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Effect of photodynamic therapy adjunct to scaling and root planing in periodontitis patients: A randomized clinical trial

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ABSTRACT

Background: A randomized split-mouth controlled clinical trial was conducted to evaluate the efficacy of photodynamic therapy (PDT) in reducing *Aggregatibacter actinomycetemcomitans* (Aa) in periodontitis patients.
Methods: Twenty patients with periodontitis were recruited for the trial. Following random allocation of either quadrants of the selected jaw to test or control treatment, conventional non-surgical periodontal therapy (NSPT) was performed. In addition, the test side received adjunct photodynamic therapy. Probing depth (PD), clinical attachment level, bleeding on probing (BoP) and plaque scores (PS%) were recorded at phase 0 (baseline), phase 1 (immediately after NSPT), phase 2 (7 days following NSPT), phase 3 (1 month following NSPT) and phase 4 (3 months following NSPT). Subgingival plaque samples for quantification of Aa by real-time polymerase chain reaction was performed at phases 0, 1, 2 and 4.
Results: There was a significant clinical improvement at phases 3 and 4 compared with baseline while BoP reduced significantly only in the test group at phase 4. However, no difference in the quantification of Aa was detected between the groups.
Conclusions: Within the limits of the study, PDT adjunct to scaling and root planing does not lead to quantitative reduction of Aa in periodontitis patients.

Keywords: *Aggregatibacter actinomycetemcomitans*, antimicrobial, non-surgical periodontal therapy, periodontitis, photodynamic therapy.

Abbreviations and acronyms: Aa = *Aggregatibacter actinomycetemcomitans*; BoP = bleeding on probing; CAL = clinical attachment level; NMMR = National Medical Research Register; NSPT = non-surgical periodontal therapy; PD = probing depth; PDT = photodynamic therapy; ROS = reactive oxygen species; SD = standard deviation; SRP = scaling and root planing.

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INTRODUCTION

Periodontitis is a chronic infection caused by pathogenic biofilm but modified and mediated by the host defence system.[1,2] Removal of the biofilm and rendering the tooth surface less amenable to its further accumulation has been the main focus towards successful periodontal management of gingivitis and periodontitis.[3,4] Though changes towards reduction of probing depth and clinical attachment loss can be attributed as a result of any non-surgical periodontal therapy (NSPT), there is a lack in sensitivity in these outcome measures unless observed over long periods of time by sequential measurements.[5–7] Quantification of putative periodontal pathogens and host inflammatory mediators has been popular to compare the effects of non-surgical therapy.[8–10]

Among NSPTs, scaling and root planing (SRP) (debridement) has been considered as the gold standard in treating periodontal diseases.[3] It has stood the test of time against challenges in various other forms of non-surgical therapies. However, even though there is a transient reduction in the quantity of periodontal pathogens, mechanical therapy alone is not successful in eliminating or preventing recolonization of the bacterial pathogens.[11] Any new or improved form of NSPT has been traditionally evaluated against SRP.[3] Antimicrobial photodynamic therapy (PDT) has been popular as an antimicrobial adjunct to SRP since the late 1990s.[9] Its action is based on the generation of reactive oxygen species (ROS) causing contact toxicity and death to bacteria.[12] Low level lasers excite certain dyes like methylene blue, toluidine blue O and malachite green to a higher energy level in the presence of oxygen to produce ROS.[9] The small radius of the effect of the cytotoxic chemically reactive molecules which are bactericidal yet safe for the host tissues makes PDT an

exciting non-invasive antimicrobial therapy with little probability of developing resistance.[13,14] Animal studies have demonstrated absence of any toxicity for the surrounding host tissues including the healing connective tissues.[15]

Several studies have shown that PDT as an adjunct to SRP was more effective in improving clinical parameters in the treatment of periodontal disease compared with SRP alone.[16–18] However, no benefits of using PDT with SRP was reported by some other studies.[19–21] The difference in conclusion of the studies could be due to a lack of consistency in the treatment protocols, different photosensitizing agents, different time of laser exposure, and variations in the severity of disease or pocket depth. Our study hypothesized that the inconsistency of results could be explained by evaluating quantitatively the reduction of key periodontal pathogens. *Aggregatibacter actinomycetemcomitans* (Aa) is considered as an important pathogen found in pockets of patients with different forms of periodontitis.[22] Hence, it was the aim of this study to compare the reduction in Aa in PDT administrated sites to the control sites in the treatment of periodontitis by using a split-mouth study design.

