Short-term effects of adjunctive antimicrobial photodynamic therapy in obese patients with chronic periodontitis: A randomized controlled clinical trial

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ARTICLE INFO

Keywords:
Antimicrobial photodynamic therapy
Dental scaling
Chronic periodontitis
Obesity

ABSTRACT

Background: The aim of the present study was to assess the effect of antimicrobial photodynamic therapy (aPDT) as an adjunct to scaling and root planing (SRP) on clinical periodontal and immunological parameters in obese patients with chronic periodontitis (CP).

Methods: Fifty-three obese with CP patients were divided into 2 groups receiving aPDT with SRP and SRP only respectively. Full-mouth plaque index (PI), bleeding on probing (BOP), pocket depth (PD) and clinical attachment level (CAL) were assessed at baseline, 6 and 12 weeks post-therapy. Gingival crevicular fluid (GCF) levels of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 were evaluated using enzyme linked immunosorbent assay at baseline and 12 weeks.

Results: There was a significant improvement in all periodontal variables in both study groups at 6 weeks and 12 weeks with respect to the baseline visit (p < 0.001). Significant reduction in PD of 4–6 mm and ≥7 mm was observed for aPDT group as compared to SRP group (p < 0.01) at both 6 weeks and 12 weeks of follow-up. At 6 weeks, a significant (p < 0.001) gain in CAL was observed in both groups, which remained stable at 12 weeks. IL-6 and TNF-α levels decreased significantly (p = 0.001) at 12 weeks after therapy in both the groups. Inter-group comparison showed significant difference for TNF-α (p = 0.024) and IL-6 (p = 0.044) levels for aPDT group at 12 week follow-up.

Conclusion: Within the limits of this clinical trial, adjunctive aPDT showed improvement in clinical and immunological parameters in obese patients with CP. Antimicrobial PDT showed additional benefit in moderate and deep periodontal pockets in obese patients with CP.

1. Introduction

Concern is growing about the rising prevalence of overweight and obesity in adults in Saudi Arabia. An estimated prevalence of 36% of adults suffer from obesity and it is projected that 41% and escalating rise of 78% by 2022 in men and women respectively [1]. Obesity is culpable to many systemic health problems such as joint diseases, endocrine and metabolic disturbances and cancers [2]. Furthermore, recent systematic review and meta-analyses have evaluated the association between obesity and chronic periodontitis (CP) [3,4] (Fig. 1).

Chronic periodontitis is the bacterial infection of the tooth supporting tissues, if untreated, may lead to tooth loss [5]. The periodontopathogenic bacteria such as Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia categorized as ‘red complex’ bacteria are significantly associated with periodontal tissue destruction [6,7]. The likely causal relationship between obesity and chronic periodontal destruction and their potential underlying biological mechanisms remain debatable, however, it has been reported that the adipose tissues in obesity express a variety of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 that are involved in inflammatory processes of both conditions [8–10]. Moreover, in a recent study by Maciel et al. [11] indicated that deep periodontal pockets in obesity have a high prevalence of periodontal pathogens. This suggests that similar inflammatory pathways are involved in the pathophysiology of obesity and CP and that certain periodontopathogenic bacteria drives these immune inflammatory response in obese patients.
Scaling and root planing (SRP) is the most commonly performed procedure for the treatment of CP [12,13]. It has been suggested that outcomes of periodontal therapy are compromised in obese patients compared with non-obese individuals; however, only a limited number of studies have evaluated this relationship with disputing results [14]. Furthermore, it has also been reported that obesity may modulate systemic and periodontal levels of proinflammatory cytokines regardless of whether SRP is performed or not [15].

Antimicrobial photodynamic therapy (aPDT) is being widely used in dentistry especially in Periodontics [16–18]. Antimicrobial PDT involves administration of nontoxic dye inside periodontal pockets, followed by illumination of visible light, which, in the presence of oxygen leads to the formation of reactive oxygen species that causes bacterial cell death [19]. The present study was based on the hypothesis that adjunctive aPDT as compared to SRP alone, improve clinical periodontal parameters, and reduce gingival crevicular fluid (GCF) TNF-α and IL-6 levels in obese patients with CP. The null hypothesis was aPDT and SRP groups will show comparable clinical and immunological outcomes. Therefore, the aim of the present study was to assess the effect of aPDT as an adjunct to SRP on clinical and immunological parameters in obese patients with CP and compare these findings with SRP alone.

