Photodiagnosis and Photodynamic Therapy 27 (2019) 388–395

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Antimicrobial photodynamic therapy (aPDT) with curcumin and LED, as an enhancement to scaling and root planing in the treatment of residual pockets in diabetic patients: A randomized and controlled split-mouth clinical trial

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ARTICLE INFO

Keywords:
Periodontitis
Diabetes mellitus
Periodontal debridement
Curcumin
Antimicrobial photodynamic therapy

ABSTRACT

Introduction: Residual pockets represent a risk factor for periodontal disease progression. Diabetes Mellitus (DM) may impair prognosis after cause-related therapy, mainly due to the chronic hyperglycemia that negatively influences tissue repair. This study evaluated the clinical efficacy of antimicrobial photodynamic therapy (aPDT) with curcumin (CUR) solution (100 mg/L) and LED irradiation (465–485 nm), as an adjunctive therapy to scaling and root planing (SRP), in the treatment of residual pockets in type 2 diabetic patients.

Methods: Individuals with type 2 DM and chronic periodontitis presenting at least one residual pocket per quadrant were selected (n = 25). In each patient, all residual pockets with probing depth (PD) ≥5 mm and bleeding on probing (BOP) were allocated to receive, according to quadrant: 1) SRP (SRP group); 2) SRP and irrigation with CUR solution (100 mg/L) (CUR group); 3) SRP and LED irradiation (InGaN, 465–485 nm, 0.78 cm^2, 78 mW, 100 mW/cm^2, 60 s) (LED group); 4) SRP, irrigation with CUR solution (100 mg/L), one minute of pre-irradiation, and LED irradiation (InGaN, 465–485 nm, 60 s) (aPDT group). Clinical parameters of PD, gingival recession (GR), clinical attachment level (CAL), BOP and visible plaque index (PI) were evaluated at baseline, three and six months post-therapies. Differences between the examination periods in each group were analyzed by Friedman's test for non-parametric data, while parametric data were submitted to analysis of variance (One-way ANOVA), followed by Tukey's test. Intergroup comparisons were performed by Kruskal-Wallis test.

Results: In an intergroup comparison, the mean values for PD, GR, CAL, BOP and PI were not different at baseline, three and six months ($p > 0.05$). The intragroup comparison evidenced reduction in PD and BOP in all treatment groups at three and six months ($p < 0.05$). Significant CAL gain was notable only for the aPDT and LED groups at three months in comparison to baseline data ($p < 0.05$).

Conclusion: Treatment of residual pockets in patients with type 2 DM through association of SRP with aPDT (CUR solution 100 mg/L and LED irradiation) or LED irradiation may yield short-term (three months) clinical benefits regarding CAL gain.

1. Introduction

Diabetes Mellitus (DM) is the most prevalent chronic metabolic disorder characterized by higher than normal blood glucose levels due to deficient management of insulin by the organism. The state of chronic hyperglycemia leads to increased levels of advanced glycation end-products (AGEs). AGEs act directly on cells, causing proinflammatory effects and oxidative stress [1]. On the other hand, AGEs may interact with their receptor, named receptor for advanced glycation end-products (RAGE), present on different cell surfaces, altering cell function. This interaction increases proinflammatory cytokine levels, interfering with tissue repair through reduced bone turnover and collagen synthesis [2,3].

A correlation between type 2 DM and periodontal disease is evidenced by the literature [1–4], supporting a risk up to three folds higher of developing periodontitis in individuals with diabetes compared to non-diabetics [2], and an increased prevalence and severity of periodontal disease for those with poor glycaemic control [2,3]. Periodontitis refers to a multifactorial inflammatory disease [5] associated with dysbiotic biofilms [6]. Periodontal tissue destruction is mainly related

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https://doi.org/10.1016/j.pdpdt.2019.07.005
Received 1 April 2019; Received in revised form 26 June 2019; Accepted 8 July 2019
Available online 10 July 2019
1572-1000/

to an inappropriate host immune-inflammatory response [4,6], influenced by genetic, epigenetic and environmental factors, such as tobacco, alcohol consumption and diabetes [6].

The conventional mechanical debridement through scaling and root planing (SRP) is an effective approach to treat periodontal disease [7,8]. In fact, limitations inherent to the technique may fail to eliminate microorganisms from anatomical structures or soft tissue, which may act as reservoirs of periodontal pathogens, enabling the re-colonization of previously treated sites [9]. Residual pockets represent a risk factor for the progression of periodontitis, especially sites with PD ≥ 6 mm after initial therapy [10,11] or multiple sites with PD ≥ 5 mm [10]. Therefore, supportive periodontal therapy (SPT) helps prevent disease recurrence and early identification of diseased sites, reducing the probability of tooth loss [12].

