UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

CAMILA AYUMI IVANAGA

Efeitos fotodinâmicos da Curcumina no tratamento de bolsas residuais de pacientes portadores de periodontite crônica e Diabetes Mellitus tipo 2: estudo de boca dividida randomizado

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Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP, como parte dos requisitos para a obtenção do título de Mestre em Periodontia.

Orientadora: Profa. Assoc. Leticia Helena Theodoro
Co-orientador: Prof. Titular Valdir Gouveia Garcia

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"The pessimist sees difficulty in every opportunity. The optimist sees opportunity in every difficulty."

Winston Churchill

Ivanaga, CA. **Efeitos fotodinâmicos da Curcumina no tratamento de bolsas residuais de pacientes portadores de periodontite crônica e Diabetes Mellitus tipo 2: estudo de boca dividida randomizado.** [Dissertação] – Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araçatuba, 2019.

RESUMO

Introdução: A presença de bolsas residuais representa um fator de risco preditor de progressão da doença periodontal. A presença de Diabetes Mellitus (DM) aumenta a prevalência de periodontite e influencia negativamente na capacidade de reparo tecidual. O objetivo do estudo foi avaliar a eficácia clínica da terapia fotodinâmica antimicrobiana (aPDT) com curcumina e LED, como terapia coadjuvante à raspagem e alisamento radicular (RAR), no tratamento de bolsas residuais de pacientes com DM tipo 2.

Métodos: Para este estudo clínico controlado randomizado de boca dividida, vinte e cinco pacientes foram selecionados. Em cada paciente, todas as bolsas residuais com profundidade de sondagem (PS) ≥5 mm e sangramento à sondagem (SS), por quadrante, foram aleatoriamente alocados para receber: 1) RAR (grupo RAR); 2) RAR e irrigação com solução de curcumina (grupo CUR); 3) RAR e irradiação com LED (grupo LED); 4) RAR e terapia fotodinâmica antimicrobiana (grupo aPDT). Para a aPDT, utilizou-se solução de curcumina (100 mg/L) seguida de irradiação com LED (InGaN, 465 - 485 nm, 100 mW/cm^2, 60 segundos). Os parâmetros clínicos de PS, recessão gengival (RG), nível de inserção clínica (NIC), SS e índice de placa visível (IP) foram avaliados no início (*baseline*), 3 e 6 meses após os tratamentos.

Resultados: Na comparação intergrupo, não houve diferença estatisticamente significante nos valores médios dos parâmetros clínicos avaliados (PS, RG, NIC, SS e IP) no início do estudo (*baseline*), aos 3 e 6 meses ($p > 0,05$). De forma semelhante, não houve diferença na média de redução de PS e ganho de NIC aos 3 e 6 meses comparados ao início (*baseline*) ($p > 0,05$). A análise intragrupo revelou que em todos os grupos de tratamento houve redução da PS e SS aos 3 e 6 meses ($p < 0,05$). Todos os grupos demonstram redução do IP, mas no grupo LED só foi estatisticamente significante aos 6 meses ($p < 0,05$). Nenhum grupo apresentou diferença na RG nos períodos avaliados ($p > 0,05$). Apenas nos grupos aPDT e LED houve melhora significativa do NIC

aos 3 meses (aPDT 4,95 ± 2,33; LED 4,41 ± 1,98) em comparação aos dados iniciais (aPDT 6,71 ± 1,85; LED 6,85 ± 1,61) ($p < 0,05$).

Conclusão: aPDT ou irradiação com LED, como coadjuvantes à RAR, promoveram benefícios clínicos a curto prazo no tratamento de bolsas residuais de pacientes portadores de diabetes tipo 2. Apesar disso, os benefícios clínicos poderiam ser relacionados apenas ao efeito fotobiomodulador após irradiação tecidual com LED.

Palavras-chave: Periodontite. Diabetes Mellitus. Raspagem Dentária. Fotoquimioterapia. Curcumina.

Ivanaga, CA. **Photodynamic effects of Curcumin in the treatment of residual pockets in patients with chronic periodontitis and type 2 Diabetes Mellitus: a randomized and controlled split mouth clinical trial.** [Dissertation] – São Paulo State University (Unesp), School of Dentistry, Araçatuba, 2019.

ABSTRACT

Introduction: Residual pockets represent a risk factor for periodontal disease progression, which is exacerbated by Diabetes Mellitus (DM) by increasing the prevalence of periodontal disease and negatively influencing healing capacity. The present study aimed to evaluate the clinical efficacy of antimicrobial photodynamic therapy (aPDT) with curcumin and LED, as an adjunctive therapy to scaling and root planing (SRP), in the treatment of residual pockets in patients with type 2 DM.

Methods: A randomized and controlled split-mouth clinical trial was conducted with twenty-five patients. In each patient, all residual pockets with probing depth (PD) ≥5 mm and bleeding on probing (BOP), per quadrant, were randomly allocated to receive: 1) SRP (SRP group); 2) SRP and irrigation with curcumin solution (CUR group); 3) SRP and LED irradiation (LED group); 4) SRP and aPDT (aPDT group). The aPDT was performed with curcumin solution (100 mg/L) followed by LED irradiation (InGaN, 465 - 485 nm, 100 mW/cm^2, 60 seconds). Clinical parameters of PD, gingival recession (GR), clinical attachment level (CAL), BOP and visible plaque index (PI) were evaluated at baseline, 3 and 6 months post-therapies.

Results: In an intergroup comparison, the mean values for PD, GR, CAL, BOP and PI were not different at baseline, 3 and 6 months ($p > 0.05$). Similarly, the mean difference in the reduction of PD and CAL gain between baseline and 3 or 6 months were not statistically different ($p > 0.05$). The intragroup comparison showed reduction in PD and BOP in all treatment groups at 3 and 6 months ($p < 0.05$). All treatment groups showed reduction in PI, but in the LED group the difference was statistically significant only at 6 months ($p < 0.05$). Mean GR did not differ in any intervention group throughout the study ($p > 0.05$). Only aPDT and LED groups showed CAL gain at 3 months (aPDT 4.95 ± 2.33, LED 4.41 ± 1.98) in comparison to baseline data (aPDT 6.71 ± 1.85, LED 6.85 ± 1.61) ($p < 0.05$).