METHODS

Study design

A split-mouth (parallel group in same person) randomized control trial was conducted in the Oral Health Centre of International Medical University to evaluate the clinical and microbial efficacy of photodynamic therapy as an adjunct to SRP among persons with periodontitis. The trial was funded and sponsored by International Medical University (project ID: IA334; Research and Ethics code: IMU 261/2012). The photosensitive dye used in the trial was gifted by CMS Dental (Malaysia). The trial was prospectively registered in the National Medical Research Register (NMMR ID: NMRR-12-1257-14411).

Sample size calculation

The sample size was calculated to detect a difference of at least 1 mm (δ) between the two groups for mean clinical attachment level (CAL) at 80% power, 95% confidence interval ($\alpha = 0.05$) and a standard deviation (SD; σ) of 1 mm. Though the calculated sample size was 16, it was boosted to 20 to account for any loss to follow up during the course of the study.

Selection of study subjects

Patients attending the University's oral health centre and diagnosed with either chronic or aggressive periodontitis were invited to take part in the trial. The other inclusion criteria were presence of a jaw with bilateral presence of periodontal pockets measuring at least 5 mm in three or more sites in different teeth of the posterior sextant, a minimum of 20 teeth, age between 18 and 60 years and good general health. Patients were excluded if they were smokers, had used antibiotics in the last 6 months or would require the use of antibiotics for periodontitis management, had received treatment for periodontitis in the last 6 months, were pregnant or lactating, or were allergic to any of the materials used for the treatment of their periodontal condition as part of the trial.

Clinical procedure and measurement

The procedure conducted is described in phases (0–4) for ease in comprehension. The selected patients were subjected to the routine oral hygiene instruction program by author SJP. One jaw, either maxillary or mandibular, having bilateral presence of periodontal pockets measuring at least 5 mm in three or more sites in different teeth of the posterior sextant was selected in the patient for study. In the event of both the maxillary and mandibular jaw being eligible for selection in a patient, a coin toss was performed for the final selection. One posterior sextant was randomly selected as the test and the other as control by author SJP. Three sites with a probing depth of 5 mm or more in each posterior sextant of the selected jaw were randomly selected at baseline for repeated microbial sampling at different phases of the study.

The time points in the study was divided into 5 phases:

Phase 0: Selection of subjects followed by SRP.
Phase 1: Immediately after NSPT wherein the test sites underwent PDT in addition to SRP.
Phase 2: Seven days following NSPT.
Phase 3: One month following NSPT.
Phase 4: Three months following NSPT.

Periodontal clinical parameters which included 6-point probing depth (mm), CAL (mm) and presence of bleeding on probing (%) measured by North Carolina periodontal probe, Hu-Friedy® (Chicago, IL, USA), and presence of plaque detected by disclosing solution as plaque score (%) were recorded for the selected quadrants and reported as mean millimetre values and percentage respectively at phases 0, 2, 3 and 4.[23–25]

PDT procedure

Following whole mouth SRP, the test sextant received a single episode of adjunctive PDT by author SJP. The photosensitive dye, methylene blue, of high viscosity was applied using a blunt tip and a disposable syringe

to the bottom of the periodontal pockets in a coronal direction. After 1 min, the pocket was rinsed with sterile water to remove the excess dye. A LED lamp emitting light in the red spectrum at a frequency of 628 Hz (Fotosan® CMS Dental, Copenhagen, Denmark) was used as the activating laser light. The manufacturer provides two tips, blunt short tip for use on the gingiva outside the pocket and a periodontal tip to be used in the pocket. Following washing the dye with sterile water, 10 s of light was exposed with the blunt tip followed by 10 s of the periodontal tip.

Microbial sampling and analysis

Quantification of Aa in the treated periodontal pocket was undertaken using real-time polymerase chain reaction (rtPCR). Subgingival plaque sampling was done by pooling from the selected pockets at phases 0, 1, 2 and 4. The periodontal pockets for sampling were selected randomly at phase 0 and the selected sites were sampled for the subsequent phases of the study. Following removal of the supragingival plaque, sterile paper points #30 were inserted to the depth of the pocket. After remaining for 30 s, all the points were pool transferred to a sterile microtube to be stored at –20°C. DNA was isolated from the sample and processed as per QIAamp® DNA Tissue kit handbook.[26] Quantification of Aa from the extracted DNA was performed using an rtPCR kit supplied by Genesiq (PrimerDesign, Chandler's Ford, UK) according to manufacturer's protocol. In brief, the cycling conditions were 95.0°C for 15 min, followed by 95.0°C for 15 s and 60 °C for 1 min. The primers and probe sequences in the kit have 100% homology to haemoglobin binding gene which was reported as a highly specific marker for Aa.[27]

Primary and secondary outcomes

The primary outcome variables were bleeding on probing (BoP), probing depth (PD) and CAL. Quantification of Aa was considered as the secondary outcome measure.

