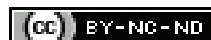


Impact of Antimicrobial Photodynamic Therapy Combined with Mechanical Debridement on Clinical Parameters and Peri-implant Microbiota in Patients with Peri-implantitis: A Prospective Clinical Study

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ABSTRACT

Introduction: Peri-implantitis is an inflammatory condition affecting tissues around dental implants, often leading to implant failure. Mechanical debridement is the standard non surgical treatment, but adjunctive therapies like antimicrobial Photodynamic Therapy (aPDT) may enhance clinical outcomes and reduce peri-implant pathogens.

Aim: To evaluate the adjunctive effect of aPDT combined with mechanical debridement on clinical parameters and peri-implant microbiota in patients with peri-implantitis.

Materials and Methods: A prospective clinical study was conducted at the Department of Periodontology, Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India, from November 2024 to March 2025, with 50 peri-implantitis patients aged 25–60 years, randomly allocated to control (Group-1) (mechanical debridement only, n=25) or test (Group-2) (mechanical debridement+aPDT, n=25) groups. Clinical parameters including

Plaque Index (PI), Gingival Index (GI), Probing Depth (PD) and Clinical Attachment Level (CAL) were recorded at baseline and three months. Subgingival plaque samples were collected for *Tannerella forsythia* quantification via Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Data were analysed using independent and paired t-tests with significance set at $p < 0.05$.

Results: Group-1 (32.56 ± 5.77 years) included 12 males and 13 females, while Group-2 (33.39 ± 4.94 years) comprised 13 males and 12 females. No significant intergroup differences in age or gender were noted ($p > 0.05$). Both groups showed significant improvements from baseline to three months in PI, GI, PD, CAL and *T. forsythia* levels ($p < 0.001$). The test group demonstrated significantly greater reductions in all parameters compared to controls at three months ($p < 0.05$).

Conclusion: aPDT significantly enhances clinical and microbiological outcomes in non surgical peri-implantitis management compared to mechanical debridement alone.

Keywords: Dental implants, Laser therapy, Photosensitising agents

INTRODUCTION

Peri-implant diseases encompass a range of pathological conditions involving inflammatory changes and tissue degradation around dental implants. Among these, peri-implantitis stands out as a destructive inflammatory process that compromises both the soft tissues and the supporting bone, often resulting in the failure of otherwise successful implant treatments. Clinically, this condition is associated with deep peri-implant pockets, bleeding on probing, suppuration and progressive bone loss, reflecting an aggressive host response to microbial biofilm accumulation [1].

The pathogenesis of peri-implantitis is multifactorial, with microbial colonisation being a principal etiological factor. The biofilm on implant surfaces harbours a diverse community of pathogenic microorganisms, many of which are also implicated in periodontitis, such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* [2,3]. *Tannerella forsythia*, an anaerobic gram-negative member of the Cytophaga-Bacteroides family [4], is frequently detected at higher levels in gingivitis, chronic and aggressive periodontitis compared to healthy sites [5]. Its presence in plaque biofilm suggests a potential role not only in sustaining microbial dysbiosis but also in influencing immunological homeostasis and modulating the progression of peri-implant inflammation [6]. The implant surface topography and its interactions with the host tissues further complicate the immune response and bacterial clearance, especially when surgical or prosthetic complications arise [7-9].

Effective management of peri-implantitis begins with thorough mechanical debridement to disrupt and reduce the microbial burden. However, mechanical instrumentation alone often proves insufficient, particularly in areas with complex surface characteristics or limited access. This limitation has prompted the exploration of adjunctive therapies to improve decontamination outcomes [10,11].

aPDT is one such adjunctive strategy that has garnered considerable interest [12]. It involves the application of a photosensitising agent that selectively binds to bacterial cells, followed by illumination with light of a specific wavelength. This interaction produces reactive oxygen species capable of destroying microbial cells without harming host tissues or contributing to antibiotic resistance [13]. aPDT offers a non invasive and targeted approach to enhance microbial eradication from implant surfaces.

Emerging evidence suggests that incorporating aPDT into conventional treatment protocols may yield favourable outcomes in peri-implant therapy, including reductions in microbial load and clinical inflammation [14]. Nevertheless, further clinical and microbiological validation is needed to establish its efficacy and optimise treatment protocols. The aim of the present study was to evaluate the adjunctive effect of aPDT combined with mechanical debridement in the management of initial peri-implantitis. The primary objective was to assess reductions in peri-implant inflammation, measured by clinical parameters including PI, GI, PD and CAL. Secondary objectives included quantifying microbiological changes, specifically targeting *Tannerella forsythia* using Real-Time

Polymerase Chain Reaction (RT-PCR) analysis of subgingival plaque samples collected from peri-implant sites.