SRP for treatment of residual pockets demonstrates feasibility preserving clinical attachment level (CAL) [7], although the literature suggests that no positive predictable results may be expected by repeating the treatment [13,14], and the effectiveness of SRP substantially decreases in sites with probing depth (PD) ≥5 mm [8]. In order to reduce microorganisms located in inaccessible sites to conventional instruments, studies have evaluated adjunctive therapies to SRP, as antimicrobial photodynamic therapy (aPDT) in type 2 DM [15–19]. The association of aPDT is based on the lethal effects on microorganisms through damage caused by reactive oxygen species (ROS) (type I reaction) or by singlet oxygen (type II reaction) [20]. Therefore, the applicability of aPDT for the treatment of residual pockets is justified by the main properties of this therapy, which include its broad spectrum of action (bacteria, fungi and protozoa) with minimal effects to the host tissue, and low probability of selection of photo resistant strains even after repeated applications [21].

Natural substances with biological properties have been evaluated, and clinical studies appointed to the therapeutic use of curcumin (CUR) as a photosensitizer in aPDT [22–24], in the form of solution for subgingival irrigation [25] or as gel for local application [26]. CUR is derived from the rhizome of *Curcuma longa*, commonly known as turmeric, and exhibits antioxidant, anti-inflammatory, antimicrobial and anticancer effects [27]. There is evidence that CUR effects are potentiated by the presence of light, and the phototoxicity is related to the free radicals and ROS produced, with restricted local effects even at low concentrations (≤5 μM) [28]. CUR has a broad spectrum of light absorption that ranges from 300 to 500 nanometers (nm) (maximum absorption 430–435 nm) [28].

in vitro studies have demonstrated the biocompatibility of CUR through the absence of cytotoxic effects on fibroblasts [29], capacity to reduce the viability of the periodontal pathogen *Aggregatibacter Actinomycetencomitans,* with strengthened effects when associated with a Light Emitting Diode (LED) [30,31]. In addition, an *in vivo* study reported that aPDT with CUR and LED, as a monotherapy, was effective in controlling alveolar bone loss and reducing the expression of RANKL (receptor activator of nuclear factor kappa-B ligand) in rats with induced periodontitis [32]. Few studies have evaluated CUR as a photosensitizer in aPDT for the treatment of periodontitis [22]. Therefore, this study aimed to investigate the photodynamic effects of CUR solution and LED irradiation, as an adjunctive therapy to SRP, in the treatment of residual pockets in patients with type 2 DM under SPT. The hypothesis of the study is that aPDT (CUR solution and LED irradiation) associated to SRP promotes significant clinical improvements over conventional mechanical debridement alone, for the treatment of residual pockets in patients with type 2 DM.

2. Material and methods

2.1. Study design

The study was designed as a split-mouth, single-blinded, randomized and controlled clinical trial (RCT). This clinical trial was approved by the local Ethics Committee of the São Paulo State University (Unesp), School of Dentistry, Araçatuba (CAAE: 69463517.8.0000.5420), registered at the "*International Clinical Trials Registry Platform* – UTN" (Protocol UTN U1111-1205-0218) and in the Brazilian platform for clinical trials "*Registro Brasileiro de Ensaios Clínicos* – REBEC" (RBR-4tq9yq). The study was conducted according to the Consort Statement [33] for clinical trials.

2.2. Sample size calculation

The sample size was calculated at 90% power to detect a significant difference of 1 mm on CAL among groups, the primary outcome variable of the study, considering a 5% significance level and 1 mm standard deviation (http://www.lee.dante.br/). A minimum sample of 21 patients would be required. However, twenty-five patients were included in the study, considering the possibility of patient loss to follow-up [34].

2.3. Study population

Twenty-five patients with medical diagnosis of type 2 DM (glycated hemoglobin (HbA1c) ≥ 6.5%), exhibiting chronic periodontitis and under SPT were recruited from patients referred to the São Paulo State University (Unesp), School of Dentistry of Araçatuba (SP, Brazil). Initially, the patients answered a questionnaire about their medical history and their daily medication intake, as well as questions related to oral hygiene habits. Patients were informed about the potential benefits and risks of their participation in the study. Blood tests were requested to confirm HbA1c level and to evaluate fasting blood glucose.

Patients were considered eligible if they met the following criteria: 1) age range 30–70 years [35]; 2) medical diagnosis of type 2 DM (HbA1c ≥ 6.5%) [36]; 3) history of chronic periodontitis [37] treated in the previous 3–6 months after cause-related therapy; 4) at least one residual pocket per quadrant with PD ≥ 5 mm, bleeding on probing (BOP) and CAL ≥ 3 mm; 5) at least 15 teeth, excluding third molars [38]. The exclusion criteria included [39]: 1) current smokers or regular smoking 12 months prior to participation in the study; 2) patients with anemia; 3) active cancer; 4) use of antibiotics within the previous 6 months; 5) use of anti-inflammatory drugs within the previous six months; 7) pregnancy; 8) patients undergoing orthodontic treatment. All patients signed a written informed consent form before enrollment in the study.