Statistical analysis

Mean and SD for the selected outcome measures were calculated. CAL, PD and BoP variable; ANOVA and Newman–Keuls test verified any differences between groups and changes over time. Bacterial log counts were compared by repeated measures of ANOVA and Newman–Keuls test. All statistical tests were considered significant at $P < 0.05$.

RESULTS

Twenty patients were recruited from April 2013 to March 2014 for the present trial with the follow up extending to December 2014. No patients reported any discomfort or complications related to the trial. The mean age of the participants in this split-mouth trial was 45.2 ± 6.7 years. Women comprised 35% of the sample. Four patients (one female, three males) withdrew from the trial at various stages for reasons related to logistics and time constraints and were excluded from analysis. Test group (N = 16) was formed by the sextant which had the additional PDT while the contralateral side formed the control group (N = 16). Mean values of all the clinical parameters compared similarly at baseline for both the groups. Periodontal treatment resulted in significant improvement in the clinical parameters over baseline at phase 3 (1 month) and phase 4 (3 months) for both groups (Table 1). However, except for BoP at phase 4, no significant difference could be found at different phases of the study when the clinical parameters were compared for the groups. Quantitative values of Aa was converted to logarithmic values for analysis. Though all samples yielded Aa, no difference in the bacterial quantity was detected for both intragroup and intergroup analysis at phases 0, 1, 2 and 4.

DISCUSSION

This split-mouth randomized controlled trial evaluated the efficacy of a single dose application of PDT as an adjunct to scaling and root planing in patients with periodontitis through clinical and microbiological analyses. The results showed that no greater benefit in improved treatment outcomes was achieved by the additional use of PDT to the conventional management of periodontitis.

The non-specific plaque hypothesis postulates that the occurrence of periodontitis can be explained by the presence of plaque on the tooth surfaces.[28] Increased accumulation by volume and time leads to greater severity in disease. However, a more recent specific hypothesis on plaque gave importance to pathogenic plaque/bacteria over other non-pathogenic bacterial florae in causing disease.[28] Aa has been regarded as an important pathogen in periodontitis.[29] Though not proven to be a truly causative species, the presence of Aa heralds active disease while difficulty in isolation from periodontal pockets signifies remission including chronic and aggressive periodontitis.[30,31] Many studies adopted Aa as a reliable marker for predicting treatment outcomes in addition to clinical parameters.[32–34] PDT is regarded as a broad antibacterial therapy and has shown efficacy against Aa when studied *in vitro*.[35–37]

All patients in our study were identified with presence of periodontitis. We precluded classifying them as chronic and aggressive periodontitis based on the recent classification of periodontal diseases.[38] The

Table 1. Mean clinical and microbiological (Aa) parameters of periodontitis patients before and following use of PDT as an adjunct to scaling and root planing

		Baseline	Phase 1 (immediate post-NSPT)	Phase 2 (7 days)	Phase 3 (1 month)	Phase 4 (3 months)
CAL (mm)	Test (N = 16)	5.46 ± 0.63	–	–	5.01 ± 0.58	4.48 ± 1.11†
	Control (N = 16)	5.76 ± 1.02	–	–	5.21 ± 1.14	4.63 ± 1.23†
PD (mm)	Test (N = 16)	4.66 ± 0.39	–	–	4.61 ± 0.65	3.56 ± 0.69†
	Control (N = 16)	4.95 ± 0.95	–	–	4.56 ± 0.65	3.93 ± 0.23†
BoP (%)	Test (N = 16)	56.84 ± 26.76	–	–	25.64 ± 20.55†	12.44 ± 20.45†*
	Control (N = 16)	52.11 ± 21.95	–	–	26.54 ± 22.35†	19.56 ± 23.35†*
PS (%)	Test (N = 16)	18.36 ± 16.35	–	–	13.65 ± 20.32	12.78 ± 19.35
	Control (N = 16)	17.32 ± 19.38	–	–	12.32 ± 19.65	11.23 ± 18.25
Aa load (log values)	Test (N = 16)	4.99 ± 1.37	4.07 ± 1.28	4.60 ± 0.89	–	4.30 ± 0.86
	Control (N = 16)	5.27 ± 1.08	4.28 ± 1.33	4.57 ± 1.01	–	4.88 ± 0.96