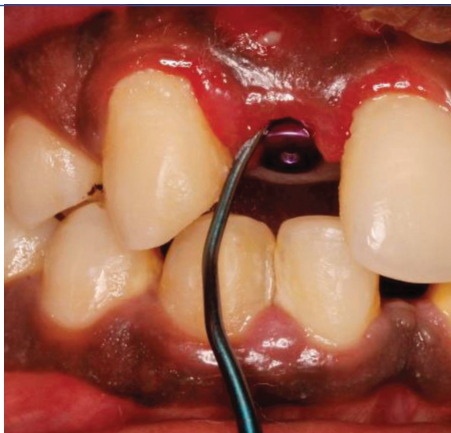
The null hypothesis was that there would be no significant difference in clinical or microbiological outcomes between mechanical debridement alone and mechanical debridement with adjunctive aPDT. The alternate hypothesis was that adjunctive aPDT would result in significantly greater improvements in these outcomes. This integrated clinical-microbiological approach allows for a comprehensive evaluation of aPDT's therapeutic potential in peri-implant disease management.

MATERIALS AND METHODS

This prospective clinical investigation was conducted at the Department of Periodontology, Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India, from November 2024 to March 2025. Fifty patients aged between 25 and 60 years, diagnosed clinically with peri-implantitis [15], were enrolled. The study adhered to the ethical principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Ethical Board (IHEC/SDC/PERIO-2303/24/272) and written informed consent was obtained from all participants prior to inclusion.

Sample size calculation: The sample size was calculated using G*Power software (version 3.1.9.4) for an independent two-sample t-test (two-tailed) with $\alpha=0.05$ and power=0.80. Based on previously published data [16] and considering a between-group difference in probing pocket depth corresponding to an effect size of $d=0.79$, a minimum of 25 subjects per group was required.

Participants were randomly divided into two groups: Group-1 (control, $n=25$) received mechanical debridement only [Table/Fig-1], whereas Group-2 (test, $n=25$) underwent mechanical debridement followed by adjunctive aPDT utilising methylene blue as the photosensitising agent [Table/Fig-2].



[Table/Fig-1]: Mechanical debridement.



[Table/Fig-2]: aPDT utilising methylene blue.

Inclusion and Exclusion criteria: Inclusion criteria comprised systemically healthy individuals aged 25 to 60 years, each with at least one functional dental implant in place for over one year, showing clinical signs consistent with peri-implantitis. All implants were commercially available bone-level fixtures with platform switching (Osstem®, Seoul, Korea), restored with porcelain-fused-to-metal crowns after a healing period of three months. Exclusion criteria included current or previous smoking habits, pregnancy or lactation, recent use of antibiotics or medications affecting bone metabolism within the last six months, systemic health conditions, history of periodontitis, or presence of mobile implants.

Study Procedure

Clinical assessment: Clinical parameters assessed included the PI and GI as described by Silness J and Löe H, in addition to PD and CAL [17]. Measurements of PD and CAL were performed at six sites per implant using a periodontal probe and the average value per implant was calculated.

Microbiological evaluation: Prior to sample collection, supragingival plaque was carefully removed using sterile curettes and the implant sites were isolated with sterile cotton rolls. Subgingival plaque samples were obtained by placing sterile paper points into the peri-implant pockets for 60 seconds. The pooled samples were stored immediately at -20°C until further analysis. For the RT-PCR assay, twenty microlitres of 1X SYBR Premix (Takara Bio Inc., Shiga, Japan), one microlitre of extracted genomic Deoxyribonucleic Acid (DNA) and universal primers (forward: 5'-GATTAGATACCCTGGTAGTCCAC-3'; reverse: 5'-TACCTTGTTACGACTT-3') were used. This assay was validated previously with conventional PCR. Species-specific primers for *Tannerella forsythia* (forward: 5'-GGGTGAGTAACGCGTATGTAACCT-3'; reverse: 5'-GCCCCATCCGCAACCAATAAA-3') were then applied for quantification. The RT-PCR was carried out using the Bio-Rad CFX96 thermal cycler with the following cycling conditions: initial denaturation at 95°C for three minutes, followed by 39 cycles of denaturation at 95°C for 10 seconds and annealing at 54°C for three minutes. Fluorescence data were collected at the end of each extension step and analysed using CFX Maestro Software (Bio-Rad, California).