2.4. Treatment protocol

The participants were submitted to clinical examination performed by a blinded examiner (CAI). Individuals presenting at least one residual pocket (PD ≥ 5 mm and BOP) per quadrant were selected. The experimental sites from each quadrant were randomly assigned to receive SRP (SRP group), irrigation with CUR solution (CUR group), LED irradiation (LED group) or aPDT with CUR and LED (aPDT group). In the pre-study phase, the professional responsible for the patients' treatment (DMJM) conducted the randomization procedure. Each treatment group was randomly assigned as group A, B, C or D, and then allocated to the four quadrants by an online randomization system (www.sealedenvelope.com). The twenty-five generated combinations were maintained in opaque sealed envelopes with no identification. According to the combination of the envelope, each quadrant randomly received the following treatments: **SRP group,** a single session of SRP [35,40] was performed (97 sites) using an ultrasonic device and periodontal curettes (Gracey Curettes, Hu-Friedy Co., Chicago, IL), and irrigation with 1 mL of saline solution; CUR group, a single session of SRP was performed (67 sites) using an ultrasonic device and periodontal curettes, followed by irrigation of the residual pockets with 1 mL of CUR solution (concentration of 100 mg/L); LED group, a single session of SRP was performed (80 sites) using an ultrasonic device and

periodontal curettes, irrigation with 1 mL of saline solution and LED irradiation (465–485 nm) for 60 s; aPDT group, a single session of SRP was performed (88 sites) using an ultrasonic device and periodontal curettes, followed by irrigation of the residual pockets with 1 mL of CUR solution (concentration of 100 mg/L) and after 1 min, LED irradiation (465–485 nm) was performed for 60 s.

2.4.1. Dye preparation (Curcumin solution)

The 100 mg/L CUR solution was obtained from the solubilization of CUR (Curcuma longa) in 99.9% of absolute ethanol and 0.1% of dimethyl sulfoxide (DMSO), and the final solution was obtained from a stock solution at 0.15% in distilled water (final concentration of 100 mg/L) [24]. The CUR was obtained commercially and processed in a compounding pharmacy (Apothicário Manipulation Pharmacy, Araçatuba, SP, Brazil). The experimental sites in the aPDT and CUR groups were irrigated with 1 mL of CUR solution using a syringe and an insulin needle (13 X 0.45 mm) (Becton Dickson Ind. Ltda., Curitiba, PR, Brazil).

2.4.2. LED protocol

After 1 min of CUR or saline solution irrigation, depending on the test site location (aPDT or LED groups), the LED tip was positioned perpendicular to the long axis of the tooth on the buccal or lingual face, for a total of 60 s per site (Fig. 1).

The irradiation was performed with a gallium and indium nitride LED (InGaN; Kon-lux Kondortech Dental Equipments Ltd., São Carlos, SP, Brazil) at a wavelength ranging from 465 to 485 nm. The LED tip used has a spot size of 0.78 cm^2, power of 78 mW and power density of 100 mW/cm^2 measured by a power meter (Power meter Demetron Research Corp. Danbury, CT, EUA) for 60 s, with a total energy density of 7.69 J $/cm^2$. The power density of the LED was measured with the power meter before each clinical procedure with the LED to ensure the standardization of the irradiation parameters.

Initially, all sites received SRP under local anesthesia and only after that, a combination with the treatment groups was revealed. All clinical procedures were performed by a single operator, who is a specialist in Periodontics (DMJM). Patients were instructed not to discuss with the examiner about the treatments received. The randomization code was not broken until all data were collected and tabulated by the examiner (CAI).

2.5. Oral hygiene program

The participants were informed about the etiology of periodontal disease and instructed regarding oral hygiene. The baseline clinical evaluation was performed 15 days after this procedure.

After the treatment of residual pockets, all subjects were recruited at 30 days for clinical evaluation to detect any alterations such as periodontal abscess, erythema, edema, pruritus, sensitivity or increase in tooth mobility, which may be related to the therapy. In addition, all patients were engaged in an oral hygiene program monthly up to 180 days post-treatment, for reinforcement of oral hygiene and professional prophylaxis with rubber cup and prophylactic paste [41].

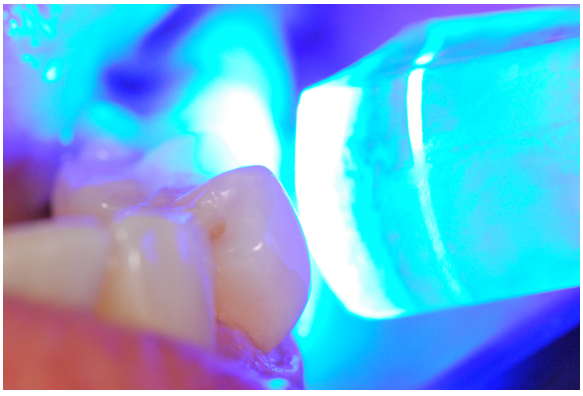

Fig. 1. LED tip positioned perpendicular to the long axis of the tooth on the buccal face.

