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Efficacy of antimicrobial photodynamic therapy in the management of chronic periodontitis: a randomized controlled clinical trial

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Abstract

Aim: To evaluate the potential of antimicrobial photodynamic therapy (aPDT) as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis.

Material and Methods: In a single-centred randomized and controlled clinical trial, 90 patients (51 females and 39 males) with untreated chronic periodontitis were randomly assigned to receive SRP with aPDT (test group) or SRP alone (control group). Clinical parameters and halitosis were recorded for 6 months after treatment by a periodontist who was blinded to the procedure.

Results: Inter-group and intra-group statistical analyses were performed. Significant difference between the two groups with respect to each variable was assessed using non-parametric Rank Order ANCOVA. Probing pocket depth and clinical attachment levels showed statistically significant reduction in the test group on evaluation at 3 months and 6 months as compared to the control group ($p < 0.05$). A statistically significant improvement in gingival index and gingival bleeding index was seen for the test group after 2 weeks and 1 month of aPDT ($p < 0.01$), whereas the improvement in gingival index and gingival bleeding index at 3 months and in plaque index at 2 weeks after aPDT was less ($p < 0.05$). Also, a significant difference was detected for the test group at 1 month in terms of halitosis ($p < 0.05$), which did not persist for long.

Conclusions: Antimicrobial photodynamic therapy acts as a beneficial adjunct to SRP in non-surgical treatment and management of chronic periodontitis in short-term. Further studies are required to assess the long-term effectiveness of aPDT.

Key words: adjunctive periodontal treatment; halitosis; methylene blue; periodontal disease; photodynamic therapy; randomized controlled trial

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Conflict of interest and source of funding statement

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The gold standard for the non-surgical treatment of periodontal disease is scaling and root planing (SRP). Although SRP has shown significant improvements in a large number of cases, it was observed to be not very effective in completely eliminating subgingival periodontal pathogens and calculus (Adriaens & Adriaens 2004, Umeda et al. 2004). Alternatively, antibiotics are widely used now-a-days to suppress periodontal pathogens and augment the effect of conventional mechanical treatment.

Nevertheless, injudicious use of antibiotics has resulted in the development of drug-resistant microorganisms. Therefore, Herrera et al. (2002) suggested that use of antibiotics should be theoretically restricted to specific groups of periodontal patients, such as those with a highly active disease or a specific microbiological profile. An alternative to systemic antibiotics in the management of chronic periodontitis is to administer antimicrobial agents directly into the periodontal pockets. However, application of this method is technically difficult in patients with generalized periodontitis and multiple deep pocket sites. Therefore, antimicrobial photodynamic therapy (aPDT) has been suggested as an alternative to eradicate the microbes in the subgingival region as it was found to be effective in killing microbes in localized topical infections (Katie et al. 2005).

Antimicrobial photodynamic therapy is a modern approach in dentistry where light of an appropriate wavelength is used in the presence of a specific photosensitizer (PS) to eradicate target cells selectively. In aPDT, the PS binds to the target bacteria and can be activated by light of a suitable wavelength within the therapeutic window (630–830 nm). On absorption of light energy, the singlet excited state of the excited photosensitizer (PS*) undergoes inter-system crossing to the triplet state. The long-lived triplet PS* then reacts with molecular oxygen to produce cytotoxic reactive oxygen species (ROSs), such as superoxide, hydroxyl and lipid-derived radicals.

Although PDT is more widely known for its application in the treatment of neoplasms, aPDT shows great potential in the treatment of periodontitis, because many oral species were reported to be killed in vitro by this approach (Komerik et al. 2003, Pfitzner et al. 2004, Wilson 2004). Gram-positive bacterial and fungal cells have been found to be more susceptible to aPDT than Gram-negative bacterial cells because neutral or anionic PS molecules can efficiently enter the cytoplasmic membrane that is surrounded by a relatively porous layer of peptidoglycan and lipoteichoic acid, or beta-glucan and chitin (Malik et al. 1990). Therefore aPDT has been proposed as an alternative approach for the inactivation of bacteria in biofilms (Schneider et al. 2012, Song et al. 2013). These findings may not always be directly applicable to a clinical situation as these studies were essentially performed in vitro on planktonic bacteria, whereas bacteria exist principally as a biofilm inside the body.

Clinical trials to evaluate the effects of aPDT as an adjunct in the management of chronic and aggressive periodontitis patients have been studied separately due to the basic differences between the two disease entities and their management strategies. While meta-analysis by Azarpazhooh et al. (2010) and Sgolastra et al. (2013) did not suggest any definitive benefit for aPDT along with SRP in the treatment of periodontitis, other clinical trials including meta-analysis by Atieh (2010) have shown additional clinical benefits when aPDT and SRP were given in combination to patients with chronic periodontitis (Braun et al. 2008, Pinheiro et al. 2010, Ge et al. 2011, Berakdar et al. 2012, Alwaeli et al. 2013), on supportive periodontal maintenance for residual pockets (Pinheiro et al. 2010, Campanile et al. 2013, Campos et al. 2013) and periodontitis with HIV infection (Noro Filho et al. 2012). In aggressive periodontitis, a more severe form of periodontitis, de Oliveira et al. (2007) demonstrated aPDT to have results similar to SRP and Novaes et al. (2012) reported aPDT to be beneficial while Arweiler et al. (2013) did not find aPDT to be more effective. However, the results of these studies need to be carefully interpreted due to differences in study designs. For example, Campos et al. (2013) carried out SRP with/without aPDT in residual pockets in chronic periodontitis patients; whereas in the study by Arweiler et al. (2013), aggressive periodontitis patients received additional administration of systemic antibiotics along with aPDT and in the study by de Oliveira et al. (2007) patients received SRP or aPDT in a split-mouth design.

Albeit the small sample size and heterogeneous nature of most of these controlled clinical studies, aPDT was found to have a huge potential in the management of periodontitis. In the light of this, the present randomized controlled clinical study was carried out to evaluate whether adjunctive use of aPDT to SRP has any short-term effectiveness in the management of patients with chronic periodontitis in terms of clinical parameters and halitosis. The null hypothesis of the study was that aPDT with SRP would produce the same results as SRP.

