RESEARCH ARTICLE

Photo-activated disinfection with light-emitting diode reduces some key periodontal pathogens in chronic periodontitis

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Abstract

The aim of the present study was to evaluate the potential of minimally invasive periodontal treatment using repeated photo-activated oral disinfection (PAD) with red light emitting diodes as an adjunct to ultrasonic scaling (US) in the treatment of chronic periodontitis. In a single-centred, randomized clinical trial involved 40 patients with untreated chronic periodontitis received US with repeated 3 episodes of PAD (test group) or US alone (control group). Clinical parameters (plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment level (CAL)) were recorded for 12 months and subgingival biofilm analyses for five periodontal pathogens (*A. actinomycetemcomitans (Aa), P. gingivalis (Pg), P. intermedia (Pi), T. forsythia (Tf), T. denticola (Td)*) were performed for 6 months. Supportive periodontal treatment (SPT) was repeated every 3 months during the clinical trial, additional one episode of PAD was also applied in the test group. Both treatment modalities improved clinical parameters after 3 months and SPT maintained favourable clinical outcomes during 12 months. There were no statistical significant differences between the treatment groups. Statistical significant reduction of *Aa, Pg, Tf, Td* ($p<0.05$) was observed in the test group. In moderate pockets significant reduction of *Pg, Tf, Td* ($p<0.05$) and in deep periodontal pockets reduction of *Pg, Pi, Td* ($p<0.05$) was observed. Within the limits of the present study it may be concluded that the addition of PAD to US significantly reduced some key periodontal pathogens, however, patients with a history of periodontitis should be on SPT every 3-4 months.

Keywords: Clinical trial, periodontology, therapy/treatment, light emitting diode

1. Introduction

Periodontal diseases are multifactorial chronic destruction of tooth supportive tissues. The main goals of periodontal therapy are to remove supra and subgingival calculus and biofilm.[1] Mechanical instrumentation may not be sufficient, therefore photodynamic therapy (PDT) is recommended due to its bactericidal and bio-stimulating effects, especially in sites that are difficult to access for mechanical instrumentation.[2] PDT as an adjunct to mechanical debridement significant reduced some of the key periodontal pathogens in subgingival biofilm.[3] However, meta-analysis has found that adjunctive PDT provides additional short-term benefits to mechanical debridement in clinical outcomes.[4,5] Therefore, it was suggested that PDT might be useful approach in supportive periodontal therapy (SPT) to maintain the health of periodontal tissues.[6]

PDT is also used in other branches of medicine. Clinical study was proven the improvement of common chronic inflammatory disorder i.e. oral lichen planus.[7] PDT was successfully used in dermatology for the treatment of multiple actinic keratosis on the scalp, rosacea, and actinic cheilitis.[8-11] Additionally, PDT can

stimulate an anti-cancer host immune response[12], so various types of skin cancers were successfully treated with this approach.[13] PDT has high specificity of action on cancer tissue and energy from excited photosensitizer resulting in cancer cell death and destruction.[12] With promising results using PDT was also reported in ophthalmology.[14,15] Diabetic foot ulcers are one of the main complications in diabetic patients with high morbidity and mortality. PDT was demonstrated to reduce the microbial loads in infected diabetic ulcers without bacterial resistance. Therefore, the authors recommended PDT for this diabetic complication.[16]

In photo-activated oral disinfection (PAD) light emitting diodes (LED) is used and these devices are less expensive compared to diode lasers,[17] as well as easier to use with longer irradiation time possible.[18,19] Study on rats demonstrated that LED light is suitable as an adjunct treatment approach for periodontitis as it reduced inflammation and induced new bone formation.[20] The use of PAD for disinfection of periodontal pockets has also been proven to cause no thermal damage to the dental pulp.[21] However, a clinical trial revealed that application of PAD did not have additional positive effects on clinical parameters in patients with chronic periodontitis compared with scaling and root planing alone.[22] In SPT adjunctive photodynamic treatment by LED light may enhance short-term clinical and microbiological outcome.[23]