2.6. Clinical parameters

The following clinical parameters were evaluated at site level: visible plaque index (PI) [42], PD, BOP, gingival recession (GR) and CAL [43]. GR was measured from the cemento-enamel junction to the gingival margin and BOP was classified as present, if bleeding was detected during the 30 s after probing. The clinical parameters were measured using a UNC 15 periodontal probe (PCPUNC-15, Hu-Friedy, Chicago, IL, USA). A single examiner (CAI), blinded to the therapies, assessed the clinical parameters at baseline and at 3 and 6 months posttreatment.

2.7. Intra-examiner calibration

In the pre-study phase, two non-study individuals were selected for intra-examiner calibration, and 170 sites were evaluated. Duplicate measurements of PD and CAL were assessed within one week. The intra-rater agreement for PD and CAL variables were obtained by intraclass correlation coefficient (ICC). The calibration was considered excellent for PD (0.8528) and CAL (0.859).

2.8. Statistical analysis

The primary outcome variable was the mean CAL value. Average and standard deviation values for the clinical parameters of PD, GR and CAL were obtained to compare treatment protocols and evaluation periods. Data of PI and BOP were transformed into percentages [41], considered at site level. Statistical analysis was performed with the software BioEstat 5.3 (BioEstat 5.3, BioEstat Software, Manaus, AM, Brazil), considering a 5% significance level.

Initially, data analysis was performed to evaluate the normality of all quantitative data by the Shapiro-Wilk test. Intragroup comparisons were performed by analysis of variance (One-way ANOVA) for variables that reached a normal distribution, followed by Tukey's test, whereas non-parametric data were compared by Friedman's test. Intergroup comparison was performed by Kruskal-Wallis test.

3. Results

A total of 25 patients were included in the study, including 17 male and 8 female patients, mean age of 55 ± 10.2 years. Two patients were excluded during follow-up: one did not complete the 90-day evaluation (female) and the other one was excluded from the 180-day evaluation (male), both related to antibiotic therapy for systemic impairment. Patient recruitment started in May 2017 and was completed by the end of March 2018. The patient recruitment process is described in Fig. 2. Treatment modalities were performed in a total of 332 sites, but only 290 sites were considered for final evaluation. Table 1 presents characteristics of subjects at baseline, number of sites treated, mean age and HbA1c level.

3.1. Adverse effects

Patients presented no adverse effects related to the therapy, nor pain or discomfort after treatment procedures.

3.2. Clinical outcomes

In the intergroup comparison, no significant differences ($p > 0.05$) were identified in the clinical parameters evaluated (PD, GR, CAL, PI and BOP) during all study periods. The mean difference in the reduction

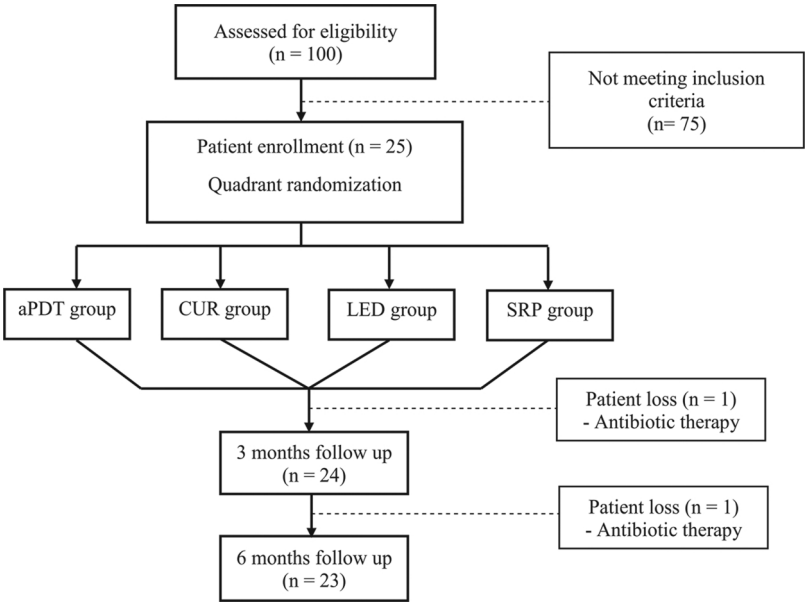

Fig. 2. Flowchart of the study.

Table 1
Subject characteristics at baseline: number of patients, test sites, age and HbA1c.