Conclusion: aPDT or LED irradiation, as adjunctive therapies to SRP, may yield short-term clinical benefits in the treatment of residual pockets in patients with type 2 diabetes. However, the clinical improvements may be related to the photobiomodulatory effects of LED irradiation.

Keywords: Periodontitis. Diabetes Mellitus. Periodontal debridement. Photochemotherapy. Curcumin.

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LISTA DE ABREVIATURAS E SIGLAS

AGEs	Advanced glycation end-products
ANOVA	Analysis of variance
aPDT	Antimicrobial photodynamic therapy
BOP	Bleeding on probing
CAL	Clinical attachment level
COX-2	Cyclooxygenase-2
CUR	Curcumin
DM	Diabetes Mellitus
DMSO	Dimethyl sulfoxide
GR	Gingival recession
HbA1c	Glycated hemoglobin
ICC	Intraclass correlation coefficient
IL-1β	Interleukin-1β
InGaN	Gallium and indium nitride
J/cm²	Joules per square centimeter
LED	Light emitting diode
mg/day	Milligram per day
mg/g	Milligram per gram
mg/L	Milligram per liter
mm	Millimeters
mW/cm²	Milliwatts per square centimeter
NF-κB	Nuclear-κB factor
nm	Nanometers
OPG	Osteoprotegerin
PBM	Photobiomodulation
PD	Probing depth
PI	Visible plaque index
RAGE	Receptor for advanced glycation end-products
RANKL	Receptor activator of nuclear factor kappa-B ligand

RCT	Randomized controlled clinical trial
ROS	Reactive oxygen species
SPT	Supportive periodontal therapy
SRP	Scaling and root planing
TNF-α	Tumor necrosis factor-α

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SUMMARY

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Title

Photodynamic effects of Curcumin in the treatment of residual pockets in patients with chronic periodontitis and type 2 Diabetes Mellitus: a randomized and controlled split-mouth clinical trial.

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Highlights

- aPDT and LED irradiation promoted CAL gain at 3 months evaluation.
- The photobiomodulation effect may be responsible for the clinical improvements.
- Only LED irradiation may yield faster healing in compromised individuals.

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 its 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 DM and periodontal disease is evidenced by the literature [1, 2, 3, 4], supporting a risk up to 3 folds for individuals with diabetes to develop periodontitis [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 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 $\geq$6 mm after initial therapy [10, 11] or multiple sites with PD $\geq$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) $\geq$5 mm [8].

Moreover, considering that the state of chronic hyperglycemia impairs tissue repair in patients with diabetes, studies have evaluated adjunctive therapies to SRP, including antimicrobial photodynamic therapy (aPDT) [15, 16, 17, 18, 19]. The aPDT has lethal effects on microorganisms through the damage caused by reactive oxygen species (ROS) (type I reaction) or by singlet oxygen (type II reaction) [20]. The main advantages of aPDT include its broad spectrum of action (bacteria, fungi and protozoa) with minimal effects to the host tissue, and absence of selection of photoresistant 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, 23, 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 and anticancer effects [27], antimicrobial property. There is evidence that CUR effects are potentiated in 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 300-500 nanometers (nm) (maximum absorption 430-435 nm).

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 associated with LED as a light source, and 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 the association of aPDT with CUR and LED promotes a significant clinical improvement compared with

conventional mechanical debridement, for 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, blinded-examiner, 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. A minimum sample of 21 patients would be required. However, considering the possibility of patient loss to follow-up, a total of 25 patients were included in the study [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, a detailed anamnesis was performed, and 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 treated in the previous 3 to 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 [37]. The exclusion criteria included [38]: 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 6 months; 7) pregnancy; 8) patients undergoing orthodontic treatment. Informed consent was obtained from all participants.

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, 39] was performed 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 using an ultrasonic device and periodontal curettes, and irrigation with 1 mL of CUR solution 100 mg/L for 1 minute;

LED group, a single session of SRP was performed using an ultrasonic device and periodontal curettes, irrigation with 1 mL of saline solution and LED irradiation;

aPDT group, a single session of SRP was performed using an ultrasonic device and periodontal curettes, irrigation with 1 mL of CUR solution 100 mg/L and LED irradiation after 1 minute.

The 100 mg/L CUR solution was obtained from the solubilization of CUR (Curcuma longa, 4-Hydroxy-3-methoxyphenyl1,6-heptadiene3,5dione, Diferuloylmethane,Diferulylmethane C1386 Sigma Aldrich, MO, USA) in 99.9% of absolute ethanol and 0.1% of dimethyl sulfoxide (DMSO). A solution with 0.15% was used to obtain the final solution with a concentration of 100 mg/L in distilled water [24]

(Apothicário Manipulation Pharmacy, Araçatuba, SP, Brazil). The experimental sites in the aPDT group 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, Brasil). After 1 minute, the LED tip was positioned perpendicular to the long axis of the tooth on the buccal or lingual face, depending on the site location, for 60 seconds.

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 465-485 nm and power density of 600 mW/cm². The LED tip used has a spot size of 0.78 cm², power density of 100 mW/cm² measured by a power meter (Power meter Demetron Research Corp. Danbury, CT, EUA) for 60 seconds, with a total energy density of 7.69 J /cm².

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 [40].

2.6 Clinical parameters

The following clinical parameters were evaluated at site level: visible plaque index (PI) [41], PD, BOP, gingival recession (GR) and CAL [42]. 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 seconds 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, 2 non-study individuals were selected for intra-examiner calibration, and 170 sites were evaluated. Duplicate measurements of PD and CAL were assessed within 1 week. The intra-rater agreement for PD and CAL variables were obtained by intraclass correlation coefficient (ICC). The calibration was considered satisfactory 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 [40], 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.

