies, the findings presented here should be interpreted as an initial step toward understanding, with further research needed to validate these results.

In terms of biological plausibility, both ozone therapy and ICG-mediated photodynamic therapy may influence periodontal healing not only through antimicrobial effects but also via modulation of the host inflammatory response. Ozone has been shown to exert oxidative antimicrobial activity and may contribute to secondary modulation of host inflammatory pathways, including downregulation of mediators such as IL-1β and PTX-3. Likewise, ICG-aPDT generates reactive oxygen species upon activation, disrupting bacterial integrity and potentially attenuating local inflammation, reflected in lower levels of biomarkers. These mechanisms provide a plausible rationale for the clinical improvements observed in the present study.

Prognostic indications for the effectiveness of periodontal therapy include shifts in the species levels or the overall pathogenic microbiota. For *Porphyromonas gingivalis*, both adjunctive therapies produced greater reductions than SRP, with SRP + OT showing the greatest early suppression at 1 month (2.78 ± 0.4) and sustaining low levels at 3 months (2.17 ± 0.09). The strong interaction effect ($p < 0.001$, $\eta^2 = 0.336$) indicates that the trajectory of microbial decline differed notably across groups, favoring the adjunctive modalities (Table 3).

For *Tannerella forsythia*, a pathogen frequently associated with persistent periodontal inflammation, adjunctive therapy, particularly SRP + OT, consistently achieved lower counts across all time points. The large group effect ($\eta^2 = 0.756$) underscores the added microbiological benefit of adjuncts for this species.

Treponema denticola demonstrated the most substantial overall reduction (time $\eta^2 = 0.925$). While both adjuncts produced greater reductions compared to SRP alone, the more modest group × time effect ($\eta^2 = 0.189$) suggests that mechanical debridement was particularly effective for this species, with adjuncts offering an additional but comparatively smaller benefit.

For *Fusobacterium nucleatum*, a bridging organism in biofilm maturation, significant group × time interactions ($\eta^2 = 0.345$) were observed, indicating a differentiated response to adjunctive therapy. The OT group showed more pronounced reductions than SRP at 3 months, while aPDT demonstrated a gradual decline over time.

In *Prevotella intermedia*, both adjuncts resulted in superior microbial suppression compared to SRP. The strongest interaction effect among all species ($\eta^2 = 0.588$) suggests that adjunctive therapies substantially altered the pattern of bacterial reduction, with both modalities showing sustained low levels at 3 months (Table 3).

Results of our study were in conjunction with the study by **A. Uraz *et al.* (2019)**[49] who observed selective reduction in the mean microbial concentrations of *Prevotella intermedia* and total bacterial counts in the subsequent 3 months of ozonated therapy.

The findings of our study align with those of **Sukumar *et al.* (2020)**[50] after multiple applications of ICG-aPDT therapy.

No study has been done so far, evaluating the microbial load by qPCR to compare the efficacy between OT and aPDT, so intergroup comparison of our study outcomes cannot be made.

The post-hoc analyses revealed distinct patterns of microbial suppression among the three treatment modalities. **SRP + OT** demonstrated significantly greater reductions than SRP alone for ***P. gingivalis*** (mean diff = 0.40, $p = 0.015$), ***T. forsythia*** (1.01, $p < 0.001$), and ***T. denticola*** (0.76, $p < 0.001$), indicating a consistent adjunctive benefit. In comparisons between **SRP and SRP + aPDT**, significant additional reductions were also observed for *T. forsythia* (0.50, $p < 0.001$), ***T. denticola*** (0.78, $p < 0.001$), ***F. nucleatum*** (−0.72, $p = 0.001$), and ***P. intermedia*** (0.49, $p < 0.001$), suggesting strong antimicrobial enhancement with aPDT for these species (Table 3).

Overall, both adjuncts improved microbial outcomes relative to SRP

alone. OT showed numerically greater early reductions for several pathogens (*P. gingivalis, T. forsythia, F. nucleatum*), whereas aPDT demonstrated strong suppression, particularly for *T. denticola* and *P. intermedia*. These differences likely reflect variations in bacterial susceptibility to oxidative stress (ozone) versus photodynamic destruction mechanisms (ICG-aPDT). The substantial reductions in red complex bacteria, *P. gingivalis, T. forsythia,* and *T. denticola*, are clinically meaningful, as these species are strongly linked with disease progression and poor therapeutic outcomes. The ability of adjunctive therapies to further shift the microbial profile toward a healthier ecological balance may contribute to improved clinical parameters observed in the corresponding treatment groups.