Study patients (*n*)	25
Male (*n*)	17
Female (*n*)	8
Test sites (*n*)	332
Age (M ± SD)	55.0 ± 10.2
HbA1c (%) (M ± SD)	8.73 ± 1.82

n sample number; *M* ± *SD* mean value and standard deviation; *HbA1c* glycated hemoglobin.

of PD and CAL gain were not statistically significant in the intergroup comparison between baseline and three months (PD: p = 0.79; CAL: p = 0.31), and baseline and six months (PD: p = 0.82; CAL: p = 0.77). The intragroup comparison revealed a reduction in PD and BOP in all treatment groups at 3 and 6 months compared to baseline ($p < 0.05$). No differences in GR were observed in any group throughout the study ($p > 0.05$). A statistically significant CAL gain was evidenced only in the aPDT and LED groups at three months (aPDT = 4.95 ± 2.33 mm; LED = 5.41 ± 1.98 mm) compared to baseline (aPDT = 6.71 ± 1.85 mm; LED = 6.85 ± 1.61) ($p < 0.05$), but it was not significant at six months (aPDT = 5.46 ± 1.98 mm; LED = 5.70 ± 1.88 mm) ($p > 0.05$). Analysis of PI in aPDT, CUR and SRP groups revealed a reduction from baseline to three and six months ($p < 0.05$), but in the LED group this difference was significant only at six months compared to baseline ($p < 0.05$). Clinical outcomes at baseline, three and six months are presented in Table 2. Data of the mean difference in PD reduction and CAL gain are presented in Table 3.

4. Discussion

The current clinical investigation revealed that no significant differences were identified between the treatment modalities. However, changes in the clinical parameters within each treatment group should be considered. For each treatment group, the adjunctive therapy evaluated effectively reduced the mean PD and BOP in residual pockets at three and six months. Similarly, the parameter of PI reduced from baseline to three and six months, but was not significant in the LED group at three-month follow-up. Significant CAL gain was identified only at three months, specifically in the aPDT group

Table 2
Clinical outcomes at baseline, 3 and 6 months.

Groups	Baseline (M ± SD)	3 Months (M ± SD)	6 Months (M ± SD)
PD (mm)			
aPDT	5.71 ± 0.92	4.33 ± 1.78*	4.47 ± 1.40*
CUR	5.71 ± 0.74	4.41 ± 1.07*	4.68 ± 1.22*
SRP	5.67 ± 0.78	4.58 ± 1.24*	4.70 ± 1.37*
LED	5.61 ± 0.77	4.29 ± 1.19*	4.55 ± 1.33*
GR (mm)			
aPDT	1.30 ± 1.27	0.88 ± 1.18	1.30 ± 1.35
CUR	1.14 ± 1.32	1.19 ± 1.44	1.12 ± 1.35
SRP	0.99 ± 1.27	1.03 ± 1.33	0.79 ± 1.12
LED	1.43 ± 1.34	1.29 ± 1.20	1.37 ± 1.30
CAL (mm)			
aPDT	6.71 ± 1.85	4.95 ± 2.33*	5.46 ± 1.98
CUR	6.68 ± 1.86	5.42 ± 2.26	5.78 ± 2.17
SRP	6.63 ± 1.66	5.54 ± 2.19	5.44 ± 1.99
LED	6.85 ± 1.61	5.41 ± 1.98*	5.70 ± 1.88
BOP (%)			
aPDT	100	42.60 ± 44.23†	34.99 ± 40.33†
CUR	100	37.03 ± 39.38†	37.33 ± 36.06†
SRP	100	48.26 ± 38.53†	30.64 ± 34.50†
LED	100	35.38 ± 36.81†	39.32 ± 39.60†
PI (%)			
aPDT	68.24 ± 38.23	33.03 ± 43.52†	29.78 ± 41.18†
CUR	69.34 ± 38.44	31.57 ± 37.82†	34.06 ± 38.43†
SRP	75.61 ± 32.22	45.11 ± 42.14†	38.75 ± 40.22†
LED	64.53 ± 36.38	42.49 ± 44.16	34.70 ± 39.60†

PD probing depth; *GR* gingival recession; *CAL* clinical attachment level; *BOP* bleeding on probing; *PI* visible plaque index; *aPDT* antimicrobial photodynamic therapy; *CUR* curcumin; *SRP* scaling and root planing; *LED* light emitting diode; *mm* millimeter.
M ± *SD* mean and standard deviation.
* Significant intragroup difference from baseline by ANOVA test ($p < 0.05$).
† Significant intragroup difference from baseline by Friedman test ($p < 0.05$).

(aPDT = 4.95 ± 2.33 mm), and in the LED group (LED = 5.41 ± 1.98 mm) ($p < 0.05$). Thus, the treatment of residual pockets in patients with type 2 DM associating a single session of aPDT (CUR solution and LED irradiation) or LED irradiation to SRP, may yield short-term (three months) clinical benefits in terms of CAL gain, the primary outcome of the study, compared to SRP alone.

According to split-mouth clinical trials, the treatment of residual

Table 3
Data (mean difference and standard deviation) for reduction in PD and CAL gain between baseline and 3 months, and baseline and 6 months post-treatment, in mm.