Because of the lack of data from clinical trials, the aim of our study was to evaluate the efficacy of repeated PAD using LED in red spectrum as an adjunct to ultrasonic scaling (test group) as an minimal invasive periodontal treatment approach in comparison to ultrasonic scaling alone (control group) in patients with chronic periodontitis. In addition, we analysed the effect of such treatment of periodontal pockets in cases not responding properly to initial therapy with SPT, where one episode of PAD was applied in test group. The null hypothesis to disprove was that statistically significant differences of the bleeding on probing and subgingival periodontal pathogens between test and control group at clinical trial follow up.

2. Material and Methods

2.1. Study design

The study was designed as a single-centred, randomized clinical trial and was conducted at the Unit of Oral Medicine and Periodontology, Division of Stomatology, University Medical Centre Ljubljana, between May 2015 and October 2016. The study was performed according to the tenets of the Declaration of Helsinki, the guidelines for Good Clinical Practice, National Medical Ethics Committee of the Republic of Slovenia approved the protocol (No: 144/02/11).

To detect a difference of 1 mm in PPD and CAL between two treatment groups (α=0.05, β=0.20, estimated SD=5 mm) the needed number of patients was calculated, and according to calculation 393 measuring sites in each group were needed. The assumption that each patient had had at least one periodontal pocket (out of six at single-rooted teeth) deeper than 4 mm yielded the number of twenty patients in each group (MedCalc statistical software). Between May and September 2015, 85 patients were screened with chronic periodontitis and selected 40 who were fit to inclusion and exclusion factors.

Supra-gingival deposits were removed in all teeth with an ultrasonic device (NSK Varios 970, NSK Europe GmbH, Germany) 14 days before inclusion in the study. All patients were instructed on oral hygiene practices and

had signed informed consent. The inclusion criteria used in selection of the study subjects were: adults between 20 and 70 years of age with at least 16 remaining teeth and a minimum of 4 teeth in each quadrant, a minimum of 4 sites with PPD ≥4 mm in each quadrant demonstrating bleeding on probing, and no periodontal treatment during the previous 6 months. The following conditions led to exclusion from the study: antibiotic treatment in the last 6 months, pregnant and nursing women, irradiation and chemotherapy, smoking, diabetes, use of immuno-depressant, anti-epileptic, and calcium antagonist medications. Forty patients completed the study.

The examiner (MP) measured the periodontal parameters at baseline and at supportive periodontal treatment (SPT). The treatment of the participants and microbiological samples were performed by another periodontal specialist (UM). At baseline the following periodontal parameters were evaluated using automatic periodontal probe »pa-on« (Orange dental GmbH & Co. KG, Biberach, Germany): periodontal probing depth (PPD), bleeding on probing (BOP) and clinical attachment level (CAL) all at 6 sites on each tooth. Plaque scores (plaque present = 1, no plaque = 0) were evaluated on 4 sites of each tooth. The kappa statistic was used to assess intra - examiner reproducibility. Periodontal examinations of 10 randomly selected individuals were carried out twice. The second measurements were repeated after 2 weeks. Reproducibility of assessing all periodontal parameters was tested. Intra - examiner calibration score was 0.85.

Initially, subgingival debridement was performed with an ultrasonic device (NSK Varios 970, NSK Europe GmbH, Germany). Afterwards, patients were randomly divided into 2 groups of 20 subjects. Random selection was performed by blindly picking a number from 1 to 40 out of a box of which the even numbers indicated the test group. Random selection was done by the dental assistant. In the test group, patients received combined treatment with an ultrasonic scaler, followed by 3 episodes of photo-activated oral disinfection (PAD) (on the first, third, and seventh day after the ultrasonic debridement). In the control group subgingival deposits were removed using the ultrasonic scaler (US) only.