Intervention protocol: Mechanical debridement in both groups was performed under local anaesthesia using titanium curettes. In the test group, aPDT was administered immediately following debridement. A total of 0.5 mL of 0.005% methylene blue solution was introduced into the peri-implant sulcus using a blunt 27-gauge needle, followed by a one-minute pre-irradiation period to facilitate adequate dye penetration and bacterial binding [18]. The 0.005% concentration was selected based on previous studies demonstrating effective microbial reduction with minimal cytotoxicity [18].

Laser irradiation was performed using a diode laser device (Pioon®, Wuhan, China) emitting red light at 650 nm in continuous wave mode with an output power of 100 mW. The irradiation was delivered via a 300- μm fibre optic tip, corresponding to a spot size of approximately 0.00071 cm^2 . The laser was applied circumferentially around the implant for 10 seconds per site, delivering an energy density of approximately 1415 J/cm^2 . These parameters were chosen in accordance with established aPDT protocols that use comparable fluence and exposure times to achieve optimal antimicrobial effects [19]. Following irradiation, the site was rinsed thoroughly with sterile saline to remove any residual dye and minimise optical interference.

All treatments were carried out by a calibrated periodontist (MT). Clinical and microbiological assessments at baseline and at three months were conducted by an independent examiner (AR), who was blinded to group allocation.

STATISTICAL ANALYSIS

Data were analysed using Statistical Package for the Social Sciences (SPSS) software (Version 23.0). The Shapiro-Wilk test

was applied to assess normality. Since the data were normally distributed, parametric tests were used. Independent t-tests compared continuous variables such as age, PI, GI, PD, CAL and *T. forsythia* levels between groups. Intra-group comparisons from baseline to three months were performed using paired t-tests. The Chi-square test was used to evaluate gender distribution between groups. Statistical significance was set at $p<0.05$.

RESULTS

Patients in Group-1 had a mean age of 32.56 ± 5.77 years. This group included 12 males and 13 females participants. Group-2 had a mean age of 33.39 ± 4.94 years. This group included 13 males and 12 females participants. No significant differences were observed between the two groups in terms of age and gender, with a p-value greater than 0.05 [Table/Fig-3].

Parameters	Group-1 (n=25)	Group-2 (n=25)	Test statistic	p-value
Age (years) (Mean±SD)	32.56±5.77	33.39±4.94	t=-0.57	0.57 ^a
Gender (M/F)	12/13	13/12	χ ² =0.08	0.78 ^b

[Table/Fig-3]: Baseline demographic characteristics of participants.
^aIndependent t-test; ^bChi-square test, $p>0.05$

Both groups showed significant improvements from baseline to three months in PI. Between-group comparisons at three months showed significantly greater reductions in Group-2 for all parameters ($p<0.05$), indicating that adjunctive aPDT provides enhanced clinical and microbiological benefits in peri-implantitis management [Table/Fig-4].

Parameters	Timeline	Group-1 (Mean±SD)	Group-1 t-value (p-value ^a)	Group-2 (Mean±SD)	Group-2 t-value (p-value ^a)	Between groups at 3 months t-value (p-value ^b)
PI	Baseline	2.45±0.19	14.2 (<0.001*)	2.49±0.17	20.5 (<0.001*)	17.2 (<0.001*)
	3 months	1.23±0.20		0.46±0.10		
GI	Baseline	2.59±0.23	12.6 (<0.001*)	2.61±0.20	18.9 (<0.001*)	32.7 (<0.001*)
	3 months	1.45±0.11		0.52±0.09		
PD (mm)	Baseline	5.83±0.04	23.1 (<0.001*)	5.86±0.02	19.4 (<0.001*)	35.6 (<0.001*)
	3 months	3.78±0.06		3.01±0.09		
CAL (mm)	Baseline	6.19±0.06	19.6 (<0.001*)	6.21±0.04	27.5 (<0.001*)	44.9 (<0.001*)
	3 months	4.52±0.11		3.47±0.04		
<i>T. forsythia</i> load (copies/μL)	Baseline	4.21±0.06×10 ²	17.9 (<0.001*)	4.24±0.04×10 ²	23.2 (<0.001*)	42.9 (<0.001*)
	3 months	2.14±0.11×10 ²		1.02±0.07×10 ²		

[Table/Fig-4]: Comparison of clinical and microbiological parameters.
^aPaired t-test; ^bIndependent t-test; *Significant at $p<0.05$

No adverse effects or patient-related complications, such as pain, swelling, or mucosal irritation, were observed in either group throughout the study period.