Evaluation periods	Groups				P-value**
	aPDT*	CUR*	SRP*	LED*	
PD					
0 – 3 month (mm)	1.38 ± 1.10	1.29 ± 1.18	1.09 ± 0.80	1.31 ± 0.93	0.79
0 – 6 month (mm)	1.23 ± 0.91	1.02 ± 0.96	0.96 ± 0.89	1.05 ± 1.13	0.82
CAL					
0 – 3 month (mm)	1.76 ± 1.29	1.25 ± 1.34	1.09 ± 1.19	1.43 ± 1.15	0.31
0 – 6 month (mm)	1.24 ± 1.03	0.89 ± 1.13	1.18 ± 1.33	1.14 ± 1.29	0.77

PD probing depth; *CAL* clinical attachment level; *aPDT* antimicrobial photodynamic therapy; *CUR* curcumin; *SRP* scaling and root planing; *LED* light emitting diode; *mm* millimeter.

* Mean difference ± standard deviation.

** p-value for aPDT vs CUR vs SRP vs LED by ANOVA test ($p < 0.05$).

pockets in normoglycemic individuals with a single session of aPDT as an adjunctive therapy resulted in similar clinical results as SRP performed alone. This was evidenced by PD reduction and CAL gain at the three-month evaluation period, even though different protocols have been adopted (photosensitizer and light source) [44–46]. Nevertheless, two of these studies considered only single-rooted teeth as study sites [45,46]. The other study reinstituted subgingival debridement at three- and six-month follow-up and reported no significant differences between SRP as a monotherapy or associated with aPDT at six months [44]. However, these authors argued that the adjunctive therapy provided a faster resolution at three months post-therapy, which may be beneficial for patients with an impaired tissue repair [44].

It is well established that diabetes is an important modifying factor of periodontitis [47] and in our study the mean value of the patients HbA1c level was 8.73% ± 1.82. Chronic hyperglycemia in individuals with DM increases the level of AGEs, which can interact with their receptor RAGE, altering cell function and increasing proinflammatory cytokine levels [2,3]. These mechanisms can impair tissue repair, interfering with the imbalance of RANKL/ OPG (osteoprotegerin) ratio, which increases bone destruction [1], and decreases osteoblast differentiation [3,48]. In addition, the collagen turnover is altered, exhibiting lower collagen synthesis by fibroblasts [2,3] and increasing matrix metalloproteinase degradation [3]. Thus, the systemic effects of chronic hyperglycemia may lead to a worse prognosis after periodontal therapy due to an exacerbated, though inefficient inflammatory response.

In this context, few clinical studies have assessed patients with type 2 DM and chronic periodontitis associated with non-surgical treatment using aPDT as an adjunctive therapy [15–19]. Overall, the studies did not evidence additional benefits in the parameters of PD and CAL, when a single session of aPDT was performed [15–18]. However, differences in the study protocols must be considered. In the previous study, the similar results at six months evaluation following ultrasonic debridement or association with aPDT may be related to the location of the study sites in single rooted teeth, which may have favorable clinical outcomes only with mechanical debridement [17]. Similarly, the results of another study at three months of follow-up, were possibly influenced by the concomitant antibiotic therapy instituted for both treatment groups (SRP × SRP + aPDT), with systemic 100 mg/day doxycycline for two weeks, after initial dose of 200 mg [18].

Regarding the clinical trials in individuals with DM, the use of systemic doxycycline (100 mg/day) or aPDT, associated with SRP, showed improvement in the clinical parameters of PD and CAL at three months [16,19]. Al-Zahrani et al., 2009 included a third group and assessed SRP as a monotherapy, but no difference between treatment modalities were identified [16]. In the other clinical study, both systemic doxycycline or multiple sessions of aPDT (0, 3, 7 and 14 days) effectively reduced PD, GR and CAL in multirooted teeth ($p < 0.05$). However, in single rooted teeth a desirable endpoint was achieved only in the aPDT group, which reduced moderate pockets (5–6 mm) at three months, suggesting that the aPDT may be an alternative to systemic antibiotics [19]. Thus, considering the comparable outcomes of systemic antibiotic therapy and aPDT, this conservative approach may be feasible to avoid the indiscriminate prescription of antibiotics, mainly related to the public concern on selection of resistant bacterial strains.

It is estimated that the non-surgical periodontal treatment by SRP results in mean BOP reduction of 45% from baseline level, mean PD reduction of 1.29 mm and CAL gain of 0.55 mm in moderate pockets (PD 4–6 mm) [8]. A mean PD reduction of 2.16 mm and 1.19 mm CAL gain are expected in deep pockets (PD ≥ 7 mm), and remarkable results at 1 to 3 months post-therapy, according to the review by Cobb, 2003 [8]. However, in the present study, no distinction was made between moderate and deep pockets, and the residual pockets were considered site-specific per quadrant. Approximately 73.57% ± 2.91 of the test sites corresponded to moderate pockets (PD 5–6 mm), and only 26.11% ± 3.48 to deep pockets (PD ≥ 7 mm).