Materials and Methods

Subjects

Ninety patients (51 females and 39 males) diagnosed with chronic periodontitis were enrolled in this study conducted over a period of 1 year (June 2011–June 2012) at the outpatient unit of Department of Periodontics, Government Dental College (GDC), Thiruvananthapuram, Kerala, India. The mean age of study population was 39.6 ± 8.7 years.

Ethical approval

The study was in accordance with the Declaration of Helsinki (as amended in Edinburgh, 2000) and was approved by the Institutional Ethics Committee (IEC) of GDC, vide IEC no. IEC/C/42-A/2011/DCT/dated 18-01-2011. The clinical trial was registered at the Clinical Trial Registry of India (CTRI) vide registration no. REFCTRI2010006105. All subjects were explained about the study protocol and their informed consent was obtained prior to the initiation of the study. The study was conducted at Department of Periodontics of GDC with the PDT unit developed at the Biophotonics laboratory of the Centre for Earth Science Studies.

Patients included in the study were those diagnosed with chronic periodontitis. Table 1 lists the sociodemographic characteristics of the patients enrolled for the trial at baseline while Table 2 shows the clinical parameters of these patients at baseline. The inclusion criteria followed for patient selection comprised of (a) probing pocket depths (PPD) between 4 and 6 mm at least in two different quadrants of the mouth, (b) a minimum of 20 teeth, (c) age between 18 and 65 years (both males and females), (d) single rooted teeth, good general health without any

Table 1. Socio-demographic characteristics of patients at baseline

Variable	Category	aPDT + SRP	SRP	*p*-value
Gender	Male (%)	22 (50.0)	15 (34.1)	0.101^{ns*}
	Female (%)	22 (50.0)	29 (65.9)	
Occupation	Agriculture/Labourers (%)	30 (68.2)	31 (70.5)	0.113^{ns*}
	Private employees (%)	9 (20.4)	10 (22.7)	
	Government employees (%)	5 (11.4)	3 (6.8)	
Education	Middle school (%)	17 (38.6)	18 (40.9)	0.176^{ns*}
	High school (%)	13 (29.6)	16 (36.4)	
	College (%)	14 (31.8)	10 (22.7)	
Income	Rs. 2000–6000 (%)	16 (36.4)	19 (43.2)	0.881^{ns*}
	Rs. 6000–10,000 (%)	20 (45.4)	17 (38.6)	
	Rs. 10,000 and above (%)	8 (18.2)	8 (18.2)	
SES	Average (%)	28 (63.6)	30 (68.2)	0.464^{ns*}
	High (%)	16 (33.4)	14 (31.8)	
Age	Mean	40.8	38.4	0.180^{ns†}
	SD	8.3	9.6	

aPDT, antimicrobial photodynamic therapy; ns, not significant; SRP, scaling and root planing.
*Chi-square test.
†Mann–Whitney *U*-test.

Table 2. Clinical parameters of patients at baseline

Baseline	Group	*n*	Median (Min–Max; IQR)	*p*-value
Gingival score	aPDT + SRP	44	2.0 (1.5–3.0; 0.5)	0.904^{ns†}
	SRP	44	2.2 (1.2–2.8; 0.5)	
Plaque score	aPDT + SRP	44	2.0 (0.5–3.0; 0.8)	0.001**,†
	SRP	44	1.2 (0.5–3.0; 1.0)	
Gingival bleeding index	aPDT + SRP	44	100 (50.0–100.0; 25.0)	0.232^{ns†}
	SRP	44	75 (50.0–100.0; 25.0)	
Probing pocket depth (mm)	aPDT + SRP	44	5.7 (5.0–6.0; 1.0)	0.363^{ns†}
	SRP	44	5.5 (4.2–6.0; 1.0)	
Recession (mm)	aPDT + SRP	44	1.0 (0.0–2.0; 1.0)	0.760^{ns†}
	SRP	44	1.0 (0.0–2.0; 1.0)	
Clinical attachment level (mm)	aPDT + SRP	44	6.5 (5.0–8.0; 1.4)	0.455^{ns†}
	SRP	44	6.0 (4.2–8.0; 1.7)	
Halitosis	aPDT + SRP	44	3.0 (2.0–4.0; 1.2)	0.204^{ns†}
	SRP	44	4.0 (2.0–4.0; 1.0)	

aPDT, antimicrobial photodynamic therapy; Min–Max; IQR, minimum–maximum; interquartile range; ns, not significant; SRP, scaling and root planing.
**$p < 0.01$.
†Mann–Whitney *U*-test.

signs of systemic disease, (e) no use of antibiotics for the past 6 months, (f) female patients were not pregnant or lactating, (g) non-smoking and (h) non-allergic to methylene/toludine blue. At the baseline, a total of 229 teeth with probing pocket depth between 4 and 6 mm (109 in the test group and 120 teeth in the control group) were included in the study. The number of teeth evaluated in patients enrolled under the test and control groups is given in Table 3.

The PPD around each tooth was assessed at four points and the site with maximum PPD for each tooth was recorded for further analysis and these same sites were probed at different evaluation intervals. In an attempt to minimize errors during periodontal probing, exclusion criteria followed for patient selection consisted of (a) third molars, (b) teeth presenting unsatisfactory restorations, (c) extensive caries lesions, or fractures, (d) teeth where the cemento-enamel junction is difficult to determine and (e) areas with great gingival morphological alterations (Fernando et al. 2009).

Study design

In this randomized controlled trial, each participant was randomly assigned either to SRP or SRP + aPDT groups according to block randomization (block size: 4). Allocation sequence was generated using a Tippet's 2-digit random number table in which the random number less than or equal to 88 was chosen and the unit was included without replacement. The allocations were concealed in opaque sealed envelopes, which were sequentially numbered. An experienced periodontist (PJ) who was blinded to the study procedure collected all clinical data at the baseline and during follow-up visits, whereas another experienced periodontist (BJ) carried out SRP and aPDT.