2. Materials and methods

2.1. Ethical guidelines

The study was performed in accordance with the Declaration of Helsinki. This study was a 12-week parallel arm, examiner-masked, randomized controlled trial (RCT) designed, conducted and reported following the Consolidation Standards of Reporting Trials (CONSORT) Statement [20]. An information sheet (written in Arabic and English) that described the purpose and methods used was given to all the study participants. Volunteering individuals were requested to read and sign a consent form.

2.2. Recruitment of study participants

Between March 2016 and February 2017, a 12-week parallel arm RCT was performed at private dental referral clinic. Obese patients with CP were randomly divided into two groups. Test group: receiving SRP and aPDT; and control group: receiving SRP only. Randomization was done by tossing a coin. Sealed non-transparent envelopes were used for allocation concealment and opened just before the interventions.

2.3. Inclusion and exclusion criteria

The following inclusion criteria were imposed: (a) obese patients with a body mass index (BMI) of ≥ 27.5 kg/m² [21] and; (b) CP (defined as bleeding on probing [BOP], probing depth [PD] ≥ 4 mm, and marginal bone loss [MBL] ≥ 3 mm in more than 30% of the sites) [22]. Self-reported tobacco smokers, individuals using smokeless tobacco products [23], habitual alcohol users and patients with systemic diseases such as acquired immune deficiency syndrome/HIV, diabetes mellitus, renal disorders and cardiovascular disorders were excluded.
2.4. Periodontal therapy

In aPDT and SRP groups, participants underwent full mouth SRP using ultrasonic scaler and hand instruments (Hu-Friedy, Chicago, IL) under local anesthesia. The end point for SRP was attained until a smooth scaled root surface was achieved. In both groups, the participants were recommended to brush their teeth using the modified Bass technique and were also encouraged to floss the teeth at least once daily. In all individuals, SRP was performed by a trained dentist (ZA). Pre- or postoperative antibiotics were not prescribed in any group.

2.5. Photodynamic therapy

In aPDT + SRP group, aPDT was performed after SRP. The protocol for aPDT is described elsewhere [24]. Briefly, using a blunt needle, 0.005% of Methylene blue was applied into the periodontal pocket and left in place for 10 s. The dye was then irradiated with a diode laser of 670 nanometers at 150 milliwatts with optic fibre diameter 0.06 mm. In each dye filled periodontal pocket, laser irradiation was performed for one minute using a flexible tip. In the present study, aPDT was performed once, at baseline, by a trained dentist.

2.6. Clinical periodontal examination

Periodontal examinations were performed at baseline, 6 weeks and 12 weeks follow-up by a trained and calibrated examiner (FV). The overall kappa value for intra-examiner reliability was 0.91. Full-mouth PI, BOP, PD and CAL were measured at six sites (mesio-buccal, mid-buccal, disto-buccal, disto-lingual/palatal, mid-lingual/palatal, and mesio-lingual/palatal) on all maxillary and mandibular teeth (excluding third molars) [25]. PD was measured to the nearest millimeter with UNC-15 periodontal probe (Hu-Friedy, Chicago, IL). In all groups, number of missing teeth (MT), excluding third molars were also recorded.

2.7. Gingival crevicular fluid sampling

In both groups, GCF was sampled 1 week after clinical examination from four deepest and non-adjacent periodontal pockets. The study sites were dried gently using air syringe and isolated with cotton rolls. Filter paper strips (PerioPaper, Oralflow, Smithtown, NY, USA) were placed inside the periodontal pockets and left for 30 s. Strips contaminated with blood were discarded. Four strips were collected from all study individuals, pooled in dried microcentrifuge tubes and stored at −70 °C until analysis. All sampling procedures were performed at baseline and 12 weeks of follow-up.