Accordingly, data reported by this investigation regarding the mean reduction of PD at three months are comparable with the values estimated for moderate pockets [8] (aPDT 1.38 ± 1.10; CUR 1.29 ± 1.18; SRP 1.09 ± 0.80; LED 1.31 ± 0.93). In a similar manner, the percentage of BOP significantly reduced at three months (aPDT 42.60 ± 44.23; CUR 37.03 ± 39.38; SRP 48.26 ± 38.53; LED 35.38 ± 36.81) and six months (aPDT 34.99 ± 40.33; CUR 37.33 ± 36.06; SRP 30.64 ± 34.50; LED 39.32 ± 39.60), compared with baseline (100%). In contrast, different data for the mean difference in CAL gain were noticed in this study at three months (aPDT 1.76 ± 1.29; CUR 1.25 ± 1.34; SRP 1.09 ± 1.19; LED 1.43 ± 1.15). Moreover, reassessment visits occurred monthly and supragingival prophylaxis was performed, reinforcing the importance of periodic maintenance in patients with chronic periodontitis.

In the present investigation, although all the treatment groups evidenced CAL gain at three and six months, it has been proven to be statistically significant only in the aPDT and LED groups restricted to the three-month evaluation (aPDT = 4.95 ± 2.33 mm; LED = 5.41 ± 1.98 mm), in comparison to baseline (aPDT = 6.71 ± 1.85 mm; LED = 6.85 ± 1.61) ($p < 0.05$). It must be emphasized that the residual pockets evaluated in this clinical trial failed to the conventional treatment through SRP. Thus, it may be assumed that the adjunctive therapies seem to benefit the periodontal treatment in diabetics. The clinical improvements in the aPDT group may be a consequence of the antimicrobial property, but it is pertinent to mention that the LED therapy offers photobiomodulatory effects, which may be beneficial to the process of tissue repair. In a previous study, a significant antimicrobial effect of aPDT using curcumin gel as photosensitizer was observed in the reduction of periodontal pathogens when compared to SRP alone and sub-gingival curcumin gel without light activation [22].

It is known that photobiomodulation (PBM) may be obtained by both low-level laser or LED irradiation, which may facilitate healing in

periodontal disease [49]. The photon absorption by cells triggers intracellular mechanisms that will lead to an increased cell proliferation and survival, and protein synthesis [49]. Furthermore, the anti-inflammatory properties are related to the reduction of edema, oxidative stress in cells, and level of pro-inflammatory cytokines [49]. Therefore, the properties of PBM following LED irradiation may be advantageous to individuals with DM.

The literature advocates that there is a "biphasic dose response", mainly related to energy density (J/cm^2) for PBM effects, characterized by opposite effects when the dose exceeds the optimal value [49]. An *in vivo* study reported that LED irradiation at a wavelength of 660 nm may lead to faster periodontal healing through a decrease in tissue inflammation, stimulating collagen synthesis and new bone apposition in rats with induced periodontitis [50]. Remarkable results were obtained at an energy density of 10 J/cm^2, rather than 15 J/cm^2 [50]. In the present study, the LED (InGaN) was used as a light source at a wavelength ranging from 465 to 485 nm and total energy density of 7.69 J /cm^2.

In addition, the aPDT is remarkable for its antimicrobial property that is related to the photooxidation of biomolecules, such as lipids, proteins and nucleic acids, through type I or type II reaction. When the photosensitizer at a ground state is illuminated by a compatible light source, it becomes highly energized (triplet state) and two different reactions may occur. In type I reaction, the excited photosensitizer reacts with an organic molecule, and the free radical species generated may interact with endogenous molecular oxygen to produce ROS (hydrogen peroxide, superoxide and hydroxyl radicals), that cause damage to the cell membrane. Type II reaction involves the direct interaction with molecular oxygen to produce singlet oxygen, which may interfere with several microbial structures [20].

Several factors may interfere with the efficacy of aPDT, such as the photosensitizer and its concentration, pre-irradiation time, light source and irradiation parameters [20]. In this clinical trial, CUR was investigated as a photosensitizer in aPDT. A recent in vivo study evaluating aPDT (CUR solution and LED irradiation) as a monotherapy, local irrigation with CUR solution or LED irradiation evidenced that all the therapies controlled alveolar bone loss in rats with induced periodontitis, but the aPDT group exhibited improved results [32].

CUR is a lipophilic molecule that alters membrane permeabilization in a similar manner in gram-positive and gram-negative bacteria, which may explain its antimicrobial property [51]. The anti-inflammatory effect may be associated with the inhibition of the nuclear-κB factor pathway (NF-κB) that is related to the expression of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) [52] and cyclooxygenase-2 (COX-2) [53] in a dose-dependent manner. In the present investigation, CUR as an adjunctive therapy did not provide clinical advantages over SRP alone.