Clinical outcome monitoring

Change in probing pocket depth (PPD) was assessed as the primary outcome following intervention using a William's graduated periodontal probe at four inter-dental sites (mesiobuccal, distobuccal, mesiolingual and distolingual). PPD was recorded in the upper and lower anterior sextant using double pass method to minimize errors; whereas, changes in clinical attachment levels (CAL), gingival index (GI; Löe, 1967), gingival bleeding index (GBI; Ainamo and Bay, 1975) and plaque index (PI; Silness and Loe, 1964) were assessed before and after treatment as secondary outcomes. A gentle probing procedure was performed by the same examiner throughout the study. In addition, halitosis as perceived by the patient, which is a significant patient-centred outcome, was also included based on self-

Table 3. Demographic data on the number of teeth evaluated in the patients enrolled under the test and control groups

	Test				Control				Total (n = 88)
	Female (n = 22)		Male (n = 22)		Female (n = 29)		Male (n = 15)		
	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	
Central incisors	16	10	20	10	12	15	13	14	110
Lateral incisors	12	8	6	4	14	9	11	5	69
Canines	9	4	7	3	6	11	3	7	50
Sub total	37	22	33	17	32	35	27	26	
Total	109				120				229

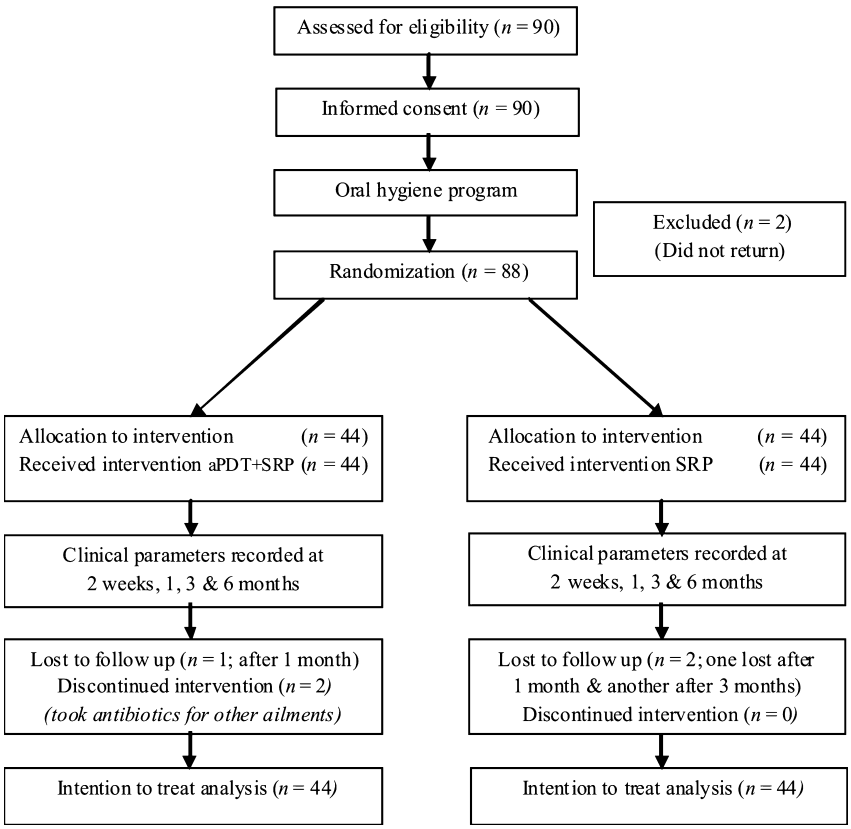

Fig. 1. CONSORT flow chart of the study.

assessment by the patient's hand on mouth technique (NS). This test was carried out at baseline, 1 month, 3 months and 6 months of treatment using Likert scale of 1–5 that categorizes patient response as strongly disagree for 1, disagree for 2, neither agree nor disagree for 3, agree for 4 and strongly agree for 5. Score 0 and 5 were not included in this study as our patients in the pilot study were unable to differentiate between 0 and 1 and also between 4 and 5. Nevertheless, the chosen scale presents symmetry of categories about a midpoint.

Treatment protocol (Interventions)

All the 88 subjects were treated by an experienced periodontist and clinical outcomes were measured by another periodontist who was blinded to patient selection and aPDT procedures as per the consort flowchart (Fig. 1). The control group (Group 1) was administered SRP by hand scalers and universal curettes (Hu-Friedy) and ultrasonic scaler (Woodpecker®). No other treatment was given to this group. Full-mouth supragingival and subgingival scaling was performed at all sites within 24 h including the evaluated sites until the operator felt that tooth and root surfaces were adequately debrided and planed. This group included 44 subjects (29 women and 15 men; mean age: 38.4 ± 9.6 years).

The test group (Group 2) included 44 subjects (22 women and 22 men; mean age: 40.8 ± 8.3 years) and was managed by aPDT in addition to SRP. The photosensitizer used consisted of freshly prepared 3,7-bis (dimethyl- amino) phenazathionium chloride trihydrate [methylene blue (MB) M9140; Sigma-Aldrich, St. Louis, MO, USA] suspended in double distilled water at a concentration of 10 mg/ml.