Photosensitizer tolonium chloride (12.7 mg/ml) was applied from the bottom of the periodontal pocket towards the crown on all teeth. After 1 min of action, the photosensitizer was exposed for 60 sec/pocket (PPD >5 mm) to the red LED light (635 nm) using »smart–pad« device with max. output power of 750 mW (Orange dental GmbH & Co. KG, Biberach, Germany) with Perio tips attached to the LED lamp. In the case of PPD ≤5 mm LED lamp was used without Perio tips with the time of exposure of 60 sec/pocket. The LED light output power was checked monthly using the power check port on the device.

Control measurements were performed at 3, 6, 9, and 12 months after the initial treatment. SPT for sites with remaining PPD (≥4 mm) and positive BOP continued every 3 months. In the PAD group a single photo-activated disinfection of complete dentition was also performed.

2.2. Microbiological assessment

The same protocol of samples collection and analysis was used as described in previous studies.[24,25] Briefly, in each of the four quadrants, subgingival plaque samples were collected by means of sterile paper points from one periodontal pocket (one per quadrant from medium pockets (4–6 mm) and one per quadrant from deep pockets (>6 mm) at baseline, 3, and 6 months after the treatment. In total, 480 samples (240

samples in each group: 120 from medium and 120 from deep periodontal pockets) were obtained. The presence of five periodontal pathogens: *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf), and Treponema denticola (Td)* was qualitatively determined in each sample by multiplex polymerase chain reaction (PCR), followed by hybridization against species-specific DNA probes using a commercially available micro-IDent test (Hain Lifescience, Nehren, Germany) according to the manufacturer's instructions.

2.3. Statistical analysis

Statistical analysis of data was performed by another researcher (BG). Demographic features of study population were analysed by Mann-Whitney test and χ^2 test. Repeated measures ANOVA followed by Post hoc Newmann-Keuls test was used to compare mean values of PI, BOP, PPD, and CAL between two treatment groups at baseline, 3, 6, 9 and 12 months after treatment. The same test was also used to compare mean values of number of single-rooted and multi-rooted teeth with medium deep (4-6 mm) and deep (>6 mm) periodontal pockets between two treatment groups at baseline and 12 months after treatment. In addition, the microbiological analysis of subgingival plaque samples before treatment, 3, and 6 months after the treatment, were compared within the groups and between the groups. The level of significance was set at $\alpha = 0.05$, and the power of the tests were set at 0.80.

3. Results

Forty patients (23 women, 17 men) with moderate to severe chronic periodontitis were recruited for the study; all of them completed the 12-month clinical trial. The mean age of patients was 50.4 ± 9.0 years (range: 23-70 years) (Table 1).

TABLE 1 Demographic features of study population

Variable	All *N* = 40		Group 1. US *N* = 20		2. PDT *N* = 20		1 vs. 2 p-value
Age (years) *M*±*SD*:	50.4 ± 9.0		51.2 ± 8.2		49.1 ± 9.1		0.748[a]
Gender:	*n*	%	*n*	%	*n*	%	
Male	17	42.5%	9	45.0%	8	40.0%	0.699[b]
Female	23	57.5%	11	55.0%	12	60.0%	

*M*ean ± Standard Deviation; [a] Mann-Whitney test; [b] χ^2 test

The most obvious clinical changes occurred during the first three months after any treatment. The distributions of mean full mouth plaque index (PI) in both treatment groups at baseline until 12 months are shown in Table 2. BOP decreased in both treatment groups. After 3 months the reduction of PPD and CAL were 0.7 mm and 0.5 mm, respectively, in the test group, and 0.5 mm for both parameters in the control group. SPT maintained favourable clinical outcomes during the 12 months of the study period. There were no statistically significant differences between the treatment groups in clinical parameters at any time point (Table 2).