2.8. Measurement of tumor necrosis factor-alpha and interleukin-6 in GCF

Gingival crevicular fluid samples for TNF-α (TNF-α Human ELISA kit, Abcam, UK) and IL-6 (Human interleukin-6, ELISA Kit, Abcam, UK) were assessed using enzyme linked immunosorbent assay (ELISA). In summary, standard curve was used to determine the concentration of TNF-α and IL-6 in the samples. Fifty microliters (μL) of the respective GCF samples was dispensed, in duplicate, into the wells coated with a specific antibody. The plates were then incubated at room temperature for 120 min following which they were washed three times with wash buffer manually. Fifty μL of biotinylated antibody was then added into the wells and allowed to incubate for 120 min. Conjugate solution (50 μL) was then added, and the plates were incubated at room temperature for another 30 min. The wells were washed once again three times with a wash solution following which 50 μL of chromogen substrate solution was added. The plates were incubated for 12 min at room temperature till the optimal blue color density was achieved. Following this, 50 μL of stop solution was added to terminate color development. Absorbance was determined by reading the plate at wavelength 450 nm on a microplate reader (VersaMax ELISA Microplate Reader, Männedorf, Switzerland). The concentration of TNF-α and IL-6 were expressed in pg/mL respectively.

2.9. Statistical analysis

Statistical analyses were performed using a software program. Data were expressed as means and standard deviations with mean percentages. Normality of distribution of the variables was tested with Shapiro-Wilk tests. Baseline and post-treatment comparisons between test (aPDT) and control (SRP) groups were accomplished by a t test or the Mann-Whitney U test for parametric and non-parametric data, respectively. For intragroup comparisons between baseline and post-treatment periods, the analyses were performed by means of a paired t test (parametric data) or Wilcoxon signed ranks test (non-parametric data). For multiple comparisons, Bonferroni post hoc adjustment test was performed. Power analysis was based on the supposition that a mean difference of 0.5 mm in PD and a standard deviation of 0.5 mm should be detected at a significance level of 0.05 and a desired study power of at least 80%. Power analysis was performed with a computer software (nQuery Advisor 5.0-Statistical Solutions, Saugus, Massachusetts). It was estimated that a sample size of at least 24 individuals per group were required.

3. Results

3.1. Characteristics of the study groups

Out of 122 screened patients, 52 patients agreed to participate in the study. In test group, 23 obese patients with CP received SRP and aPDT while in control group, 29 obese patients with CP received SRP only. In aPDT and SRP groups, the mean age of obese patients was 51.84 and 48.68 years respectively. In aPDT and SRP groups, the mean BMI of obese patients was 34.8 kg/m² and 31.4 kg/m² respectively. Mean fasting blood glucose levels were 107.2 mg/dl and 119.4 mg/dl in aPDT and SRP groups respectively (Table 1). None of the patients recruited in the study reported adverse outcomes with either of the two therapies used.

3.2. Clinical periodontal parameters

There was a significant improvement in all periodontal variables in both study groups at 6 weeks and 12 weeks after periodontal treatment with respect to the initial visit (Table 2). At 6 weeks, the PI had significantly reduced (p < 0.001) from 88 ± 18.5% to 15 ± 12% in aPDT group and 83 ± 16% to 16 ± 7% in SRP groups and the BOP had significantly (p < 0.001) reduced from 85 ± 20% to 17 ± 12% in aPDT group and 83 ± 18% to 13 ± 6% in SRP group. At 12 weeks, both indexes remained below 15% in both groups.

At 6 weeks, the percentage of sites with PD ≤ 3 mm had significantly increased (p < 0.001) from 26 ± 21% to 83 ± 16% in aPDT group and from 23 ± 19% to 81 ± 7% in SRP group and the percentage of sites with PD of 4–6 mm and ≥7 mm was also

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>aPDT + SRP group (n = 23)</th>
<th>SRP group (n = 29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years</td>
<td>51.84 ± 9.02</td>
<td>48.68 ± 10.63</td>
<td>0.76</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>81.4</td>
<td>86.6</td>
<td>0.43</td>
</tr>
<tr>
<td>Body mass index in kg/m²</td>
<td>34.8 ± 2.1</td>
<td>31.4 ± 2.8</td>
<td>0.58</td>
</tr>
<tr>
<td>Mean fasting blood glucose in mg/dl</td>
<td>107.2 ± 7.5</td>
<td>119.4 ± 8.5</td>
<td>0.087</td>
</tr>
</tbody>
</table>
### Table 2