The initial data analysis was performed to evaluate if a parametric or non-parametric distribution was reached (Shapiro-Wilk test). All variables were submitted to intergroup comparison by Kruskal-Wallis test. The intragroup comparisons at different evaluation periods were performed by Analysis of variance (ANOVA) using a mixed-model approach for variables that reached a normal distribution, followed by Tukey's test. Data presenting non-normal distribution were analyzed by Friedman's test in intragroup comparison.

3. Results

A total of 25 patients were included in the study, including 16 male and 7 female patients. 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. Data from these patients were excluded from the statistical analysis. Patient recruitment started in May 2017 and was completed by the end of March 2018. The patient recruitment process is described in figure 1. 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 of PD and CAL gain were not statistically significant in the intergroup comparison between baseline and 3 months (PD: $p = 0.79$; CAL: $p = 0.31$), and baseline and 6 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 significant difference in CAL was detected only in the aPDT and LED groups at 3 months (aPDT=4.95 $\pm$ 2.33 mm; LED=4.41 $\pm$ 1.98 mm) compared with baseline (aPDT=6.71 $\pm$ 1.85 mm; LED=6.85 $\pm$ 1.61) ($p < 0.05$). Analysis of PI in aPDT, CUR and SRP groups revealed a reduction from baseline to 3 and 6 months ($p < 0.05$), but in the LED group this difference was significant only at 6 months compared to baseline ($p < 0.05$). Clinical outcomes at baseline, 3 and 6 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 all treatment modalities were effective in reducing the mean PD and BOP in residual pockets at 3 and 6 months. The parameter of PI reduced from baseline to 3 and 6 months in all treatment groups, but a statistically significant difference was not identified in the LED group at 3 months follow-up. Specifically, the association of aPDT to SRP promoted a significant CAL gain at 3 months, and similar clinical results were observed in the LED group. Thus, a single session of aPDT with CUR and LED or LED irradiation as adjunctive therapies may yield short-term (3 months) clinical benefits in the treatment of residual pockets in patients with type 2 DM.

According to split-mouth clinical trials in normoglycemic individuals, the adjunctive treatment of residual pockets with a single session of aPDT also demonstrated similar clinical results as SRP performed alone, evidenced by PD reduction and CAL gain at the 3-month evaluation period [43, 44, 45]. Nevertheless, two of these studies considered only single-rooted teeth as study sites [44, 45] and one reinstituted subgingival debridement at 3 and 6 months follow-up [43]. Goh *et al*., 2017 reported no significant differences between conventional debridement or association with aPDT, with significant improvement in mean PD reduction and CAL gain at 6 months of evaluation [43]. However, these authors noticed that the adjunctive therapy provided a faster resolution at 3 months post-therapy, which may be beneficial for patients with an impaired tissue repair [43].

It is well established that diabetes is an important modifying factor of periodontitis [46]. It is pertinent to mention that in the present study the mean value of HbA1c was 8.73% $\pm$ 1.82, which categorizes patients as patients with decompensated diabetes. Individuals with DM have a deficient healing capacity, mainly related to the increased levels of AGEs, as an effect of chronic hyperglycemia. The impairment in tissue repair may be associated with the imbalance of RANKL/ OPG (osteoprotegerin) ratio, which increases bone destruction [1], in association with a decrease in osteoblast differentiation [3, 47]. In addition, the collagen turnover is altered, exhibiting lower collagen synthesis by fibroblasts [2, 3], and increased susceptibility to degradation by matrix metalloproteinases [3].

In this context, few studies have evaluated the clinical efficacy of aPDT as an adjunctive therapy to SRP, in the nonsurgical treatment of chronic periodontitis in patients with type 2 DM [15, 16, 17, 18, 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, 16, 17, 18]. However, differences in the study protocols must be taken into account. In the study of Castro Dos Santos *et al*., 2016, the similar results at 6 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 Macedo *et al*., 2014 at 3 months follow-up, possibly have been influenced by the concomitant antibiotic therapy instituted for both treatment groups (SRP X SRP+aPDT), with systemic 100 mg/day doxycycline for 2 weeks, after initial dose of 200 mg [18].

Regarding these clinical trials in individuals with DM, those who compared systemic doxycycline (100 mg/day) versus aPDT as adjunctive therapies to SRP, identified that both treatments were effective and improved the clinical parameters of PD and CAL at 3 months [16, 19]. Al-Zahrani *et* al., 2009, in addition to the comparison of systemic doxycycline or aPDT as adjunctive therapies, also evaluated SRP as a monotherapy, and did not evidence differences between treatment modalities [16]. According to Ramos *et al*., 2016, although both systemic doxycycline or multiple sessions of aPDT (0, 3, 7 and 14 days) effectively reduced PD, GR and CAL ($p < 0.05$) and aPDT therapy reduced moderate pockets (5-6 mm) in single rooted teeth at 3 months, the authors suggested that adjunctive aPDT may be an alternative to systemic antibiotics [19]. Thus, considering the comparable outcomes with systemic antibiotic therapy and aPDT, this approach may be feasible to avoid the indiscriminate use of antibiotics, mainly related to the public concern on selection of resistant bacterial strains.

It is estimated that a non-surgical treatment for periodontitis 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]. On the other hand, mean PD reduction of 2.16 mm and 1.19 mm CAL gain are expected in deep pockets (PD $\geq$7 mm), with 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 3 months are comparable with the values estimated for moderate pockets, as reported by Cobb, 2003 [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 3 months (aPDT 42.60 ± 44.23; CUR 37.03 ± 39.38; SRP 48.26 ± 38.53; LED 35.38 ± 36.81) and 6 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 found in this study at 3 months (aPDT 1.76 ± 1.29; CUR 1.25 ± 1.34; SRP 1.09 ± 1.19; LED 1.43 ± 1.15). Nevertheless, only the aPDT and LED group promoted a significant CAL gain at 3 months compared with baseline.