TABLE 2 Periodontal parameters at baseline and in maintenance period for 12 months, ultrasonic scaling followed by photo-activated disinfection (PAD), ultrasonic scaling (US)

Therapy mode	Time (months)	PPD (mm)	CAL (mm)	BOP (%)	PI (%)
PAD	0	3.1±0.4	4.1±0.9	35.4±16.9	16.5±10.7
	3	2.4±0.3	3.6±0.6	24.2±12.7	7.4±5.0
	6	2.2±0.5	3.2±0.7	23.9±11.5	6.5±3.5
	9	2.0±0.7	3.0±1.1	19.8±8.8	7.4±5.7
	12	2.1±0.7*	3.0±1.1*	17.5±7.6*	6.9±5.1*
US	0	3.1±0.5	3.8±0.8	34.9±17.5	18.2±7.3
	3	2.6±0.6	3.3±0.8	25.4±14.2	13.4±5.5
	6	2.4±0.6	3.3±0.8	24.6±13.3	8.9±5.2
	9	2.4±0.7	3.3±0.8	21.5 ±10.3	7.9±4.4
	12	2.4±0.6*	3.2±0.7*	20.5±10.7*	7.9±4.3*

PPD probing pocket depth; CAL clinical attachment level; BOP bleeding on probing; PI plaque index. Mean ± Standard Deviation; *Different from Baseline, $p<0.01$

The total number of teeth in the test group was 493 (2.958 measured sites for each parameter: BOP, PPD, CAL), of which 337 were single-rooted teeth and 156 multi-rooted teeth. The total number of teeth in control group was 499 (2.994 measured sites for each parameter), of which 335 were single-rooted teeth and 164 multi-rooted teeth.

After 3 months, the average number of teeth with PPD 4-6 mm in both treatment groups decreased significantly from baseline values ($p=0.00002$). There was also statistical difference between the treatment groups ($p<0.0006$). Similar results were found for deep periodontal pockets (PPD >6 mm). In the test and control groups the number of teeth decreased significantly from baseline to 3 months ($p=0.00002$, $p<0.0002$, respectively). There were no statistical differences between the treatment groups (Table 3).

TABLE 3 Number of teeth with medium deep (4-6mm) and deep (>6mm) periodontal pockets (PPD) from baseline till 12 months after treatment

PPD (mm)	Time (months)	ALL	PAD	US
4-6mm	Baseline	42.0 ±15.4	41.6 ±14.7	42.4 ±16.7
	3	27.6 ±18.7*	22.9 ±9.1*,§	32.4 ±24.3*
	6	22.8 ±19.0	19.0 ±10.5	26.6 ±24.7
	9	20.1 ±18.9	15.7 ±9.1	24.4 ±24.9
	12	19.3 ±19.1	14.9 ±9.6	23.7 ±24.9
>6mm	Baseline	10.8 ±9.8	10.0 ±4.7	10.2 ±13.5
	3	5.5 ±7.7*	4.4 ±4.7*	6.8 ±9.8**
	6	4.4 ±7.6	2.9 ±4.1	5.9 ±9.9
	9	3.9 ±7.7	2.2 ±4.2	5.5 ±9.9
	12	3.6 ±7.6	1.9 ±3.9	5.3 ±9.8

Mean ± Standard Deviation; *p=0.00002 vs. Baseline; **p=0.0002 vs. Baseline; §p=0.0006 vs. US