To the best of our knowledge, only one clinical study assessed the efficacy of aPDT with CUR gel (10 mg/g) and LED irradiation, as an adjunctive therapy to SRP in the treatment of sites with PD ≥ 5 mm, in individuals with untreated chronic periodontitis [22]. Sreedhar et al., 2015 compared four treatment groups in a split-mouth design: 1) SRP; 2) SRP + CUR gel for 5 min; 3) SRP + aPDT at day 0; 4) SRP + aPDT at 0, 7 and 21 days. The authors reported that all treatment modalities were effective in reducing PI, sulcus bleeding index and CAL at three-month follow-up, but improved results were obtained after multiple sessions of aPDT revealed by a mean difference of 0.76 mm in CAL gain [22]. In contrast, the current investigation evidenced a mean difference of 1.76 ± 1.29 in CAL gain following a single session of aPDT, in the same evaluation period. The presented results are consistent with a recent meta-analysis of the effects of aPDT adjunctive to SRP in the non-surgical treatment of chronic periodontitis [54]. The long-term clinical effects are questionable, and short-term effects may be expected with 0.36 mm CAL gain at three-month follow up [54].

However, differences between study protocols must be considered. Sreedhar et al., 2015 [22] also adopted the split-mouth design, but the treatment allocation was pre-established according to quadrants. Differently, the treatment allocation in this study was performed after debridement of all quadrants, through combinations sealed in opaque envelopes without identification. Although both studies considered patients diagnosed with chronic periodontitis as study subjects, the author evaluated untreated individuals, while the current study assessed residual pockets as study sites. Moreover, the authors [22] evaluated CUR gel (10 mg/g), 5 min of pre-irradiation, followed by LED irradiation (470 nm) and power density of 620 mW/cm^2 for 5 min. In our study, a CUR solution (100 mg/L) was used with one minute of pre-irradiation, followed by LED irradiation (465–485 nm) with power density of 100 mW/cm^2, for 60 s (total energy density of 7.69 J /cm^2).

To guarantee the validity of studies with split-mouth design, several requirements must be met, including treatment randomization, blinding of professionals, adequate statistical analysis and sample size calculation [55]. Potential problems related to the study design implicates the difficulty in patient recruitment presenting similar disease patterns among quadrants [56]. Considering the small sample size necessary for this type of study, losses to follow-up are more relevant and must be considered in sample size calculation [55]. In this investigation, two patients were excluded from the statistical analysis related to antibiotic therapy for systemic impairment, but the sample calculation was performed considering potential loss to follow-up. Indeed, as the study aimed to evaluate residual pockets during SPT, mean PD, CAL, GR, BOP and PI were not statistically different between groups at baseline ($p < 0.05$), which may not limit the external validity of the results by restricting patient recruitment [56].

Moreover, the applicability of the split-mouth design is based on the principle that the influence of inter-subject characteristics are subtracted, which increases the power of the study [55,56], but requires that the therapies and their effects must be localized [55]. Considering the local effects of aPDT, mainly related to the short lifespan of singlet oxygen as a result of type II reaction, the cellular damage on bacteria, protozoa, viruses and fungi are restricted to the therapy site [20]. Accordingly, the applicability of the split-mouth design in this clinical trial was feasible to evaluate the local effects of aPDT that show low risk of carry-over effects, and to reduce inter-subject variance, considering the variability of the host immune response in diabetic individuals.

The persistence of residual pockets after the non-surgical treatment represents a clinical challenge, considered as sites at risk for additional tissue destruction. Therefore, the clinical parameters evaluated in this clinical trial were analyzed and reported as site-specific, considering the local patterns of the periodontal disease. Because this was a split-mouth clinical trial, a minimum amount of residual pockets per quadrant was established as inclusion criteria (at least one residual pocket per quadrant with PD ≥ 5 mm) due to the difficulty in recruiting patients with similar disease patterns per quadrant, which limited the number of sites evaluated per treatment group. In addition to the split-mouth design, a variable that can not be controlled refers to the diversity of interactions between our oral microbiome and the distinct environments present in the mouth [6]; also, one therapy may possibly influence the clinical response to other therapies [55,56]. Although a reduction in the level of periodontopathogens may be obtained following periodontal therapy, the microbial recolonization is likely to occur, but the microbiological profile was not assessed in the current investigation.

Individual susceptibility to periodontal disease is strongly influenced by behavioral risk factors, genetic predisposition, and variation in the immune response as shown by diabetics [6]. To the best of our knowledge, this was the first clinical trial to evaluate aPDT (CUR solution and LED irradiation) for the treatment of residual pockets in patients with type 2 DM, under SPT. This split-mouth clinical trial supports that adjunctive therapies to SRP may positively influence the reduction in PD and CAL gain in patients with type 2 DM, after three and six months. Among these therapies, the aPDT (CUR solution and LED irradiation) and LED irradiation seem to be superior in promoting

CAL gain at three months.

5. Conclusion

Specifically in patients with type 2 DM, the treatment of residual pockets with aPDT (CUR solution 100 mg/L and LED irradiation) or LED irradiation as adjunctive therapies to SRP, may yield short-term (three months) clinical benefits regarding CAL gain.

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