DISCUSSION

With the rapid advancements and growing interest in oral implantology [20-22], current investigation evaluated the efficacy of aPDT as an adjunct to mechanical debridement in patients with peri-implantitis. The results demonstrated significant clinical improvements in PI, GI, PD and CBL among individuals receiving aPDT along with mechanical debridement compared to mechanical debridement alone. Microbiological analysis further revealed a greater reduction in the prevalence of *Tannerella forsythia* in the aPDT group.

These findings align with the in-vitro study by Tonin MH et al., who demonstrated that both Low-Level Laser Therapy (LLLT) and aPDT significantly reduced colony-forming units of periodontal biofilm and *Staphylococcus aureus*, with aPDT showing superior efficacy [23]. Similarly, Hayek RR et al., validated the microbial reduction potential of aPDT in an experimental model of peri-implantitis, highlighting reductions in *Prevotella*, *Fusobacterium* and *Streptococcus beta-haemolyticus*, comparable to conventional mechanical methods [24].

Moreover, Ohba S et al., confirmed the clinical safety and short-term effectiveness of aPDT in reducing purulent discharge from peri-implant pockets. Their randomised trial showed a significant decrease in pus exudation in the aPDT group compared to saline irrigation alone, supporting its potential in inflammation control [25]. Wang H et al., further expanded the understanding of aPDT's microbiological effects by profiling gingival crevicular fluid microbiota using 16S ribosomal Ribonucleic Acid (rRNA) sequencing. Their study revealed a significant post-treatment shift in microbial composition, particularly among low-abundance pathogenic genera, suggesting that aPDT may restore microbial balance in the peri-implant sulcus [26].

Systematic review by Alasqah MN have also underscored the antimicrobial efficacy of aPDT on dental implants, noting significant reductions in bacterial load without inducing surface alterations [27]. Bahrami R et al., concluded that aPDT is effective in reducing clinical indicators such as PI, bleeding on probing and PD, though they emphasised the need for standardisation across studies and more robust trials to validate long-term benefits [28].

The present study results are corroborated by the meta-analysis conducted by Fonseca VC et al., which confirmed that aPDT leads to significant improvements in PI, GI and notably, PD reduction when used adjunctively with mechanical debridement [29]. This is further echoed by Zhao Y et al., who compared aPDT with systemic antibiotics and reported comparable improvements in clinical parameters, including PI, bleeding scores, probing pocket depth and CAL, alongside superior suppression of red-complex pathogens [30]. Fraga RS et al., also emphasised the microbiological

benefits of aPDT, reporting significant reductions in *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Their findings reinforce the therapeutic potential of aPDT in disrupting periopathogenic biofilms implicated in peri-implantitis [31].

In summary, the outcomes of the present study align with and add to the growing body of literature supporting aPDT as an effective adjunctive modality in the management of peri-implantitis. Its clinical benefits, coupled with notable microbiological improvements-particularly in reducing specific anaerobic pathogen- highlight its potential role in comprehensive non surgical treatment strategies. A key strength of this study lies in its prospective design and the integration of both clinical and microbiological endpoints, offering a holistic assessment of therapeutic efficacy. The inclusion of a well-defined patient population and standardised aPDT protocol further enhances the reliability of the results. As the between-group comparisons showed statistically significant improvements with adjunctive aPDT, the null hypothesis was rejected, confirming that aPDT provides superior clinical and microbiological outcomes compared to mechanical debridement alone.

Future studies with larger cohorts, extended follow-up durations and molecular-level assessments are warranted to validate the long-term stability of clinical improvements and elucidate the underlying

mechanisms by which aPDT modulates peri-implant microbiota and host response. Additionally, incorporating standardised reporting parameters and exploring the integration of aPDT into personalised maintenance protocols may further enhance its clinical applicability and help minimise disease recurrence.

Limitation(s)

The relatively short follow-up period may not fully capture the long-term stability of treatment outcomes, though adequate for preliminary assessment, may limit the generalisability of the findings.

CONCLUSION(S)

The present study demonstrated that adjunctive aPDT, when combined with mechanical debridement, significantly improved both clinical parameters and microbial reduction in the non surgical management of peri-implantitis. These findings underscore the potential of aPDT as a safe, effective and minimally invasive adjunctive treatment to enhance peri-implant health and control pathogenic biofilms. However, further long-term, multicentre studies are needed to validate these results and establish standardised clinical protocols for its routine use.

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