The PDT device used was built using a diode laser (CNI Opto-electronics Tech. Co. Ltd, Changchun, China) operating at 655 nm with a CW output power of 1 W (CSP). Two millilitre of MB was applied topically to sites with 4–6 mm pocket depth for 60 s using a syringe (Fig. 2). After 3 min., the site was irrigated with distilled water to flush out excess MB as it can act as an optical shield during laser irradiation. The laser beam was guided through a flexible fibre-optic cable (200 μm dia.) terminated in a custom-designed stainless steel (SS) hand piece (Fig. 2c,d). The outer diameter of the SS probe tip was 0.5 mm, which facilitated easy access to periodontal pockets. The aPDT treatment was performed at a continuous laser power density of 60 mW/cm^2, with top-hat energy distribution for 60 s, at each mesiobuccal, distobuccal, mesiolingual or distolingual site with 4–6 mm pockets on selected teeth. aPDT along

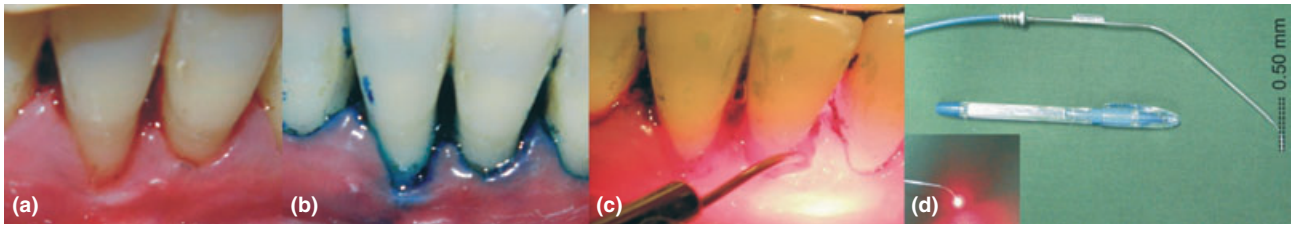

Fig. 2. Antimicrobial PDT procedure; (a) before scaling and root planing (SRP), (b) after application of methylene blue, (c) during antimicrobial photodynamic therapy (aPDT) with the 655 nm diode laser probe inserted into the periodontal pocket and (d) handpiece of the aPDT probe.

with SRP was performed in a single session in each patient, within 24 h. During aPDT, both clinicians and patients were provided with the appropriate safety eyewear. Clinical outcome was measured at 2 weeks, 1 month, 3 months and 6 months after treatment.

Sample size calculation

The sample size was determined based on probing depth (PD) as the primary outcome measure assuming normality.

Atleast 37 subjects in each group (total 74 patients) were required to detect a moderate difference between the before- and after-treatment groups with an effect size of 0.5 and standard deviation of 0.6. [$Z\alpha = 1.96$, $Z\beta = 1.28$ and $(Z\alpha + Z\beta)^2 = 13$]. Therefore, a total of 90 patients (20% was added to compensate for loss during follow up) were assessed for eligibility and enrolled in the oral hygiene program. Size calculation was based on the formula given below.

$$n = \frac{2(\text{Standard deviation})^2}{(\text{effect size})^2} \times (Z\alpha + Z\beta)^2 \quad (1)$$

Intra-examiner reproducibility

Five patients, not related to the study, each with at least two pairs of contralateral teeth and probing depths >5 mm on at least one aspect of each tooth, were used as calibration for the examiner. The examiner inspected the patients on two separate occasions, 48 h apart. Calibration was accepted if the results of measurements at baseline and at 48 h were the same in more than 90% of the cases.

To ensure a sufficient level of plaque control, all subjects were initially enrolled in an oral hygiene program and were given oral hygiene instructions that included twice daily brushing before they returned for treatment after 1 week.

Data analysis

A subject-level analysis was performed (BKV) statistically for each of the parameters using SPSS software for Windows, Version 16.0. (SPSS Inc., Chicago, IL, USA). Intention to treat analysis was done where the primary clinical outcome variable for patients was change in median of PPD at various recall visits ($n = 88$). Median (minimum to maximum; inter-quartile range) for the clinical variables were calculated for each treatment.

As the data distribution of clinical parameters in this study did not obey the Gaussian law by Kolmogorov-Smirnov test ($p < 0.05$), non-parametric methods were used for analysing the data. Significant difference between the test and control groups with respect to categorical data was assessed using Chi-square test, whereas Mann-Whitney *U*-test was used for continuous variable. Likewise, Wilcoxon's Signed Rank Test was used for finding significant changes from baseline to various intervals within the test and control groups. As some confounding factors also could be responsible for the statistically significant treatment results, analysis of covariance was used rather than Student's *t*-test, as it adjusts for imbalances between groups by eliminating confounding variables (Quade 1967). Distribution-free or non-parametric procedures have been developed in equivalence to the classical parameteric ANCOVA which require normality assumption. However, in the absence of normality assumption, non-parametric methods have been developed in parallel with ANCOVA. One such non-parametric rank order ANCOVA was developed by Puri and Sen (1969) as has been presented in simple form by Quade (1967). Using this method, the observations are ranked ignoring the grouping variable and these ranked data were used to develop a general linear hypothesis (Quade 1967, Lawson 1983).

Results

Among the 90 patients enrolled in the oral care program, two patients did not return for randomization and hence were excluded. The remaining 88 patients were randomized into test and control groups according to block randomization. In the test group, one patient was lost to follow-up, one did not respond to aPDT treatment and two patients reported to have taken antibiotics for other ailments. Figure 3a–f shows the appearance of gingiva at baseline, 2 weeks and 3 months of aPDT in two typical cases studied. In the control group, two patients were lost to follow up and two did not respond to treatment. Intention to treat analysis was done for all the 88 patients who were randomized and missing data were handled by last observation carried forward (LOCF) method. Healing was uneventful in all cases and no adverse effects, such as discomfort, burning sensation, or pain related to the laser irradiation, were reported by any of the subjects.