The antimicrobial property of aPDT 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 radicals 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]. Moreover, it is important to mention that the LED therapy alone may be beneficial to tissue repair as a consequence of its photobiomodulatory effect, associated with photon absorption by cells that trigger intracellular mechanisms that will lead to an increased cell proliferation and survival, and protein synthesis [48]. Furthermore, the anti-inflammatory properties are related to the reduction of edema, oxidative stress in cells, and level of pro-inflammatory cytokines [48].

In the present investigation, only aPDT and LED groups resulted in significant CAL gain, although restricted to the 3-month evaluation (aPDT=4.95 ± 2.33 mm; LED=4.41 ± 1.98 mm), in comparison to baseline (aPDT=6.71 ± 1.85 mm; LED=6.85 ±

1.61) ($p < 0.05$). Thus, it may be presumed that the similar outcomes are mainly related to the photobiomodulatory effects promoted by the LED light, than a real effect of the aPDT. It is known that the photobiomodulation (PBM) effects when using a low-level laser or LED may accelerate tissue repair in periodontal disease [48]. Therefore, the PBM by the LED may yield a faster tissue repair, which can be advantageous to individuals with DM.

A recent systematic review evidenced that when low-level laser therapy is associated with SRP, a reduction in PD may be expected only in the short-term (1 to 2 months), considering the methodological weakness of the few clinical studies assessed [49]. Specifically, the clinical trial of Demirturk-Gocgun *et al*, 2016 in patients with type 2 DM, evidenced a significant reduction only in BOP from deep pockets following low-level laser therapy at 1, 2 and 7 days after SRP, but restricted to 1 month post-therapy [50].

It is well established that there is a “biphasic dose response”, mainly related to energy density (J/cm²) for PBM effects, characterized by opposite effects when the dose exceeds the optimal value [48]. 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 [51]. Remarkable results were obtained with an energy density of 10 J/cm² [51]. In the present study, the LED (InGaN) was used as a light source at a wavelength ranging from 465-485 nm and total energy density of 7.69 J /cm².

It is important to mention that 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, resulting in significant CAL gain at 3 months, like the LED group. Additionally, a recent *in vivo* study demonstrated that the monotherapy with aPDT (CUR and LED), CUR irrigation and LED irradiation were effective in controlling alveolar bone loss in rats with induced periodontitis, but improved results were obtained in the aPDT group [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 [52]. 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) [53] and cyclooxygenase-2 (COX-2) [54] in a dose-dependent manner. In the present investigation, CUR as an adjunctive therapy did not provide clinical advantages compared with SRP alone.

To the best of our knowledge, only one clinical study assessed the efficacy of aPDT with CUR 10 mg/g and LED, as an adjunctive therapy to SRP in the treatment of sites with PD ≥5 mm, in individuals with 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 3 months follow-up, but multiple sessions of aPDT provided greater results, with a mean difference of 0.76 mm in CAL gain [22]. In contrast, the present study revealed that a single session of aPDT promoted a mean difference of 1.76 ± 1.29 in CAL gain, in the same evaluation period.

However, differences between study protocols must be considered. Sreedhar *et al*., 2015 also adopted the split-mouth design, but the treatment allocation was pre-established according to quadrants [22]. Differently, the treatment allocation in this study was performed after debridement of all quadrants, through combinations in opaque sealed envelopes with no identification. Moreover, Sreedhar *et al*., 2015 evaluated CUR gel (10 mg/g), 5 minutes of pre-irradiation, followed by LED irradiation with a wavelength of 470 nm and power density of 620 mW/cm² for 5 minutes [22]. In our study, a CUR solution (100 mg/L) was used with 1 minute of pre-irradiation, followed by LED irradiation (465-485 nm) with power density of 100 mW/cm², for 60 seconds (total energy density of 7.69 J /cm²).

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], although 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.

Therefore, this split-mouth clinical trial revealed that aPDT using CUR as photosensitizer and LED as light source, or LED irradiation as adjunctive therapies to SRP were effective in reducing PD and promoting CAL gain in residual pockets of patients with DM, after 3 months. Although the interference of the level of HbA1c was not the object of the present study, the association of these conservative approaches to conventional debridement may yield an improved tissue response in patients with decompensated diabetes. In addition, these results indicate that the clinical advantages may be obtained only with LED irradiation through cellular and molecular PBM effects. The LED therapy features easy application and low cost, which may be relevant in patients with DM that exhibit healing capacity impairment. Moreover, reassessment visits occurred monthly and supragingival prophylaxis was performed, reinforcing the importance of periodic maintenance in patients with chronic periodontitis.

5. Conclusion

The conventional mechanical debridement associated with a single session of aPDT (CUR 100 mg/L and LED) or LED irradiation, for the treatment of residual pockets in patients with type 2 DM, promoted significant CAL gain in the short-term (3 months). However, aPDT did not demonstrate clinical advantages over LED irradiation. It is assumed that the PBM effects through LED irradiation are responsible for the faster tissue repair observed in this study, and this conservative therapeutic approach may be important to improve the healing capacity in these patients.

References

[1] J.J. TAYLOR, P.M. PRESHAW PM, E. LALLA, A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes, J Clin Periodontol. 40 (14) (2013) S113–S134.

[2] F. LLAMBÉS, S. ARIAS-HERRERA, R. CAFFESSE, Relationship between diabetes and periodontal infection, World J Diabetes 6 (7) (2015) 927-35.

[3] B. L. MEALEY, T. W. OATES, Diabetes mellitus and periodontal diseases, J Periodontol. 77 (8) (2006) 289-303.

[4] I. L. CHAPPLE, R. GENCO, Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases, J Periodontol. 84 (4) (2013) S106-12.