<table>
<thead>
<tr>
<th>Periodontal parameters</th>
<th>Baseline</th>
<th>6-weeks</th>
<th>12-weeks</th>
<th>Δ (6w-baseline)</th>
<th>Δ (12w-baseline)</th>
<th>Δ (12w-6w)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bleeding on probing (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRP + aPDT</td>
<td>85.4 ± 20.3</td>
<td>82.7 ± 17.7</td>
<td>16.8 ± 12.4</td>
<td>13.0 ± 6.4</td>
<td>13.6 ± 8.6</td>
<td>11.3 ± 5.5</td>
</tr>
<tr>
<td>SRP</td>
<td>68.6 ± 17.4*</td>
<td>69.7 ± 15.5*</td>
<td>71.8 ± 14.9*</td>
<td>71.4 ± 15.1*</td>
<td>3.2 ± 7.6</td>
<td>-1.7 ± 3.3</td>
</tr>
<tr>
<td><strong>Sites 4 mm probing depth (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SRP + aPDT</td>
<td>5.9 ± 2.9*</td>
<td>6.1 ± 3.1</td>
<td>2.2 ± 2.5</td>
<td>0.6 ± 4.6</td>
<td>1.7 ± 1.4</td>
<td>0.9 ± 1.9</td>
</tr>
<tr>
<td>SRP</td>
<td>3.7 ± 2.7*</td>
<td>0.5 ± 0.7</td>
<td>0.4 ± 1.4*</td>
<td>0.9 ± 1.9</td>
<td>0.5 ± 0.5</td>
<td>0.7 ± 1.0</td>
</tr>
<tr>
<td><strong>Probing depth (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRP + aPDT</td>
<td>4.0 ± 0.4</td>
<td>2.8 ± 0.2</td>
<td>3.2 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>3.1 ± 0.4</td>
<td>0.6 ± 0.7</td>
</tr>
<tr>
<td>SRP</td>
<td>1.0 ± 0.3*</td>
<td>1.1 ± 0.3*</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td><strong>Clinical attachment level (mm)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>SRP + aPDT</td>
<td>5.0 ± 0.7</td>
<td>4.5 ± 0.7</td>
<td>4.4 ± 0.7</td>
<td>4.4 ± 0.7</td>
<td>4.4 ± 0.7</td>
<td>4.4 ± 0.7</td>
</tr>
<tr>
<td>SRP</td>
<td>1.0 ± 0.3*</td>
<td>1.1 ± 0.3*</td>
<td>0.4 ± 0.2*</td>
<td>0.4 ± 0.2*</td>
<td>0.4 ± 0.2*</td>
<td>0.4 ± 0.2*</td>
</tr>
</tbody>
</table>

* Significantly different at different time points within the group at p < 0.001.
† Significantly different between groups at p < 0.001.

### Table 3

Gingival crevicular fluid levels of TNF-α and IL-6 in aPDT and SRP groups at baseline and 12 weeks.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Baseline</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRP</td>
<td>13.12 ± 3.52</td>
<td>13.34 ± 2.03</td>
</tr>
<tr>
<td>+ aPDT</td>
<td>13.34 ± 2.03</td>
<td>13.34 ± 2.03</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRP</td>
<td>4.17 ± 1.98*</td>
<td>4.17 ± 1.98*</td>
</tr>
<tr>
<td>+ aPDT</td>
<td>4.17 ± 1.98*</td>
<td>4.17 ± 1.98*</td>
</tr>
</tbody>
</table>

* Statistically significant from baseline at p < 0.001.
† Significant difference between groups at p < 0.05.

Significantly reduced in both the groups. At 12 weeks, a reduction in the percentage of sites with PD was again observed, although the difference with the findings at 6 weeks was not significant (p > 0.001).

### Discussion

The present study was based on the hypothesis that adjunctive aPDT as compared to SRP alone, improve clinical periodontal parameters, and reduce gingival crevicular fluid (GCF) TNF-α and IL-6 levels in obese patients with CP. The present result showed that adjunctive aPDT showed improvement in clinical and immunological parameters in obese patients with CP. Antimicrobial PDT showed additional benefit in moderate and deep periodontal pockets in obese patients with CP.