In the final tally of 88 patients at baseline, no statistically significant differences were present between the test and control groups with respect to socioeconomic status ($p > 0.05$; Table 1). Similarly, no significant differences were found (Table 2) between the test and control group of

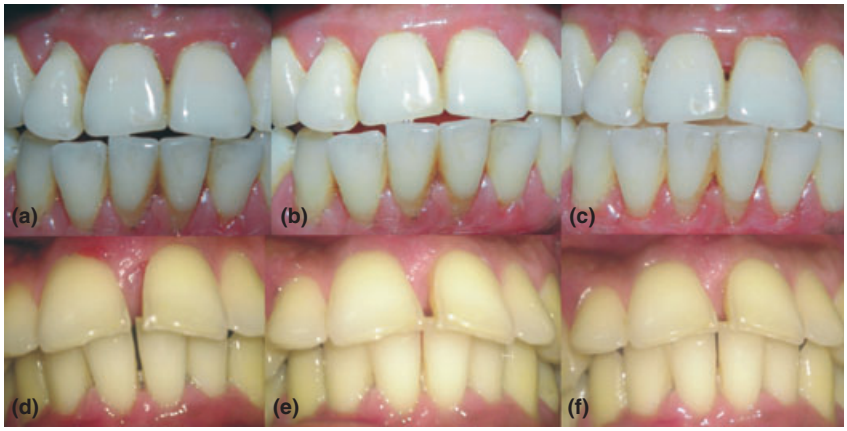

Fig. 3. Appearance of the gingiva before and after antimicrobial photodynamic therapy (aPDT); in case 1 at (a) baseline, (b) 2 weeks and (c) 3 months of PDT and in case 2 at (d) baseline, (e) 2 weeks and (f) 3 months of aPDT.

patients with regard to the baseline values of clinical parameters ($p > 0.05$), except for the plaque index ($p < 0.01$). Table 4 summarizes the changes in PPD, recession and CAL for test and control groups, which are expressed as median with minimum, maximum and inter-quartile range (IQR). Table 5 shows changes in GI, PI and GBI for the test and control groups at various intervals of treatment in terms of median with minimum, maximum and (IQR).

Table 6 shows the changes in halitosis in both the test and control groups at various intervals of treatment (baseline, 1 month, 3 months and 6 months).

As compared to control group, PPD and CAL showed statistically significant reduction in the test group at 3 months and 6 months ($p < 0.05$). A statistically significant improvement in gingival index and gingival bleeding index was seen for the test group ($p < 0.01$) after 2 weeks and 1 month of aPDT, whereas the improvement in gingival index and gingival bleeding index at 3 months and in plaque index at 2 weeks after aPDT was found to be lesser ($p < 0.05$). Also, a significant difference was detected at 1 month between SRP and SRP + aPDT in terms of halitosis ($p < 0.05$), which did not persist for long. No statistically significant change was observed in terms of recession during the evaluations. The changes observed at various intervals in both groups are detailed in Tables 2–4.

Discussion

To the best of our knowledge this is the first randomized and controlled clinical trial reported in Indian population to evaluate the efficacy of aPDT in the management of chronic periodontitis. Similar randomized control trial results were published by various international groups to understand the efficacy of aPDT in the treatment of chronic periodontitis as a primary mode of treatment or as an adjunct to SRP (Andersen et al. 2007, Braun et al. 2008, Christodoulides et al. 2008, Ge et al. 2011, Berakdar et al. 2012, Balata et al. 2013, Alwaeli et al. 2013).

Effect of aPDT on primary outcome (PPD)

Our study results show statistically significant reduction in the PPD when the test group was compared with the control group at 3 months and 6 months of aPDT. These results suggest additional clinical benefit of aPDT along with SRP in reducing PPD and are comparable to the results of Braun et al. (2008), Pinheiro et al. (2010), Ge et al. (2011), Berakdar et al. (2012) and Alwaeli et al. (2013) where probing

Table 4. Differences in probing pocket depth, recession and clinical attachment level at baseline, 1 month, 3 months and 6 months of treatment

Parameter	Baseline	1 month	3 months	6 months	*p*-values		
	Median (Min–Max; IQR)	Median (Min–Max; IQR)	Median (Min–Max; IQR)	Median (Min–Max; IQR)	0–1 month	0–3 months	0–6 months
PPD (mm)							
Test	5.7 (5.0–6.0; 1.0)	4.0 (2.0–6.0; 1.0)	3.3 (2.0–6.0; 1.0)	3.0 (2.0–6.0; 1.0)	<0.01**	<0.01**	<0.01**
Control	5.5 (4.2–6.0; 1.0)	4.7 (3.0–6.0; 1.0)	3.9 (2.0–6.0; 1.0)	4.0 (2.0–6.0; 1.0)	<0.01**	<0.01**	<0.01**
p-values	0.363^{ns}	0.392^{ns}	0.023*	0.016*			
REC (mm)							
Test	1.0 (0.0–2.0; 1.0)	1.0 (0.0–2.0; 1.0)	1.0 (0.0–2.0; 0.3)	1.0 (0.0–2.0; 1.0)	0.046*	0.132^{ns}	0.206^{ns}
Control	1.0 (0.0–2.0; 1.0)	1.0 (0.0–3.0; 1.0)	1.0 (0.0–2.0; 1.0)	1.0 (0.0–3.0; 1.0)	0.025*	0.257^{ns}	0.013*
p-values	0.760^{ns}	0.626^{ns}	0.493^{ns}	0.237^{ns}			
CAL (mm)							
Test	6.5 (5.0–8.0; 1.4)	5.1 (3.0–7.2; 2.2)	4.0 (2.0–7.0; 1.4)	4.0 (2.6–7.0; 2.0)	<0.01**	<0.01**	<0.01**
Control	6.0 (4.2–8.0; 1.7)	5.1 (4.0–8.0; 1.0)	4.4 (2.0–7.0; 1.9)	4.5 (2.0–7.0; 2.0)	<0.01**	<0.01**	<0.01**
p-values	0.455^{ns}	0.831^{ns}	0.037*	0.021*			

CAL, clinical attachment level; Min–Max; IQR, minimum–maximum; inter-quartile range; PPD, probing pocket depth; REC, recession; ns, not significant.
*$p < 0.05$.
**$p < 0.01$.