[5] P.N. PAPAPANOU, M. SANZ, N. BUDUNELI, et al., Periodontitis: Consensus report of Workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, J Periodontol. 89 (1) (2018) S173–S182.

[6] M. KILIAN, I.L. CHAPPLE, M. HANNIG, et al., The oral microbiome – An update for oral healthcare professionals, Br Dent J. 221 (10) (2016) 657-666.

[7] C. L. DRISKO, Periodontal debridement: still the treatment of choice, J Evid Based Dent Pract. 14 (2014) 33-41.

[8] C.M. COBB, Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing, J Clin Periodontol. 29 (2) (2002) 6-16.

[9] A. MOMBELLI, Microbial colonization of the periodontal pockets and its significance for periodontal therapy, Periodontol 2000. 76 (1) (2018) 85-96.

[10] G. MATULIENE, B.E. PJETURSSON, G.E. SALVI, et al., Influence of residual pockets on progression of periodontitis and tooth loss: Results after 11 years of maintenance. J Clin Periodontol. 35 (8) (2008) 685–695.

[11] S. RENVERT, G.R. PERSSON, A systematic review on the use of residual probing depth, bleeding on probing and furcation status following initial periodontal therapy to predict further attachment and tooth loss, J Clin Periodontol. 29 (3) (2002) 82–89.

[12] R.E. COHEN, Research, Science and Therapy Commitee, Position paper: periodontal maintenance, J Periodontol. 74 (9) (2003) 1395-401.

[13] W.M. JENKINS, S.H SAID, M. RADVAR, D.F. KINANE, Effect of subgingival scaling during supportive therapy, J Clin Periodontol. 27 (8) (2000) 590–596.

[14] I. LALEMAN, S. CORTELLINI, S. DE WINTER, et al., Subgingival debridement: end point, methods and how often? Periodontol 2000, 75 (1) (2017) 189–204.

[15] F.I. BARBOSA, P.V. ARAÚJO, L.J.C. MACHADO, Effect of photodynamic therapy as an adjuvant to non-surgical periodontal therapy: Periodontal and metabolic evaluation in patients with type 2 diabetes mellitus, Photodiagnosis Photodyn Ther. 22 (2018) 245-250.

[16] M.S. AL-ZAHRANI, S.O. BAMSHMOUS, A.A. ALHASSANI, M.M. AL-SHERBINI, Short-term effects of photodynamic therapy on periodontal status and glycemic control of patients with diabetes, J Periodontol. 80 (10) (2009) 1568-1573.

[17] N.C. CASTRO DOS SANTOS, N.M. ANDERE, C.F ARAUJO, et al., Local adjunct effect of antimicrobial photodynamic therapy for the treatment of chronic periodontitis in type 2 diabetics: split-mouth double-blind randomized controlled clinical trial, Lasers Med Sci. 31 (8) (2016) 1633-1640.

[18] O. MACEDO Gde, A.B. Jr. NOVAES, S.L. SOUZA, M. Jr. TABA, D.B. PALIOTO, M.F. GRISI, Additional effects of aPDT on nonsurgical periodontal treatment with doxycycline in type II diabetes: a randomized, controlled clinical trial, Lasers Med Sci. 29 (3) (2014) 881-6.

[19] U.D. RAMOS, L.G. AYUB, D.M. REINO, et al., Antimicrobial photodynamic therapy as an alternative to systemic antibiotics: results from a double-blind, randomized, placebo-controlled, clinical study on type 2 diabetics, J Clin Periodontol. 43 (2) (2016) 147–155.

[20] E. MIELCZAREK-BADORA, M. SZULC, Photodynamic therapy and its role in periodontitis treatment, Postepy Hig Med Dosw (online). 67 (2013) 1058-65.

[21] G. JORI, C. FABRIS, M. SONCIN, et al., Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications, Lasers Surg Med. 38 (5) (2006) 468–481.

[22] A. SREEDHAR, I. SARKAR, P. RAJAN, et al., Comparative evaluation of the efficacy of curcumin gel with and without photo activation as an adjunct to scaling and root planing in the treatment of chronic periodontitis: A split mouth clinical and microbiological study, J Nat Sci Biol Med. 6 (1) (2015) S102-9.

[23] D.P. Jr. LEITE, F.R. PAOLILLO, T.N. PARMESANO, et al., Effects of photodynamic therapy with blue light and curcumin as mouth rinse for oral disinfection: a randomized controlled trial, Photomed Laser Surg. 32 (11) (2014) 627-32.

[24] H.A. RICCI DONATO, S. PRATAVIEIRA, C. GRECCO, et al., Clinical comparison of two photosensitizers for oral cavity decontamination, Photomed Laser Surg. 35 (2) (2017) 105-110.

[25] S.N. GOTTUMUKKALA, S. KONERU, S. MANNEM, et al., Effectiveness of sub gingival irrigation of an indigenous 1% curcumin solution on clinical and microbiological

parameters in chronic periodontitis patients: A pilot randomized clinical trial, Contemp Clin Dent. 4 (2) (2013) 186-91.

[26] M. BHATIA, S.S. UROLAGIN, K.B. PENTYALA, et al., Novel therapeutic approach for the treatment of periodontitis by curcumin, J Clin Diagn Res. 8 (12) (2014) ZC65-69.

[27] S. PRASAD, S.C. GUPTA, A.K. TYAGI, B.B. AGGARWAL, Curcumin, a component of golden spice: from bedside to bench and back, Biotechnol Adv. 32 (6) (2014) 1053-64.

[28] E.M. BRUZELL, E. MORISBAK, H.H. TONNESEN, Studies on curcumin and curcuminoids. XXIX. Photoinduced cytotoxicity of curcumin in selected aqueous preparations, Photochem Photobiol Sci. 4 (7) (2005) 523-30.

