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Periodontology

PHOTODYNAMIC THERAPY USING FOTOSAN 630 AS AN ADJUNCT TO SCALING AND ROOT PLANING FOR TREATMENT OF CHRONIC PERIODONTITIS - A CLINICAL AND MICROBIOLOGICAL STUDY

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ABSTRACT

Background: Persistent pockets with incomplete eradication of microorganisms following scaling and root planing (SRP), remain as risk factor for progression of chronic periodontitis. Thus, the goal of the present study is to assess the efficiency of light-emitting diode (LED) in reducing probing pocket depth (PPD), clinical attachment level (CAL) along with lethal photosensitization of Porphyromonas gingivalis (P. gingivalis) and also as an adjunct to SRP for treatment of chronic periodontitis.

Methods – Sixty sites were randomly divided into two groups of 30 each. The test sites received SRP with photodynamic therapy (Fotosan LED + toluidine blue as photosensitizer). Control sites received only SRP. Oral hygiene status was assessed using plaque index (PI) and gingival bleeding index (GBI) along with clinical parameters like PPD and CAL were measured at baseline, 3 and 6 months. Subgingival plaque samples are collected from both sites to evaluate quantitative analysis of P. gingivalis in relation to 16S rRNA using Real-Time polymerase chain reaction (rT-PCR) at baseline and 3 months.

Results – Statistically significant improvement was seen in plaque and gingival bleeding scores with significant reduction in PPD and CAL in the test sites when compared to the control sites. Similar results were obtained for quantitative analysis of P. gingivalis between both the groups.

Conclusion – PDT applying LED as the light source, is an easy-to-use anti-infective therapy for the daily practice and can be used as an effective adjunct to SRP in improving the clinical and microbiological parameters.

KEYWORDS : Non-surgical periodontal therapy; Chronic Periodontitis; Light-activated disinfection; Photodynamic therapy

INTRODUCTION

Chronic periodontitis is a polymicrobial disease that is characterized by host-mediated inflammation which eventually results in loss of periodontal attachment. Evidence suggests that the presence of pathogenic microflora in the plaque biofilm is essential for the causation of the disease. [1], [2] Disruption of this adherent, complex architecture of the plaque biofilm to subdue the pathogenic microflora in order to restore the periodontium to health has been the major goal of periodontal therapy. [2],[3]

Over the years, scaling and root planing has evolved as the gold standard and principal approach for treatment of chronic periodontitis.[4] Regardless of the overall clinical improvement following scaling and root planing, recolonization of persistent pockets have been observed. This has been attributed to the choice of treatment protocol, diverse distribution patterns of the pathogenic microflora in the oral cavity or presence of untreated inaccessible sites that could form new niches for recolonization.[5]

In order to overcome these issues, various adjunctive therapies, such as local drug delivery, application of topical antiseptics, systemic antibiotics and lasers have been successfully researched at various levels to improve the SRP outcome in the management of periodontal disease.[6,7] However, conflicting results have been reported regarding the effectiveness of adjunctive therapies, and there is no consensus on the best method to improve the outcome of mechanical treatment.[8], [9], [10]

Photodynamic therapy (PDT), a type of non-invasive phototherapy was introduced as early as 1904 that uses low intensity light with the capacity to selectively target the bacteria without endangering the host tissues. [11] This is based on the principle that binding of a photoactivable substance or a photosensitizer to the target cells following light activation of suitable wavelength, results in production of cytotoxic products such as singlet oxygen that are extremely toxic to certain cells and bacteria. [12] The activity of singlet oxygen molecules is limited by their short lifespan (0.04µs) and short radius of action (0.02µm) and thus is localized to the site of application of the photosensitizer. [13] Thus, cytotoxic action is produced against the cells or bacteria in the area without jeopardizing the distant cells or organs.

However, conflicting results exist on the clinical as well as microbiological outcomes of PDT as adjunct to non-surgical periodontal therapy. [14],[15],[16] Therefore, the aim of our study is to assess the effects of PDT using LED on improving the clinical and microbiological parameters as well as an adjunct to SRP for treatment of chronic periodontitis.

MATERIAL AND METHODS

The present study is a single-blind, randomized controlled clinical study.

Subjects and sites -

The study enrolled fifteen outpatients diagnosed with chronic periodontitis who reported to the Department of Periodontics, V.S. Dental College and Hospital, Bengaluru, Karnataka, India from September 2017 to August 2018. A simple randomization was followed to divide a total of 60 sites with mild to moderate chronic periodontitis in these patients into two groups of 30 each. Ethical clearance was procured from the institutional review board. All subjects received oral and written explanation of the purpose of the study and signed an informed consent.