Table 5. Differences in gingival index, plaque index and gingival bleeding index at baseline, 2 weeks, 1 month, 3 months and 6 months of treatment

Parameter	Baseline	2 weeks	1 month	3 months	6 months	*p*-values			
	Median (Min–Max; IQR)	Median (Min–Max; IQR)	Median (Min–Max; IQR)	Median (Min–Max; IQR)	Median (Min–Max; IQR)	0–2 weeks	0–1 month	0–3 months	0–6 months
GI									
Test	2.0 (1.5–3.0; 0.5)	1.0 (0.0–3.0; 0.5)	0.75 (0.0–3.0; 0.7)	0.8 (0.0–3.0; 0.8)	1.0 (0.0–3.0; 1.0)	<0.01**	<0.01**	<0.01**	<0.01**
Control	2.2 (1.2–2.8; 0.5)	1.5 (0.5–2.8.0; 1.0)	1.0 (0.0–2.8; 0.8)	1.0 (0.0–2.8; 0.7)	1.5 (0.2–2.8; 1.0)	<0.01**	<0.01**	<0.01**	<0.01**
p-values	0.904^{ns}	<0.01**	<0.01**	0.021*	0.135^{ns}				
PI									
Test	2.0 (0.5–3.0; 0.8)	0.8 (0.0–2.5; 0.5)	0.5 (0.0–2.5; 1.0)	0.5 (0.0–2.5; 1.0)	1.0 (0.0–2.5; 1.0)	<0.01**	<0.01**	<0.01**	<0.01**
Control	1.2 (0.5–3.0; 1.0)	1.0 (0.0–2.0; 0.5)	0.5 (0.0–1.5; 0.6)	0.5 (0.0–1.5; 0.7)	0.5 (0.0–2.0; 0.5)	<0.01**	<0.01**	<0.01**	<0.01**
p-values	0.001**	0.015*	0.326^{ns}	0.426^{ns}	0.457^{ns}				
GBI									
Test	100.0 (50.0–100.0; 25.0)	25.0 (0.0–100.0; 50.0)	25.0 (0.0–100.0; 25.0)	25.0 (0.0–100.0; 25.0)	25.0 (0.0–100.0; 56.2)	<0.01**	<0.01**	<0.01**	<0.01**
Control	75.0 (50.0–100.0; 25.0)	50.0 (0.0–100.0; 75.0)	25.0 (0.0–100.0; 68.7)	25.0 (0.0–100.0; 50.0)	50.0 (0.0–100.0; 50.0)	<0.01**	<0.01**	<0.01**	<0.01**
p-values	0.232^{ns}	<0.01**	<0.01**	0.019*	0.351^{ns}				

GBI, gingival bleeding index; GI, gingival index; Min–Max; IQR, minimum–maximum; inter-quartile range; inter-quartile range; PI, plaque index; ns, not significant.
*$p < 0.05$.
**$p < 0.01$.

depth reductions were reported in aPDT + SRP *versus* SRP alone. In our study, SRP was given to both the two groups as it would be unethical to deny the conventional treatment to anyone. When compared with baseline data, PPD showed higher improvement in the test group than control group at recall visits of 3 months and 6 months. This points to the positive effect of aPDT in PPD reduction, which could be due to the usage of a narrow probe tip (0.5 mm diameter) that facilitates easy probing of deep periodontal pockets with top-hat distribution of radiation.

Effect of aPDT on secondary outcomes

Statistically significant reduction in GI was observed in the test group as compared to the control group after 2 weeks and 1 month ($p < 0.01$) and also after 3 months of aPDT ($p < 0.05$). Similar changes in GI were reported by de Oliveira et al. (2007) and Yilmaz et al. (2002) after aPDT as compared to SRP. However, reduction in gingival inflammation is well documented following SRP alone. PI also showed significant reduction after 2 weeks for the test group ($p < 0.05$). Another notable effect was the statistically significant reduction in GBI in the test group after 2 weeks and 1 month ($p < 0.01$) and the reduction seen after 3 months ($p < 0.05$). The reduction in GBI after aPDT correlated well with other studies although bleeding on probing (BOP) data was reported differently by various authors in entirely two disease entities, namely, aggressive and chronic periodontitis (de Oliveira et al. 2007, Braun et al. 2008, Ge et al. 2011). SRP and aPDT have also been demonstrated to have effects on crevicular proinflammatory cytokines tumour necrotic factor-α (TNF-α) and receptor activator of nuclear factor kappa-β ligand (RANKL) levels in patients with periodontitis (de Oliveira et al. 2009).

Effect of aPDT on halitosis - a tangible patient based outcome

Although organoleptic measurement is the most commonly used method for assessing halitosis, we have used hand on mouth method primarily

Table 6. Differences in halitosis score at baseline, 1 month, 3 months and 6 months of treatment

Parameter	Baseline	1 month	3 months	6 months	*p*-values		
	Median (Min–Max; IQR)	Median (Min–Max; IQR)	Median (Min–Max; IQR)	Median (Min–Max; IQR)	0–1 month	0–3 months	0–6 months
Halitosis							
Test	3.0 (2.0–4.0; 1.0)	3.0 (2.0–4.0; 1.0)	2.5 (2.0–4.0; 1.0)	3.0 (2.0–4.0; 1.0)	<0.01**	<0.01**	<0.01**
Control	4.0 (2.0–4.0; 1.0)	3.0 (2.0–4.0; 2.0)	2.0 (2.0–4.0; 1.0)	3.0 (2.0–4.0; 1.0)	<0.01**	<0.01**	<0.01**
p-Values	0.204^{ns}	0.015*	0.212^{ns}	0.624^{ns}			

Min–Max; IQR, minimum–maximum; inter-quartile range; ns, not significant.
*$p < 0.05$.
**$p < 0.01$.

because we wanted to evaluate the change in halitosis over time as perceived by the patient, which is a tangible effect. Further, in organoleptic method the examiner uses his nose to determine halitosis, which could involve the risk of spreading airborne infections from the patient. Following treatment, halitosis scores in Likert scale showed statistically significant change after 1 month in the test group ($p < 0.05$), but this did not last long. Probably, repeated application of aPDT could have resulted in improved control of halitosis.