[29] J.E. GOMES-FILHO, G. SIVIERI-ARAUJO, C.R. SIPERT, et al., Evaluation of photodynamic therapy on fibroblast viability and cytokine production, Photodiagnosis Photodyn Ther. 13 (2016) 97-100.

[30] D. SAITAWEE, A. TEERAKAPONG, N.P. MORALES, et al., Photodynamic therapy of Curcuma longa extract stimulated with blue light against Aggregatibacter actinomycetemcomitans. Photodiagnosis Photodyn Ther. 22 (2018) 101–105.

[31] S. NAJAFI, M. KHAYAMZADEH, M. PAKNEJAD, et al., An in vitro comparison of antimicrobial effects of curcumin-based photodynamic therapy and chlorhexidine, on *Aggregatibacter actinomycetemcomitans*, J Lasers Med Sci. 7 (1) (2016) 21-5.

[32] L.H. THEODORO, M.L. FERRO-ALVES, M. LONGO, et al., Curcumin photodynamic effect in the treatment of the induced periodontitis in rats, Lasers Med Sci. 32 (8) (2017) 1783–1791.

[33] D. MOHER, S. HOPEWELL, K.F. SCHULZ, et al., CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials, Int J Surg. 10 (2012) 28-55.

[34] M. SAGLAM, S. KOSEOGLU, I. TASDEMIR, et al., Combined application of Er:YAG and Nd:YAG lasers in treatment of chronic periodontitis. A split-mouth, single-blind, randomized controlled trial, J Periodontal Res. 52 (5) (2017) 853-862.

[35] F. MATARAZZO, L.C. FIGUEIREDO, S.E. CRUZ, et al., Clinical and microbiological benefits of systemic metronidazole and amoxicillin in the treatment of smokers with chronic periodontitis: a randomized placebo-controlled study, J Clin Periodontol. 35 (10) (2008) 885-96.

[36] AMERICAN DIABETES ASSOCIATION, Classification and diagnosis of Diabetes: Standards of medical care in Diabetes – 2018, Diabetes Care. 41 (1) (2018) S13-S27.

[37] L.H. THEODORO, N.Z. ASSEM, M. LONGO, et al., Treatment of periodontitis in smokers with multiple sessions of antimicrobial photodynamic therapy or systemic antibiotics: A randomized clinical trial, Photodiagnosis Photodyn Ther. 22 (2018) 217-222.

[38] E.E. EVANGELISTA, C.M. FRANÇA, P. VENI, et al., Antimicrobial photodynamic therapy combined with periodontal treatment for metabolic control in patients with type 2 diabetes mellitus: study protocol for a randomized controlled trial, Trials. 16 (2015) 229.

[39] P. RIBEIRO EDEL, S. BITTENCOURT, I.C. ZANIN, et al., Full-mouth ultrasonic debridement associated with amoxicillin and metronidazole in the treatment of severe chronic periodontitis, J Periodontol. 80 (8) (2009) 1254-64.

[40] L.H. THEODORO, S.P. SILVA, J.R. PIRES, et al., Clinical and microbiological effects of photodynamic therapy associated with nonsurgical periodontal treatment. A 6-month follow up, Lasers Med Sci. 27 (4) (2012) 687-93.

[41] J. AINAMO, I. BAY, Problems and proposals for recording gingivitis and plaque, Int Dent J. 25 (4) (1975) 229-35.

[42] G.C. ARMITAGE, The complete periodontal examination, Periodontol 2000. 34 (2004) 22-33.

[43] E.X. GOH, K.S. TAN, Y.H. CHAN, L.P. LIM, Effects of root debridement and adjunctive photodynamic therapy in residual pockets of patients on supportive periodontal therapy: a randomized split-mouth trial, Photodiagnosis Photodyn Ther. 18 (2017) 342-348.

[44] G.N. CAMPOS, S.P. PIMENTEL, F.V. RIBEIRO, et al., The adjunctive effect of photodynamic therapy for residual pockets in single-rooted teeth: a randomized controlled clinical trial, Lasers Med Sci. 28 (1) (2013) 317–24.

[45] M.G. CORREA, D.H. OLIVEIRA, C.H. SARACENI, et al., Short-term microbiological effects of photodynamic therapy in non-surgical periodontal treatment of residual pockets: a split-mouth RCT, Lasers Surg Med. 48 (10) (2016) 944-950.

[46] S. JEPSEN, J.G. CATON, J.M. ALBANDAR, et al., Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, J Periodontol. 89 (1) (2018) S237–S248.

[47] D.T. GRAVES, R. LIU, M. ALIKHANI, et al., Diabetes-enhanced inflammation and apoptosis - impact on periodontal pathology, J Dent Res. 85 (1) (2006) 15-21.

[48] M.R. HAMBLIN, Mechanisms and applications of the anti-inflammatory effects of photobiomodulation, AIMS Biophys. 4 (3) (2017) 337-361.

[49] C. REN, C. MCGRATH, L. JIN, et al., The effectiveness of low-level laser therapy as an adjunct to non-surgical periodontal treatment: a meta-analysis, J Periodontal Res. 52 (1) (2017) 8-20.

[50] O. DEMIRTURK-GOCGUN, U. BASER, G. AYKOL-SAHIN, et al., Role of low-level laser therapy as an adjunct to initial periodontal treatment in type 2 diabetic patients: a split-mouth, randomized, controlled clinical trial, Photomed Laser Surg. 35 (2) (2017) 111-115.

[51] P.C. CHANG, L.Y. CHIEN, Y. YE, M.J. KAO, Irradiation by light-emitting diode light as an adjunct to facilitate healing of experimental periodontitis in vivo, J Periodont Res. 48 (2) (2013) 135-43.

[52] P. TYAGI, M. SINGH, H. KUMARI, et al., Bactericidal activity of curcumin I is associated with damaging of bacterial membrane, PLoS ONE. 10 (3) (2015) e0121313.