The collective findings of this study underscore the potential clinical value of adjunctive ozone therapy and antimicrobial photodynamic therapy in enhancing the non-surgical management of chronic periodontitis. The significant reduction in inflammatory biomarkers (IL-1β and PTX-3) suggests effective modulation of the host immune-inflammatory response, leading to improved periodontal healing and reduced tissue breakdown. Furthermore, the marked suppression of key periodontopathogens such as *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum,* and *Prevotella intermedia* reflects a favorable ecological shift within the subgingival biofilm. This dual impact on both microbial and host inflammatory components indicates that these adjunctive modalities not only target the etiologic biofilm but also mitigate the downstream inflammatory cascade. Such approaches may be particularly valuable in reducing dependence on systemic antibiotics and addressing concerns related to antimicrobial resistance. Collectively, these results support the integration of ozone therapy and photodynamic therapy as promising, minimally invasive, and biologically driven adjuncts to conventional scaling and root planing in clinical periodontal practice.

17. Strengths and limitations

The strengths lie in its **comprehensive, multi-dimensional evaluation** of ozonated water therapy (OT) and ICG-mediated antimicrobial photodynamic therapy (aPDT) as adjunctive treatments for chronic periodontitis. This effectively integrated **clinical, microbiological, and immunological assessments**, alongside inflammatory biomarkers (IL-1β and PTX-3) and microbial quantification using qPCR. This study not only tracked the **temporal variations** across 1- and 3-month intervals but also compared the **specific pathogen loads**, providing detailed insight into the microbial shifts associated with each treatment. However, this study has certain limitations that cannot be overlooked, including a small sample size and a short-term re-evaluation period; longer-term studies are required to confirm the durability of these effects. The possibility that subtle biochemical or microbial differences may not have been detected due to the limited sample size. Additionally, the split-mouth design was not used because of the most critical issue, the cross-arch or carry across effect; intervention on one side of the mouth may affect outcomes on the other, violating the assumption of independence between test and control sites. However, a split-mouth design could have minimized inter-individual variability factors such as general health, age, and oral hygiene habits, all of which may influence the study's outcomes. Moreover, the lack of a universally accepted protocol for ozonated water therapy in periodontitis treatment results in variations in application methods, concentration, and duration, potentially leading to inconsistent clinical outcomes.

18. Conclusion

The present study indicates that both OT and aPDT are good adjuncts to non-surgical management of periodontitis. The reductions in inflammatory biomarkers such as IL-1β and PTX-3, alongside meaningful decreases in anaerobic bacterial loads, affirm these adjunctive therapies in routine periodontal treatment protocols. The therapeutic value of these interventions, notably OT, demonstrated significant immediate effects in microbial reduction for several key pathogens, while aPDT offered pathogen suppression, particularly for specific microorganisms such as *P. intermedia and F. nucleatum*. Additionally, advanced sequencing and metagenomic analyses, such as 16S rRNA gene profiling, shotgun metagenomics, and meta-transcriptomics, could uncover specific post-treatment microbial shifts and host response, offering a better understanding of ecological changes and potential for microbiome-based personalized therapy.

Ethical approval statement

The study received ethical approval from the **Institutional Ethics Committee of King George's Medical University, U.P., Lucknow (IEC No. 554/Ethics/2024; Ref Code: XIX-PGTSC-IIA/P4).**

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Dr. Shilpi Mangal: Conceptualization, investigations, methodology, writing-original draft, and data curation.

Prof. (Dr.) Shalini Kaushal: Project administration, supervision, methodology, writing–review, and editing.

Prof. (Dr.) Nand Lal: Methodology, validation, writing–original draft, and writing–review and editing.

Prof. (Dr.) Anjani Kumar Pathak: Investigation and writing–review and editing.

Prof. (Dr.) Bhaskar Agarwal: Resources and writing–review and editing.

Dr. Suruchi Shukla: Resources**,** Data curation, and formal analysis.

Clinical trial registration

This clinical trial is filed at ClinicalTrials.gov under the code CTRI/2024/08/072049, and the reference number is REF/2024/04/082112.

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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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