INCLUSION CRITERIA -

1. Age range of 20-55 years
2. Subjects diagnosed with mild to moderate chronic periodontitis showing at least three to four sites with periodontal pocket depth ranging from 3-5mm and clinical attachment loss of ≥ 3mm but not extending to root apex.

EXCLUSION CRITERIA -

1. Subjects with history of any known allergies or on local or systemic antibiotic therapy for the last six months or have received SRP, two months prior to baseline examination.
2. Smokers, pregnant and lactating women, history of extensive subgingival restorations, crowns, partial dentures, or implants.
3. All subjects with any known systemic condition that would affect the course of periodontal disease also were excluded.

Treatment protocol -

At baseline, both sites received adequate instrumentation through SRP with ultrasonic instruments (EMS scaler, Universal Pvt Ltd, India) and Gracey curettes (Hu-Friedy, Chicago, IL, USA) till a hard and smooth root surface was felt. In addition, test sites received PDT using 0.1% toluidine blue as photosensitizer, that was inserted into the depth of the pocket using a disposable syringe (Figure 1a & b). Activation of TBO was done by FotO2San LED (CMS Dental ApS, Copenhagen,

Denmark) of wavelength 630nm, programmed for 1 cycle of 30 seconds per site using perio tips of diameter 0.5mm-1mm. (Figure 1c, d)

Clinical parameters –

Oral hygiene status was assessed using plaque index (Silness P & Loe H, 1964). Clinical parameters like PPD, bleeding on probing (Ainamo & Bay, 1975) and CAL were measured at baseline, 3 and 6 months. All clinical measurements were made with a UNC-15 probe (University of North Carolina – No.15) and a custom-made acrylic stent (Figure 1a) to ensure reproducibility at subsequent measurements.

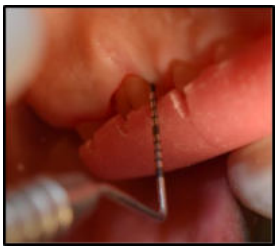


Figure 1a) measurement of PPD and CAL at baseline visit

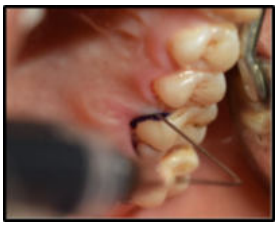


Figure 1b) application of TBO using a syringe

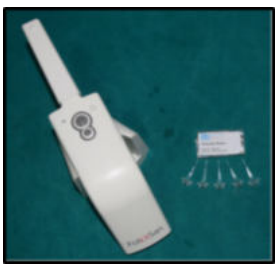


Figure 1c) FotO2San Light Emitting Diode (LED pen) for photoactivation of TBO

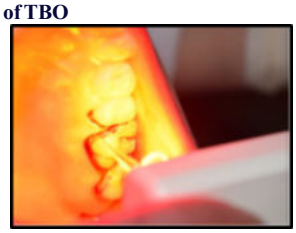


Figure 1d) Photoactivation using FotoSan LED pen with perio tip

Microbiological evaluation -

Subgingival plaque samples were carefully curetted from the test and control sites respectively and transported in TE – Buffer media for evaluating relative quantification of Porphyromonas gingivalis in relation to 16S RNA using Real-Time PCR (Applied Biosystems, India) at baseline and 3 months follow up (Figure 2). Oral hygiene measures were instructed and repeated at all appointments.

Figure 2: Cartridges filled with TE buffer media for transporting subgingival plaque samples.

Primers used in the PCR procedure

Custom SYBR® Green assay reagents (Applied Biosystems, India) were used in this study. The primer sequences are as follows:

For P. gingivalis-

- Forward primer 3'-TGCAACTTGCCTTACAGAGGG -5'
- Reverse primer 5'-ACTCGTATCGCCCGTTATTC- 3'
- **16S RNA-**
- Forward Primer: 3'-TCCTACGGGAGGCAGCAGT-5'
- Reverse Primer: 5'-

GGACTACCAGGGTATCTAATCCTGTT-3'

PCR Protocol:

A solution composed of SYBR® Green Universal PCR Master Mix (10µl), a forward primer (1µl) and reverse primer (1µl) for P. gingivalis, extracted DNA of the subgingival plaque sample (3µl) and RNAase free water taken together to make 20µl volume of final reaction mixture.