[53] D. CHEN, M. NIE, M.W. FAN, Z. BIAN, Anti-inflammatory activity of curcumin in macrophages stimulated by lipopolysaccharides from Porphyromonas gingivalis, Pharmacology. 82 (4) (2008) 264-9.

[54] P. HU, P. HUANG, M.W. CHEN, Curcumin attenuates cyclooxygenase-2 expression via inhibition of the NF-kB pathway in lipopolysaccharide-stimulated human gingival fibroblasts, Cell Biol Int. 37 (5) (2013) 443–8.

[55] A.A. ANTCZAK-BOUCKOMS, J.F. TULLOCH, C.S. BERKEY. Split-mouth and cross-over designs in dental research, J Clin Periodontol. 17 (1990) 446-53.

[56] E. LESAFFRE, B. PHILSTROM, I. NEEDLEMAN, H. WORTHINGTON, The design and analysis of split-mouth studies: what statisticians and clinicians should know, Stat Med. 28 (2009) 3470-82.

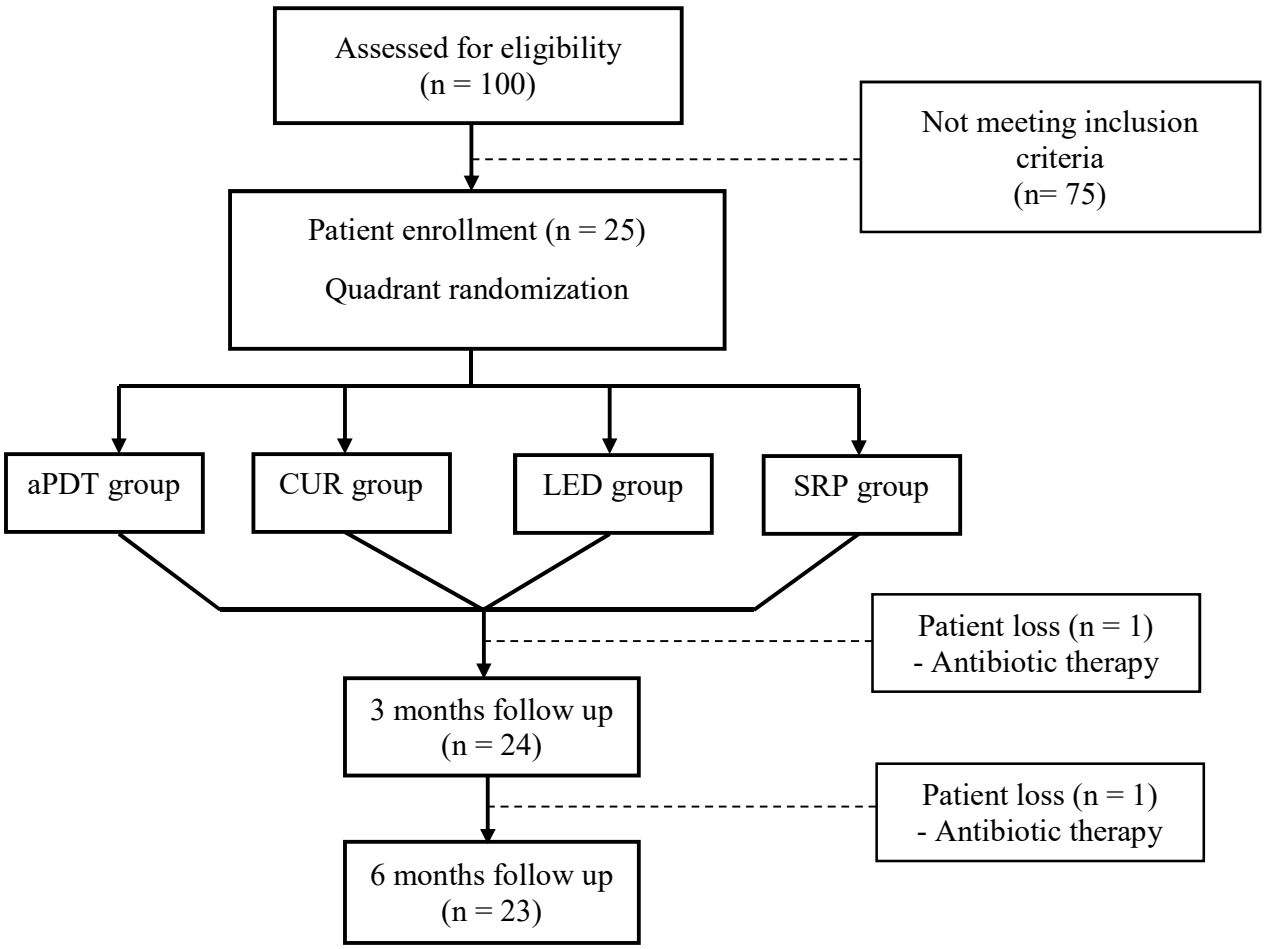

Figure 1 - Flowchart of the study design.

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.

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	4.41 ± 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†

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 ($p < 0.05$).

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

* Mean difference ± standard deviation.
** p-value for aPDT vs CUR vs SRP vs LED by ANOVA test ($p < 0.05$).

APPENDIX

Appendix A – Certificado do Comitê de Ética em Pesquisa.

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos Fotodinâmicos da Curcumina no tratamento da periodontite crônica em pacientes portadores de Diabetes Mellitus tipo 2: estudo clínico controlado randomizado.

Pesquisador: Leticia Helena Theodoro

Área Temática:

Versão: 1

CAAE: 69463517.8.0000.5420

Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.116.716

Apresentação do Projeto:

O presente estudo terá caráter experimental para avaliação clínica da terapia fotodinâmica antimicrobiana (aPDT) utilizando a curcumina como agente fotossensibilizador e irradiação com Light Emitting Diode (LED), como terapia coadjuvante à raspagem e alisamento radicular no tratamento de bolsas residuais em pacientes diabéticos tipo 2 com periodontite crônica.