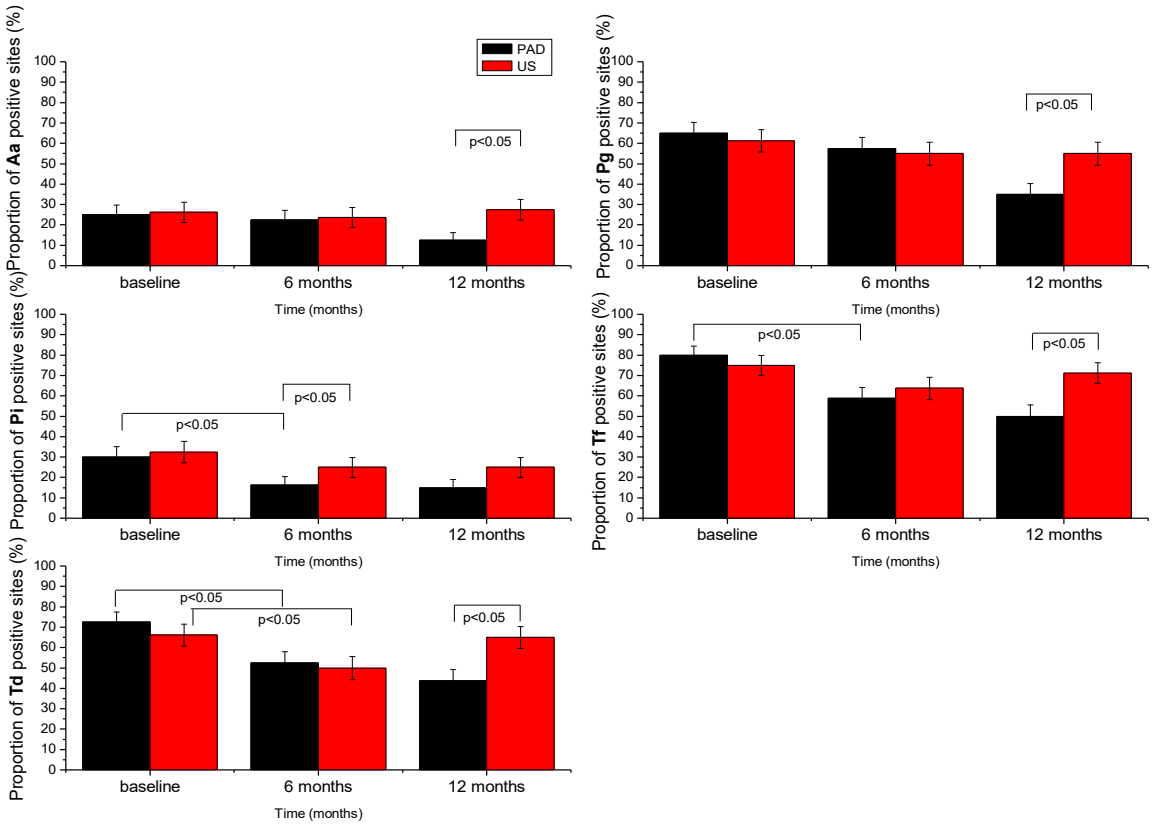

Figure 1. Microbiological analysis of subgingival plaque samples for five periodontal pathogens (*A. actinomycetemcomitans (Aa), P. gingivalis (Pg), P. intermedia (Pi), T. forsythia (Tf), T. denticola (Td)*) at baseline, 3, and 6 months after initial and supportive periodontal treatment. At six months statistical significant reduction of *Aa, Pg, Tf, Td* (p<0.05) between groups was observed.

Statistical significant reduction of *Aa*, *Pg*, *Tf*, and *Td* (p<0.05) was observed in the test group compared with control group (Fig. 1). In medium pockets significant reduction of *Pg*, *Tf*, *Td* and in deep periodontal pockets reduction of *Pg*, *Pi*, *Td* was observed (p<0.05) (Fig. 2).