The conditions for Real-Time PCR were as follows:

- Holding stage at 950°C for 10 seconds followed by 40 cycles of shuttle heating at 950°C for 15 seconds and at 600°C for 1 minute.
- The melt curve stage was at 950°C for 15 seconds, 600°C for 1 minute and 950°C for 15 seconds.

16S RNA was used as an endogenous control. (SYBR® Green assay reagents, Applied Biosystems, India). Relative Quantification (RQ) of P. gingivalis was based on the Ct (the number of PCR cycles necessary to obtain the threshold signal of fluorescence) values. All the calculations were done using Applied Biosystems Software.

STATISTICAL ANALYSIS:

The clinical data is represented using descriptive statistics (mean ± standard deviation) and inferential statistical methods were used to determine statistical significance. Intragroup comparisons were performed using repeated measures ANOVA while a non-parametric Mann-Whitney test was used for intergroup comparisons of clinical parameters like plaque index, gingival bleeding index, PPD and CAL. Following the repeated measures ANOVA, post hoc, multiple comparisons Tukey test was performed to control the Type I error for the family of comparisons.

For relative quantification of Porphyromonas gingivalis in relation to 16sRNA data, paired t-test was used to compare the intragroup differences and a non-parametric Mann-Whitney test was used to compare the intergroup differences.

The normality of the data was assessed by Shapiro-Wilk test wherever necessary. The level of significance was set at 95% confidence interval. *P-value* < 0.05 was considered statistically significant. All the analyses were performed with GraphPad Prism version 7.10 (GraphPad Software, Inc., California, USA).

RESULTS:

All subjects completed the study. There were no adverse effects reported in both the groups.

Clinical parameters:

Significant improvement was seen in the plaque scores from baseline to the end of 6 months within the test group (p = 0.0209) with adjunctive use of PDT. However, plaque scores were not statistically significant in the control group. Intergroup comparison of plaque index showed significant improvement in the test group at 6 months (*p=0.0432*; Figure 3a). However, no statistically significant improvement was noted at 3 months between both the groups.

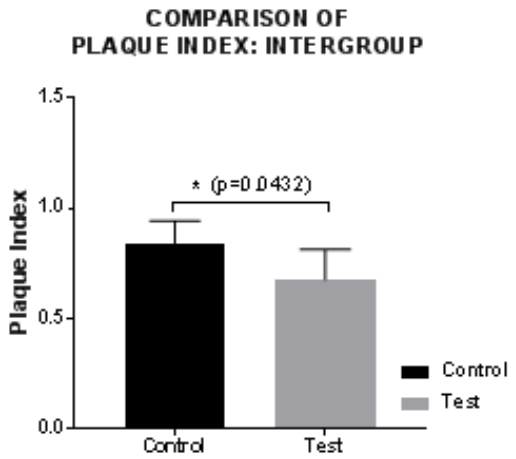
The severity of gingivitis was assessed by gingival bleeding scores. Intragroup comparison of bleeding scores showed statistically significant reduction in both test and control groups from baseline to 3 and 6 months respectively (83.19 ± 6.65 to 35.25 ± 8.134; 88.83 ± 6.197 to 49.55 ± 14.0). On intergroup comparison, statistically significant reduction was noted in the test sites as compared to control sites at 3- and 6-month intervals (*p=0.0006, p=0.0494*) respectively (Table 1, Fig 3b).

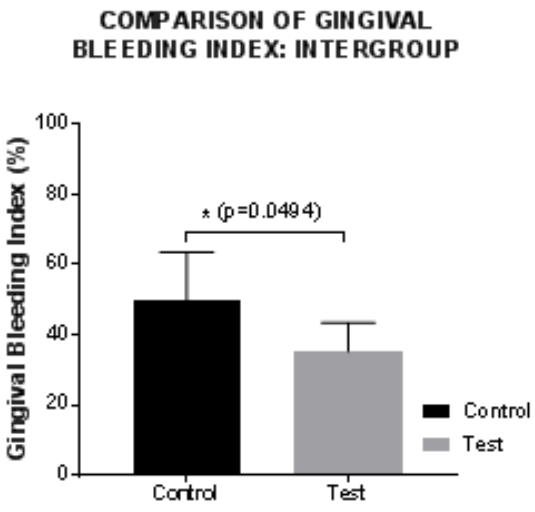
Intragroup comparison of PPD and CAL improved significantly at all time intervals. Adjunctive use of PDT in the test group, contributed significantly in improving the PPD and CAL when compared from baseline to 6 months between the groups (0.0008, 0.0011).