Strengths and Limitations of the Study

A randomized and controlled study design was adopted in this trial to evaluate the effectiveness of the adjunctive use of aPDT to SRP in the management of chronic periodontitis patients. This has been done with adequate sample size to detect moderate differences between test and control groups before and after treatment. Statistically significant variations were observed between the two groups on analysis of covariance and comparisons between groups for imbalances by eliminating confounding variables. As our data did not obey the Gaussian Law, non-parametric ANCOVA or Rank Order ANCOVA was applied (Quade 1967) to determine the efficacy of aPDT along with SRP.

A limitation of this study was that the test and control groups differed significantly at the baseline in terms of PI ($p < 0.01$), which could possibly explain the lack of statistically significant improvement in PPD at 1 month. It is well-known that plaque is a major contributory factor for periodontitis. The mean PI of test group was 1.7 ± 0.6 and that of control group was 1.4 ± 0.5. Despite these limitations, the overall improvement of clinical parameters in the test group suggests that aPDT with SRP is indeed effective in the management of periodontitis. It may also be noted that pressure calibration was not made for assessing PPD.

Interpretations and implications

Few systematic reviews (Atieh 2010, Azarpazhooh et al. 2010, Sgolastra et al. 2013) and randomized controlled trials in patients with chronic and aggressive periodontitis and those on supportive periodontal therapy (Andersen et al. 2007, de Oliveira et al. 2007, Braun et al. 2008, Pinheiro et al. 2010, Ge et al.2011, Arweiler et al. 2013, Balata et al.2013, Campos et al. 2013) have been published regarding the efficacy of aPDT in the management of periodontitis. Nonetheless, this study is novel in its own ways and adds to the available evidence. The analysis used in this study (non-parametric ANCOVA) is a novel idea and is different from other studies as it takes into account the confounding factors in the interpretation of the results. It can be seen in this study that aPDT has a positive effect on patient care, mainly due to the considerably fast resolution of overt inflammation in the gingival tissues, which is supported by the significant reduction in GI, PI and GBI. It is noteworthy that reduction in BOP is the most consistent finding in almost all the clinical trials. A plausible explanation for improvement in GI and GBI in test group patients could be due to bacterial load reduction and inactivation of bacterial virulence factors and cytokines when the methylene blue is irradiated with laser (Braham et al. 2009).

Conclusions

Within the scope of this study, our results have shown that aPDT has an important role to play in improving clinical outcomes obtained through SRP. Single application of MB-mediated aPDT, evaluated over a period of 6 months was found to be effective in reducing gingival inflammation and probing pocket depth. In view of the encouraging results obtained, it would be worthwhile to repeat aPDT at frequent intervals to obtain a more definitive cure. The study could further be extended to cover generalized chronic periodontitis and aggressive periodontitis patients.

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References

Adriaens, P. A. & Adriaens, L. M. (2004) Effects of nonsurgical periodontal therapy on hard and soft tissues. *Periodontology 2000* **36**, 121–145.

Ainamo, J. & Bay, I. (1975) Problems and proposals for recording gingivitis and plaque. *International Dental Journal* **25**, 229–235.

Alwaeli, H. A., Al-Khateeb, S. N. & Al-Sadi, A. (2013) Long-term clinical effect of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. *Lasers in Medical Science*. doi: 10.1007/s10103-1426-y.

Andersen, R., Loebel, N., Hammond, D. & Wilson, M. (2007) Treatment of periodontal disease by photo disinfection compared to scaling

and root planing. *The Journal of Clinical Dentistry* **18**, 34–38.

Arweiler, N. B., Pietruska, M., Skurska, A., Dolińska, E., Auschill, T. M. & Sculean, A. (2013) Nonsurgical treatment of aggressive periodontitis with photodynamic therapy or systemic antibiotics. *Schweizer Monatsschrift für Zahnmedizin* **123**, 532–544.

Atieh, M. A. (2010) Photodynamic therapy as an adjunctive treatment for chronic periodontitis: meta-analysis. *Lasers in Medical Science* **25**, 605–613.

Azarpazhooh, A., Prakesh, S., Tenenbaum, H. & Goldberg, M. (2010) The effect of photodynamic therapy for periodontitis: a systematic review and meta-analysis. *Journal of Periodontology* **81**, 4–14.

Balata, M. L., de Andrade, L. P., Santos, D. B., Cavalcanti, A. N., Ribeiro, E. D. & Bittencourt, S. (2013) Photodynamic therapy associated with full-mouth ultrasonic debridement in the treatment of severe chronic periodontitis: randomized-controlled clinical trial. *Journal of Applied Oral Science* **21**, 208–214.

Braham, P., Herron, C., Street, C. & Darveau, R. (2009) Antimicrobial photodynamic therapy may promote periodontal healing through multiple mechanisms. *Journal of Periodontology* **80**, 1790–1798.

Berakdar, M., Callaway, A., Eddin, M. F., Ross, A. & Willershausen, B. (2012) Comparison between scaling-root-planing (SRP) and SRP/photodynamic therapy: six-month study. *Head & Face Medicine* **8**, 12.

Braun, A., Dehn, C., Krause, F. & Jepsen, S. (2008) Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. *Journal of Clinical Periodontology* **35**, 877–884.

Campanile, V. S. M., Giannopoulou, C., Campanile, G., José, A. & Mombelli, A. (2013) A Single or repeated antimicrobial photodynamic therapy as adjunct to ultrasonic debridement in residual periodontal pockets: clinical, microbiological, and local biological effects. *Lasers in Medical Science*. doi: 10.1007/s10103-013-1337-y.

Campos, G. N., Pimentel, S. P., Ribeiro, F. V., Casarin, R. C. V., Fabiano, C. R. & Márcio, C. Z. (2013) The adjunctive effect of photodynamic therapy for residual pockets in single-rooted teeth: a randomized controlled clinical trial. *Lasers in Medical Science* **28**, 317–324.

Christodoulides, N., Nikolidakis, D. & Chondros, P. (2008) Photodynamic therapy as an adjunct to non-surgical periodontal treatment: a randomized, controlled clinical trial. *Journal of Periodontology* **79**, 1638–1644.