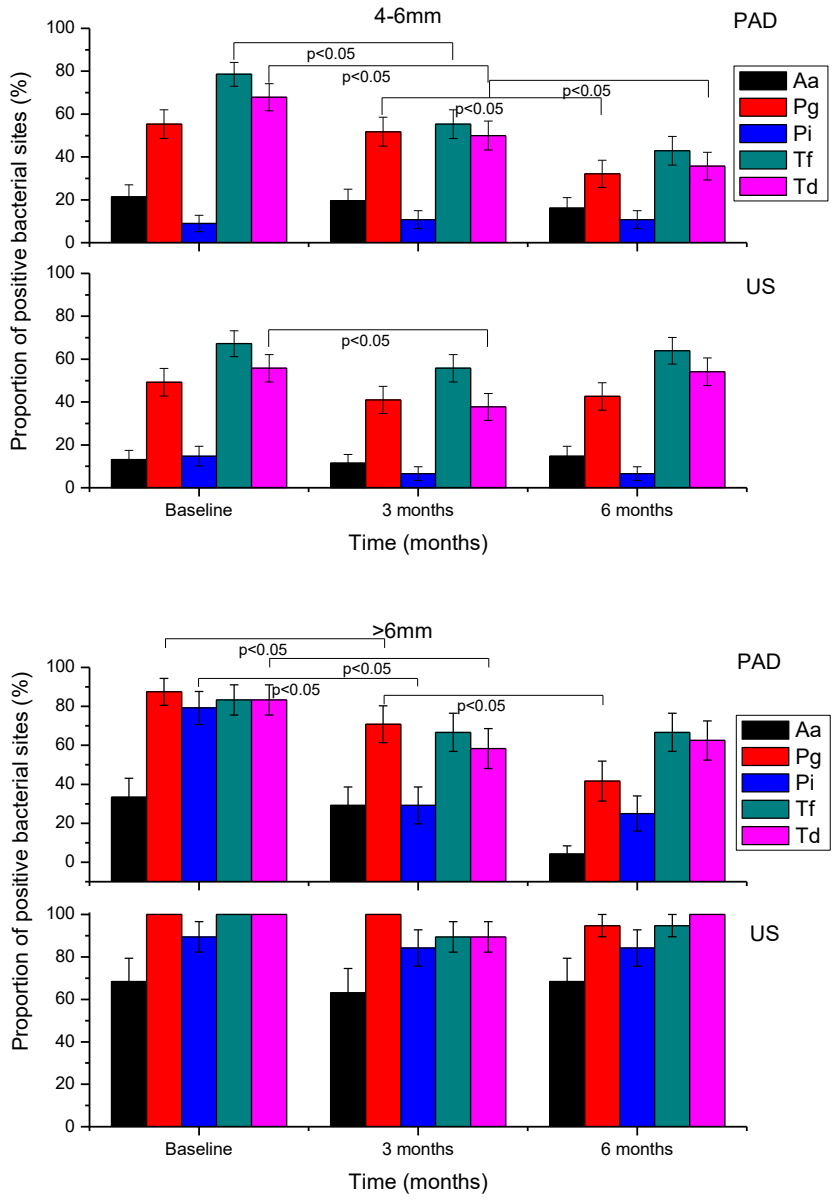

Figure 2. Proportion of positive sites of periodontal pathogens *(A. actinomycetemcomitans (Aa), P. gingivalis (Pg), P. intermedia (Pi), T. forsythia (Tf), T. denticola (Td))* in moderate (4-6 mm) and deep periodontal pockets (>6 mm) at baseline, 3, and 6 months after the initial and supportive treatment. In moderate pockets (4-6 mm) statistical significant reduction of *Tf, Td* (p<0.05) at 3 months and *Pg, Td* (p<0.05) at 6 months periods in the test group was evaluated. In the control group statistical significant reduction was observed for *Td* (p<0.05) at 3 months. In deep periodontal pockets reduction of *Pg*, *Pi*, and *Td* (p<0.05) were observed at 3 months and reduction of *Pg* (p<0.05) at 6 months in test group. In the control group the proportion of pathogens remained about the same at baseline, 3 and 6 months.

4. Discussion

The present study evaluated the effect of LED light in red spectrum when used after application of a photosensitizer using a commercially available system. The results revealed that in patients with moderate to severe periodontitis, repeated treatment with PAD in adjunct to ultrasonic debridement resulted in clinical improvements that were not significantly different from those following ultrasonic scaling alone. Additionally, PAD appliance to US statistical significant reduced some of the key periodontal pathogens.