Table 1: Inter comparisons of Plaque Index, Gingival Bleeding Index, Probing Pocket Depth and Clinical Attachment Loss.

Timeline	Control	Test	*p-value* Control vs Test
Plaque Index			
3 Months	0.9326 (±0.1025)	0.8131 (±0.1687)	0.2643
6 Months	0.839 (±0.1039)	0.6761 (±0.1362)	0.0432
Gingival Bleeding Index			
3 Months	60.15 (±14.37)	31.83 (±8.925)	0.0006
6 Months	49.55 (±14.0)	35.25 (±8.134)	0.0494
Probing Pocket Depth			
3 Months	4.233 (±1.135)	3.60 (±1.404)	0.0287
6 Months	3.90 (±1.185)	2.90 (±1.423)	0.0008
Clinical Attachment Loss			
3 Months	4.567 (±1.104)	3.967 (±1.65)	0.0254
6 Months	4.267 (±1.112)	3.333 (±1.605)	0.0011

***p<0.05* is considered statistically significant for intragroup comparison**





Microbiological outcomes

On intragroup comparison, statistically significant reduction was obtained in the relative quantification levels of P. gingivalis in relation to 16sRNA in both the control and test groups from baseline to 3-month intervals (*p=0.0097, p=0.0021*) respectively. However, intergroup comparison did not show any statistical significance from baseline and 3 months (Table 2, Fig 4).

Table 2: Intergroup comparison of relative quantification of Porphyromonas gingivalis in relation to 16sRNA.

Group	Control	Test	*p-value* Control vs Test
Porphyromonos Gingivalis			
Baseline	30.23 (±39.35)	17.08 (±17.59)	0.5125
3 Months	0.4998 (±0.0172)	0.0066 (±0.0172)	0.4050

***p<0.05* is considered statistically significant for intergroup comparison**

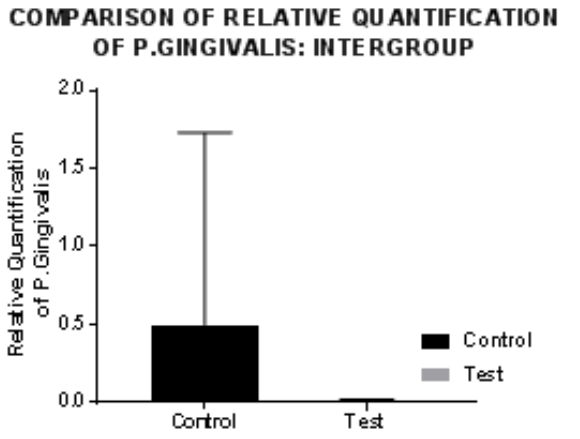


Figure 4) Intergroup comparison and representation of relative quantification of P. gingivalis in relation to 16sRNA at 3 months

DISCUSSION:

The goal of periodontal therapy is to arrest progressive attachment loss, and hence to prevent further disease progression, and eventually tooth loss. The focus of the current study was to evaluate the efficacy of photodynamic therapy as an adjunct to scaling and root planing on clinical and microbiological parameters.

Both groups have shown significant improvement in regard to oral hygiene status and gingival bleeding scores from baseline to the end of 6 months. However, greater reduction in bleeding scores from 31.2% and 35.3% from baseline to 3 months and 6 months respectively (*p<0.0001*) were seen in the sites supplemented with PDT.

The results of the present study are consistent with a number of recent clinical investigations by Chondros et al that reported significantly reduced bleeding scores by the adjunctive use of PDT.[13] Similarly, Christodoulides et al reported a statistically significant improvement of full-mouth bleeding scores at 3 and 6 months for subjects in the test group who received a single episode of PDT.[17] In patients with aggressive periodontitis, enrolled in a split-mouth design clinical trial, de Olivera et al. also observed a significant reduction in BOP values at sites treated by PDT.[18] In support of the above literature, Dmitry et al also concluded that Photo activated Disinfection using LED not only helps in decreasing the intensity of the inflammation but also keeps the periodontal tissues intact by normalizing the oxygenic metabolism in the tissues.[19] Systematic review by Garcia Canas et al reported that change in bleeding on probing was the most common secondary outcome among the clinical parameters in investigations carried out as adjuncts to SRP.[20] Also, from the results of the current study, it can be stated that oral hygiene had been maintained by the subjects throughout the study.