Objetivo da Pesquisa:

Objetivo Primário:

Constitui objetivo do presente estudo, avaliar clinicamente a influência de um protocolo de terapia fotodinâmica antimicrobiana, associado à raspagem e alisamento radicular, no nível de inserção clínica de bolsas residuais, em pacientes diabéticos tipo 2 não controlados com periodontite crônica.

Objetivo Secundário:

Avaliação clínica do índice de placa visível, sangramento à sondagem, recessão gengival e profundidade de sondagem decorridos 90 e 180 dias do tratamento periodontal e avaliação microbiológica (Porphyromonas gingivalis (Pg) e Prevotella intermedia (Pi).

Endereço: JOSE BONIFACIO 1193
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Continuação do Parecer: 2.116.716

Avallação dos Riscos e Benefícios:

Riscos:

Os riscos referentes a esta pesquisa são médios, visto que serão realizados procedimento de Raspagem e Aplainamento Radicular (RAR). Entretanto, estes serão executados por especialistas na área. A RAR é um procedimento rotineiro na odontologia, em que se remove os microrganismos presentes supra e subgengivalmente, sendo uma terapia conservadora. Já com relação à aPDT esta é uma técnica atraumática, indolor, localizada, específica, que tem o intuito de eliminar os microrganismos presentes nas bolsas periodontais moderadas e profundas.

Benefícios:

Todos os pacientes que participarão desta pesquisa deverão ser diagnosticados com diabetes mellitus tipo 2 e periodontite. Receberão tratamento periodontal conservador, considerado padrão ouro na periodontia, associado à aPDT. A associação da aPDT como terapia coadjuvante é justificada pelo fato de pacientes diabéticos apresentarem complicações sistêmicas, sendo que as alterações observadas na cicatrização desses pacientes tem grande influência no âmbito periodontal. Além disso, a curcumina a ser utilizada como agente fotossensibilizador não apresenta toxicidade e conta com diversas propriedades biológicas, como atividade antioxidante, anti-inflamatória, antimicrobiana e anticarcinogênica (Nagpal e Sood, 2013). Com o tratamento da periodontite em pacientes diabéticos tipo 2, pode haver uma melhora no controle glicêmico, uma vez que há indícios de uma relação bidirecional entre essas duas alterações crônicas. A DM tipo 2 aumenta a propensão do desenvolvimento da doença periodontal, enquanto a doença periodontal influencia negativamente no controle glicêmico desses pacientes.

Comentários e Considerações sobre a Pesquisa:

Objetivos são claros e bem definidos.

A metodologia proposta é capaz de atender os objetivos do estudo.

Considerações sobre os Termos de apresentação obrigatória:

Os termos obrigatórios foram apresentados

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

Não havendo pendências, recomenda-se a aprovação do projeto.

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Considerações Finais a critério do CEP:

Não havendo pendências, o CEP propõe a aprovação do projeto de pesquisa salientando que, de acordo com a Resolução 466 CNS de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 01/01/2018. O CEP reitera a necessidade de entrega de uma via (não cópia) do TCLE ao sujeito participante da pesquisa e solicita ao pesquisador responsável leitura da carta circular 003/2011 CONEP/CNS antes do início do projeto.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_921000.pdf	08/06/2017 08:16:09		Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DE_PESQUISA_AUXILIO.pdf	07/06/2017 14:53:06	Leticia Helena Theodoro	Aceito
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TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	14/05/2017 18:33:21	Leticia Helena Theodoro	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

ARACATUBA, 13 de Junho de 2017

Assinado por:
André Pinheiro de Magalhães Bertoz
(Coordenador)

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Appendix B - Guide for authors (Manuscript submission - “Photodiagnosis and Photodynamic Therapy”).

PHOTODIAGNOSIS AND PHOTODYNAMIC THERAPY

ELSEVIER

AUTHOR INFORMATION PACK

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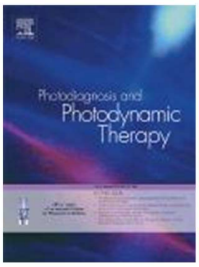

ISSN: 1572-1000

DESCRIPTION

Official Journal of the European Platform for Photodynamic Medicine Affiliated with the International Photodynamic Association
Also affiliated with the British Medical Laser Association and the Polish Society for Photodynamic Medicine

INDEXED in MEDLINE/PubMed, SciSearch/Science Citation Index Expanded, Current Contents/Clinical Medicine.

Aims and Scope:
Photodiagnosis and Photodynamic Therapy is an international journal for the dissemination of scientific knowledge and clinical developments of **Photodiagnosis** and **Photodynamic Therapy** in all medical specialties. The journal publishes original articles, review articles, case presentations, "how-to-do-it" articles, Letters to the Editor, short communications and relevant images with short descriptions. All submitted material is subject to a strict peer-review process.

AUDIENCE

Professionals in all medical disciplines with an interest in medical and biological applications of lasers and light sources, and photodiagnosis/photodynamic therapy in the treatment of human disease.

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INTRODUCTION

Scope

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List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:

[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
[2] Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000. Reference to a chapter in an edited book:
[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304. Reference to a website:
[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).
Reference to a dataset:
[dataset] [6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/xwj98nb39r.1.

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Appendix C – CONSORT 2010 checklist.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	20
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	11
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	22
	2b	Specific objectives or hypotheses	23
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	25
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	25
	4b	Settings and locations where the data were collected	25
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	26
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	28
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	25
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	26
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	26
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	26
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	26
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	28

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	28
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	-
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	44
	13b	For each group, losses and exclusions after randomisation, together with reasons	44
Recruitment	14a	Dates defining the periods of recruitment and follow-up	29
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	45
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	29
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	46
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	47
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	34
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	32
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	35
Other information			
Registration	23	Registration number and name of trial registry	25
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	-

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.