The purpose of non-surgical periodontal treatment is to completely remove calculus and biofilm from the root surfaces. However, a number of studies have demonstrated that this purpose is frequently not achieved by mechanical debridement alone. It was showed that over 50% of root surfaces had residual calculus after subgingival scaling and root planning.[26] Complete removal of microbial biofilm is also impossible.[27] Although chronic periodontitis is the infection of periodontal tissue, the use of antibiotics may not be recommended due to the increasing resistance of periodontal pathogens.[28] Therefore, an additional approach to non-surgical treatment using PAD seems reasonable. It was found that PAD using LED is effective against single microbial species of periodontal pathogens but multi-species biofilms are less sensitive.[27] In another *in vitro* study it has been proven that the photosensitizer toluidine blue was active also on bacteria in the biofilm.[29]

The principle of PDT is that the photosensitizer binds to the outer membrane of the target bacteria followed by activation with specific wavelength of light and reactive oxygen species are released.[30] However, the low pH in the periodontal pocket and proteins that are present in gingival fluid and saliva may reduce the photosensitizer activity.[31] In addition, less oxygen is present in the periodontal pocket,[32] so the photosensitizer activation could be lower. Therefore, some researchers combine hydrogen peroxide, as a source of oxygen with the photosensitizer and thus increase its effectiveness in the periodontal pocket.[33-35]

In the present study, minimally invasive non-surgical treatment approach with ultrasonic scaler was used, which reportedly results in comparable clinical and microbial outcomes to using hand instruments or ultrasonic scalers for mechanical debridement.[36] Microbiological evaluation of subgingival biofilm were analysed and in test group compared to control group statistical reduction of *Aa*, *Pg*, *Tf*, *Td* was observed. Previously published clinical trial using PDT with diode laser (660 nm, 60 mW/cm^2) and evaluating proportions of the same five periodontal pathogens revealed that all bacterial species were decreased more effectively after PDT than after US alone, which could explain also greater BOP reduction. However, the combination of PDT with US had no additional effect on other evaluated clinical parameters such as PPD and CAL.[24] The same conclusion was found in another study where PDT was compared with SRP.[37]

Currently, there has been no established protocol for the treatment of chronic periodontitis with PAD proposed yet, therefore, the same protocol was used in the present study as in previous clinical trials in which the diode laser was used.[24,25] US has been followed by three episodes of PAD, namely on the first, third, and seventh day after mechanical debridement to disturb the subgingival biofilm formation. The possible inhibitory effect of proteins from the blood to the photosensitizer activity was reduced

by the fact that the first episode of PAD was carried out the following day after the US. In most studies PDT was applied just once,[38-42] however, in meta-analysis it was found that using PDT several times is more effective.[43] In our study 3 episodes of PAD at baseline and a single episode during SPT had additional effect on the key pathogens compared to US alone.

The results of present study indicate that most clinical changes occurred during the first 3 months after any treatment and improvements have remained about the same during the maintenance period. One of the disadvantages of periodontal treatment is that it is not possible to completely and permanently remove all etiological and risk factors.[44] Patients with a history of periodontitis have a high risk for recurrence of the disease and therefore need a carefully planned maintenance care. The presence of residual deep periodontal pockets after initial periodontal therapy may influence on further disease progression and tooth survival.[45-46] Our patients were monitored every 3 months for 1 year of the clinical trial, when ultrasonic debridement was done and followed by PAD of whole dentition in the test group.

5. Conclusion

In conclusion, we were able to demonstrate that minimally invasive treatment procedure for chronic periodontitis using the ultrasonic scaler can improve pockets depths, reduce bleeding on probing and result in clinical attachment gain, but the emphasis of the successful periodontal treatment is on the maintenance care. It is advisable to repeat the ultrasonic debridement every 3 to 4 months in patients with moderate to severe periodontitis to maintain the low level of full oral plaque index and stable condition of periodontal tissues.

Within the limits of this study it may be concluded that the addition of PAD to US during SPT significantly reduced some of the key periodontal pathogens. The clinical trial disproved the first hypothesis that there were not statistically significant differences of the bleeding on probing between groups and partially confirmed the second one that PAD reduced some of the key pathogens. Long-term studies are necessary to establish the most effective protocol of adjunctive photodynamic therapy in the treatment of chronic periodontitis and further studies are needed concerning the acceptable antibacterial efficiency against oral pathogens in subgingival environment.

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