*Significant intergroup difference 5% (Newman–Keuls test).
†Significant intragroup difference related to baseline (Newman–Keuls test).

clinical distinction between the two entities is not clear cut and no difference in treatment protocol would be necessitated.[39,40]

Though Aa is always associated with aggressive periodontitis, research shows that presence of any particular pathogen is a poor distinguisher between the two forms of periodontitis.[41] This has also led to the conduct of trials on patients diagnosed with periodontitis *per se* compared with any type of periodontitis.[33,42] We observed improvement in all clinical parameters during the course of follow up in both test and control groups. BoP reduced to less than 20% while PS reduced to less than 13% at 3 months in both the groups. This is in accordance with good plaque control as recommended to be observed in clinical settings for predicable healing.[43] Similar reductions in BoP and PS were observed by Kolbe *et al.* following adjunctive use of PDT in patients with chronic periodontitis.[24] However, no difference in the improvements were identified between test and controls except for BoP (Table 1). The BoP reduced similarly at 1 month (phase 3) following respective therapy by at least 50% for both groups. However, the test group had a significantly greater reduction at 3 months (phase 4). A similar trend where only BoP showed improvement was demonstrated in the research by Chondros *et al.* and Cappuyns *et al.* following a single application of PDT along with ultrasonic/hand debridement.[44,45] This differential improvement of BoP could be due to the positive effect of PDT on the host immune or inflammatory pathways and needs further exploration.[46] This could also potentially lead to discovery of other non-bacterial mechanisms of action by PDT with improved treatment outcomes in affected persons. Mongardini *et al.* showed improved pocket reduction in a week whereas another study by Müller Campanile *et al.* showed improvement of probing depth only after 3 months due to PDT.[18,47] However, other clinical trials failed to realize an increased benefit by the use of PDT in terms of clinical improvement of attachment levels.[9,19] The difference in results may be attributed to the frequency of application of PDT. Though a consensus has not been achieved, Lulic *et al.* showed superiority of results by increasing the frequency of PDT regimens.[16] Müller Campanile *et al.*, however, did not find any significant difference in clinical improvement with either a single or double application of PDT within a week.[18]

Aa reduction

All subjects had presence of Aa in the sampled sites at all the phases of the study. Aa was found to be detectable 7 days following SRP in a trial on Chinese subjects while we found presence immediately after SRP (phase 0).[48] Another study in the UK also found presence of Aa following NSPT in periodontal sites of patients treated for periodontitis.[49] No reduction of Aa was observed in our study at 7 days and at 3 months following respective treatments over baseline. All patients had detectable presence of Aa but this finding did not translate to quantitative changes among different treatment time points. Carvalho *et al.* also found Aa in the periodontal pockets of all his subjects, though no quantitative difference was observed relative to the use of PDT.[9] Moreira *et al.* revealed significant reduction of Aa in the intragroup comparison of the group treated with PDT.[50] However, the magnitude of reduction was similar in test and control groups. In our study, no significant reduction of Aa occurred over the follow up in either of the groups.

Various *in vitro* studies have shown significant killing ability of PDT for various periodontal pathogens and also the ability to inactivate bacterial proteases.[50–52] The inconsistency of our study with the *in vitro* results could primarily be attributed to the interference of the host and its environment in which the bacterial pathogens coexist. Aa in a live active

environment may be protected by many aspects of the host defence as well as the biofilm it is part of.[53]

The severity of the clinical condition, the varied host response, photosensitizer concentration and the retention time allowed, the pH variation and fluid flow in the local environment are a few of the factors that could influence the results apart from the varying protocols of PDT.[54] The positive effects of PDT on the host tissues and its response to healing and inflammation have not been studied. The patients recruited were not classified based on the current accepted classification of periodontitis, namely, chronic and aggressive. However, as this was a split-mouth study, the different forms of periodontitis had its own control in the same patient. Thus, this may not affect the conclusion of our trial but needs further investigation. Thus, standardizing an effective protocol of PDT and research on the immune/inflammatory mechanisms affected by the therapy could be future areas of research. Selection of only three sites for microbiological sampling can also be a limitation as the depth or location of the pockets might not have been similar in test and controls even though a 5-mm threshold depth in the selection of sites was maintained. Aa was not measured in phase 3 which could have precluded the identification of any decrease followed by regrowth or recolonization in phase 4. Within the limits of our study, we did not find any additional benefits to recommend the use of PDT as an adjunct to conventional scaling and root planing as a non-surgical therapy for periodontitis.

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