Fernando, O. C., Alessandra, N. G., Luís, O. M. C., André, L. P., Takeshi, K. S., Sheila, C. C. & José, E. C. (2009) Impact of different periodontitis case definitions on periodontal research. *Journal of Oral Science* **51**, 199–206.

Ge, L., Shu, R., Li, Y., Li, C., Luo, L., Song, Z., Xie, Y. & Liu, D. (2011) Adjunctive effect of photodynamic therapy to scaling and root planing in the treatment of chronic periodontitis. *Photomedicine and Laser Surgery* **29**, 33–37.

Herrera, D., Sanz, M., Jepsen, S., Needleman, I. & Roldan, S. (2002) A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. *Journal of Clinical Periodontology* **29** (Suppl. 3), 136–159.

Katie, O., Akilov, O. E. & Hasan, T. (2005) The potential for photodynamic therapy in the treatment of localized infections. *Photodiagnosis and Photodynamic Therapy* **2**, 247–262.

Komerik, N., Nakanishi, H., MacRobert, A. J., Henderson, B., Speight, P. & Wilson, M. (2003) In vivo killing of Porphyromonas gingivalis by toluidine blue-mediated photosensitization in an animal model. *Antimicrobial Agents and Chemotherapy* **47**, 932–940.

Lawson, A. (1983) Rank analysis of covariance: alternative approaches. *Journal of the Royal Statistical Society. Series D (The Statistician)* **32**, 331–337.

Löe, H. (1967) The gingival index, the plaque index and the retention index systems. *Journal of Periodontology* **38**, 610–616. (Supplement).

Malik, Z., Hanania, J. & Nitzan, Y. (1990) Bactericidal effects of photoactivated porphyrins – an alternative approach to antimicrobial drugs. *Journal of Photochemistry and Photobiology. B, Biology* **5**, 281–293.

Noro Filho, G. A., Casarin, R. C., Casati, M. Z. & Giovani, E. M. (2012) PDT in non-surgical treatment of periodontitis in HIV patients: a split-mouth, randomized clinical trial. *Lasers in Surgery and Medicine* **44**, 296–302.

Novaes, A. B. Jr, Schwartz-Filho, H. O., de Oliveira, R. R., Feres, M., Sato, S. & Figueiredo, L. C. (2012) Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: microbiological profile. *Lasers in Medical Science* **27**, 389–395.

de Oliveira, R. R., Schwartz-Filho, H. & Novaes, A. Jr (2009) Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: cytokine profile in gingival crevicular fluid, preliminary results. *Journal of Periodontology* **80**, 98–105.

de Oliveira, R. R., Schwartz-Filho, H. O., Novaes, A. B. Jr & Taba, M. Jr (2007) Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: a preliminary randomized controlled clinical study. *Journal of Periodontology* **78**, 965–973.

Pfitzner, A., Sigush, B. W., Albrecht, V. & Glockmann, E. (2004) Killing of periodonto-pathogenic bacteria by photodynamic therapy. *Journal of Periodontology* **75**, 1343–1349.

Pinheiro, S. L., Donegá, J. M., Seabra, L. M. S., Adabo, M. D., Lopes, T., do Carmo, T. H. D., Riberio, M. C. & Bertolini, P. F. R. (2010) Capacity of photodynamic therapy for microbial reduction in periodontal pockets. *Lasers in Medical Science* **25**, 87–91.

Puri, M. L. & Sen, P. K. (1969) Analysis of covariance based on general rank scores. *Annals of Mathematical Statistics* **40**, 610–618.

Quade, D. (1967) Rank analysis of covariance. *Journal of the American Statistical Association* **62**, 1187–1200.

Schneider, M., Kirfel, G., Berthold, M., Frentzen, M., Krause, F. & Braun, A. (2012) The impact of antimicrobial photodynamic therapy in an artificial biofilm model. *Lasers in Medical Science* **27**, 615–620.

Sgolastra, F., Petrucci, A., Gatto, R., Marzo, G. & Monaco, A. (2013) Photodynamic therapy in the treatment of chronic periodontitis: a systematic review and meta-analysis. *Lasers in Medical Science* **28**, 669–682.

Silness, J. & Loe, H. (1964) Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **22**, 112–135.

Song, H. H., Lee, J. K., Um, H. S., Chang, B. S., Lee, S. Y. & Lee, M. K. (2013) Phototoxic effect of blue light on the planktonic and biofilm state of anaerobic periodontal pathogens. *Journal of Periodontal & Implant Science* **43**, 72–78.

Umeda, M., Takeuchi, Y., Noguchi, K., Huang, Y., Koshy, G. & Ishikawa, I. (2004) Effects of nonsurgical periodontal therapy on the microbiota. *Periodontology 2000* **36**, 98–120.

Wilson, M. (2004) Lethal photosensitisation of oral bacteria and its potential application in the photodynamic therapy of oral infections. *Photochemical and Photobiological Sciences* **3**, 412–418.

Yilmaz, S., Kuru, B., Kuru, L., Noyan, U., Argun, D. & Kadir, T. (2002) Effect of gallium arsenide diode laser on human periodontal disease: a microbiological and clinical study. *Lasers in Surgery and Medicine* **30**, 60–66.

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Clinical Relevance

Scientific rationale for the study: The effect of aPDT in the management of chronic periodontitis has not been reported conclusively. This study explores the use of aPDT as an adjunct to conventional SRP in treating chronic periodontitis patients.

Principal findings: This study shows that aPDT can cause a statistically significant reduction in GI and GBI after 2 weeks and 1 month of treatment ($p < 0.01$) and also significant improvement in PPD and CAL 3 and 6 months after aPDT ($p < 0.05$).

Practical implications: Results of SRP can be enhanced by aPDT, thereby reducing gingival inflammation and bleeding on probing following treatment. The results of the study has shown that clinically significant reduction in gingival inflammation and bleeding on probing can be achieved by application of aPDT as an adjunct to SRP in routine clinical practice.