Because gain in clinical attachment level was clinically relevant, increase in the clinical attachment level occurred as a result of reduction in probing depth and not as a result of change in the clinical recession (data not shown) [26]; this was probably due to the formation of long epithelial adherence and removal of underlying causal factors (i.e. subgingival plaque and calculus), that followed reduced inflammation [27]. The mean decrease in probing depth 4–6 mm and ≥7 mm was again observed, although the difference with the findings at 6 weeks was not significant (p > 0.001).

At 6 weeks, the mean PD of the whole mouth was reduced by 1 mm in both the groups: a significant reduction (p < 0.001) from 4.0 ± 0.4 to 2.8 ± 0.2 mm in aPDT group and from 4.2 ± 0.5 to 3.2 ± 0.4 mm in SRP group. At 12 weeks, the mean PD of the whole mouth was significantly reduced (p < 0.01) in comparison with the 6-week findings.

At 6 weeks, a significant (p < 0.001) gain in clinical attachment level for the whole mouth improved from 5.0 ± 0.7 to 4.4 ± 0.7 mm in aPDT subjects and from 5.5 ± 0.6 to 5.1 ± 0.7 mm in SRP group.
improvement [29]. Furthermore, improvement in the percentage of plaque index at week 6 follow-up was greater in the SRP + PDT group. This is likely because PDT reduces biofilm reorganization [30]. However, it may be postulated that significant improvement in plaque index in aPDT group may be as a result of greater oral hygiene care due to the Hawthorn effect [31].

Obesity itself produces a pro-inflammatory state characterized by the release of cytokines into the gingival tissue [10]. Pro-inflammatory cytokines, including IL-6 and TNF-α stimulate alveolar bone resorption [32], although the mechanism underlying such increased resorption in obesity is unclear. In our study, SRP lowered TNF-α and IL-6, providing indication of the effectiveness of this approach for lowering down inflammation that was expected. The observed decreases in TNF-α and IL-6 concentrations were similar between the SRP and aPDT groups, reaching statistical significance between the groups thus indicating that PDT does have a beneficial effect on the systemic inflammatory component. Reduction in local periodontal inflammation in obesity corroborates with the study by Duzagac et al. [33] that showed similar reduction by SRP. However, there has been no study that assessed reduction in periodontal inflammation through aPDT in obesity. Because increases in TNF-α is related to enhanced IL-6 production [34], a decrease in TNF-α could be expected to result in lower IL-6 levels. Our results showed significant changes in IL-6 concentrations, thus suggesting that the regulation of IL-6 secretion may under the exclusive control of TNF-α in obesity.

It is noteworthy from the included study participants, the mean diabetic status of obese patients in both group ranged from 107 to 119 mg/dl. These levels correspond to prediabetic state. It is well-documented that similar to diabetes mellitus, prediabetes is also an established risk factor for increased periodontal tissue destruction [35,36]. It has been reported that hyperglycemia is associated with excessive formation of accumulated glycation end products (AGEs) in the tissues and AGES are coupled with impaired fibroblastic growth and increased production of proinflammatory cytokines (including TNF-α and IL-6) [37]. Moreover, results by Manoucher-Pour et al. [38] showed that chronic hyperglycemia impairs the chemotactic and phagocytic function of neutrophils (which prevent destruction of bacteria in periodontal pockets), thereby increasing periodontal destruction. Therefore it may be speculated that the unfavorable clinical and immunological outcomes seen in SRP group may be associated with low levels of hyperglycemic state.

The findings of the present study indicates significant reduction of local inflammation by aPDT application in obesity. However, future trials may be performed to assess the reduction of systemic inflammatory burden (C-reactive protein levels) by controlling local inflammation with the use of aPDT. This may help to understand the association of local inflammation (chronic periodontitis) that is modified by systemic inflammatory burden (obesity) [10,39]. Furthermore, bacterial counts should be investigated to assess the decrease in the red complex counts. A recent study suggests that deep periodontal pockets in overweight and obese patients have a high prevalence of periodontal pathogens that may induce negative impact on periodontal therapy [11]. In addition, long follow-up RCTs are required in order to evaluate the long term effects of aPDT use in obese patients.

5. Conclusion

Within the limits of this clinical trial, adjunctive aPDT showed improvement in clinical and immunological parameters in obese patients with CP. Antimicrobial PDT showed additional benefit in moderate and deep periodontal pockets in obese patients with CP.

Conflict of interest statement

The authors declare that they have no conflict of interest and all authors have read and approved the final draft.