On intergroup comparison, PDT sites showed significant reduction in PPD over the control sites. (2.90±1.423 vs 3.90±1.185; p = 0.0008). Notable clinical attachment gain has also been observed with the test sites (3.333±1.605) as compared to the control sites (3.967±1.65) at the end of 6 months.

The above findings are in line with the systematic review by Joseph et al that outlined the additional benefits of PDT in terms of clinical, microbiological, immunological, and patient-based outcomes and, hence, should be included in the routine treatment protocol of patients with periodontitis.[21] Pooled evidence has been described in a systematic review by Xue et al indicating an additional clinical improvement in the maintenance of residual pockets in favor of SRP+PDT compared with SRP alone.[22] Campos et al. and Mongardini et al. found a significant reduction of PPD following a single application of adjunctive PDT at sites with residual pockets, as compared with control. However, these 2 studies were of a shorter follow-up period, being 3 months and 1 weeklong respectively.[23][2]

This observation was contrasted by Chondros et al. where authors could not demonstrate any additional effect on PPD reduction in sites treated by mechanical debridement and PDT compared to mechanical debridement alone.[13] Azarpazhooh et al. conducted a systematic review and concluded that photodynamic therapy as an independent

therapy or as an adjunct to SRP was not superior to control treatment.[24] However, although long-term benefits of PDT have been questionable, meta-analysis performed by Sgolastra et al. suggested that the use of PDT as an adjunct to conventional treatment provides short-term benefits in terms of CAL gain and pocket depth reduction (at 3 months after treatment) thereby confirming the safety of PDT.[25] However, these discrepancies are possibly due to variations in the conditions of PDT application like the type of light source used, type and quantity of the photosensitizer, depth of penetration of light and photosensitizer, the frequency of application of PDT or even to the experimental design.

The microbiologic response is one of the main goals of periodontal therapy. Socransky et al. demonstrated that bacteria are frequently found in microbial complexes in periodontal lesions.[26] In the present study, relative quantification of P. gingivalis has also been evaluated using real time PCR. On intragroup comparison significant reduction of P. gingivalis was observed in both groups from baseline to 3 months. Data from different in vitro studies showed that it is possible to kill bacteria sensitised with an appropriate photosensitizer and irradiated with a low-power laser light in a correlated spectrum. The findings of the present study correlate with those of Zanin et al who demonstrated up to 99 % killing efficacy after photosensitization of biofilms.[27] Long term follow-up on the proportions of periodontopathogens in patients with chronic periodontitis has also been evaluated by Theodoro et al that demonstrated a significant reduction in the proportion of sites positive for periodontopathogens at 60, 90 and 180 days compared to baseline ($p<0.05$).[28]

Difference in P. gingivalis counts between the groups was not statistically significant ($p = 0.4050$). This indicates that adjunctive use of PDT is quite comparable with mechanical debridement in reducing the oral microbial counts. Similarly, Polansky et al. could not find any statistically significant difference in the clinical and microbiological parameters, except in bleeding on probing, when non-surgical periodontal treatment of chronic periodontitis patients was implemented by a single cycle of PDT.[29]

The lethal photosensitization of these microorganisms must involve changes in membranes and/or plasma membrane proteins and DNA damage mediated by singlet oxygen. Several studies have demonstrated that gram-positive bacteria are susceptible to photodynamic inactivation, but gram-negative bacteria are significantly resistant to many photosensitizers used in PDT.[29] In the present study, TBO was used as the photosensitizer because of its known interaction with lipopolysaccharides present in the cell membrane of gram-negative bacteria more significantly than methylene blue.

The changes in bacterial composition after scaling are the basis for periodontal healing expressed as reductions in PD and gains in CAL.[30] The microbiological findings were not reflected in clinical outcomes probably because reductions in PD and gains in CAL after nonsurgical treatment depend on initial PD, with a greater probability of success if the PD of deeper pockets is reduced, independent of treatment type.

Also, several conditions such as drug ion concentration, period of retention of the drug within the tissue, time for biological response, pH of the environment (tissue/tooth interface), presence of exudates and gingival fluid, and mode of drug application (irrigation, slow-release gel) may influence the biological response to PDT. These could be considered some of the limitations of the current study.

CONCLUSION:

Within the limits of this study and a wide range of heterogeneity in the included studies, all indicated that PDT has the potential to be an effective adjunct in the treatment of chronic periodontitis. However, long-term, multicentre studies with larger sample sizes are needed before PDT can be recommended as an effective treatment modality.

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