Non-surgical periodontal treatment supplemented with photodynamic therapy

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Abstract

The objective was to prove if non-surgical therapy supplemented with photodynamic therapy results more efficient than mechanical debridement alone.

This study included 50 systemically healthy patients with at least one site with periodontal pocket ≥4 mm and with clinical attachment level ≥3 mm in two or more sites in all four quadrants of the mouth. Quadrants were divided using split-mouth design to choose one of the treatment options: scaling and root planning (SRP), SRP + photodynamic therapy (PDT), SRP + low-level-laser-therapy (LLLT) and basic therapy. Periodontal parameters taken at the baseline, four and eight weeks following the treatment included: periodontal probing depth - PPD, clinical attachment level - CAL, gingival index - GI, gingival recession - GR, and bleeding-on-probing - BOP. A blinded-to-the-treatment-procedure examiner did the periodontal examinations and performed sampling from each quadrant, using sterile paper point and transferred it in a Schaedler terrain for cultivation. The culture was incubated for 48h and read with anaerobic ID cards in the Vitek-2© machine.

We found a significant reduction of PPD, CAL, GI and BOP for the PDT treated quadrants, with similar results for LLLT and SRP-treated sites. The PDT group showed significantly higher PPD reduction than the other two study groups after four and eight weeks (P<.01 and P<.001, respectively). The difference in microbiological findings was found only between the basic therapy group and the photodynamic group only at four weeks after treatment.

PDT, as an adjunct to the non-surgical method, may enhance mechanical debridement.

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Introduction

Periodontitis as an inflammatory disease is induced by the bacteria and represents one of the leading causes of tooth loss.[1] Some systemic disorders (cardiovascular disease, preterm low birth weight[3,4]) show association with periodontal disease. There are distinct and diverse microbial types present in diseased periodontal sites. The periopathogen called *Porphyromonas gingivalis* frequently colonizes these sites and has a leading role in protease production.[5,6] Modern photodynamic therapy (PDT) had its initial use in the treatment of a dermatological tubercular condition.[7] There are three components required for PDT: the light source, the photosensitizer, and oxygen. The photosensitizer is a solution with the ability to react after the irradiation with light of a particular wavelength, and it becomes excited to a singlet state, causing a rapid and selective damage to the target cells.[8] It shouldn't be toxic, but it should display local toxicity only after activation. Dyes from the porphyrin chlorine platform and furocoumarins represent the greater part of the sensitizers.[9,10]

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There was no significant improvement reported in patients with residual periodontal pockets after standard therapy of scaling, and a diode laser and PDT as alternative methods.[11] Treatment of class II furcations with PDT, as an addition to scaling and root planning, did not promote clinical improvement, but it confirmed a reduction in local levels of cytokines and periopathogens.[12] A small number of *in vitro* studies showed selective suppression of bacteria while preserving mammalian cells. *Streptococcus sanguis* got annihilated by toluidine blue and red light, with no influence to the human gingival fibroblasts and keratinocytes.[13] Several cutaneous microbial species were eliminated using methylene blue-mediated PDT, while human keratinocytes survived.[14]

Gómez did find in a short-term study (four and eight weeks) significant decreases both in IL-1β as in TNF-α when using Nd:YAG as an adjuvant to SRP versus SRP alone.[15]

The evaluation of the PDT benefits as an adjunctive method to the conventional periodontal therapy was the aim of this study.

Materials and methods

This prospective longitudinal double-blind, randomized study, recruited fifty patients from the Periodontology and Oral Medicine Department of the University of Prishtina Dentistry School.
The University of Prishtina Medical Faculty's Ethics Committee approved this study. Periodontal parameters included: periodontal probing depth-PPD, clinical attachment level-CAL, gingival recession –GR, gingival index (Loe-Sillnes)-GI, and bleeding on probing-BOP.

Measurements of PPD, CAL, and GR were done at six sites per tooth. General and local exclusion criteria were: systemic diseases, systemic antibiotics and periodontal treatment in the last six months, lidocaine allergy, pregnancy, and lactation, and less than three teeth in a quadrant. Each patient signed a consent form after they accepted to participate in the study.

The study design included the split-mouth methodology, using four quadrants of the mouth as study/control groups. Eligibility for assigning a patient for this study was the presence of a periodontal pocket 4 or more millimeters in at least one site and CAL 3 or more millimeters in at least one tooth of each quadrant. Each quadrant randomly assigned to a treatment group. The control group was assigned the quadrant receiving only basic therapy. The quadrant receiving scaling and root planning (SRP) was assigned to test group 1. The quadrant receiving SRP and low-level laser therapy (LLLT) was assigned to test group 2. The quadrant receiving SRP and single-episode photodynamic therapy (PDT) was assigned to test group 3. All the quadrants received basic therapy. Three quadrants received SRP, except the control quadrant. After receiving SRP, two random quadrants were assigned to undergo either LLLT or PDT (groups 2 and 3, respectively) while the third quadrant with SRP received no further treatment (group 1). A blinded-to-the-treatment periodontist recorded periodontal parameters at the baseline, and after four and eight weeks following the treatment. The working hypothesis was that PDT after SRP would produce significantly better results than SRP alone.

Medical diode laser HELBO TheraLite® laser (HELBO mini laser 2075 F dent; HELBO, Walldorf, Germany) that emits continuous laser radiation was used. The laser light was monochromatic with wavelength 660 nm and power 100 mW. Helbo 3D Pocket Probe® insert (HELBO, Walldorf, Germany) was used to apply this light into the periodontal pocket, providing power density 60 mW/cm^2. The total radiation surface of the insert working length (8 mm) was 0.17 cm^2 (Figure 1).

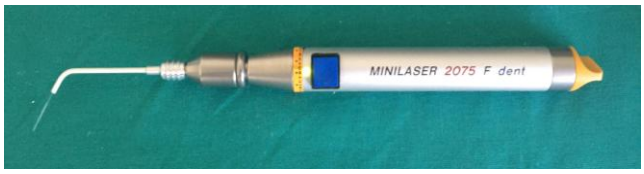

Figure 1. The diode laser with insert.

The photosensitizer dye was phenothiazine chloride containing 1% phenothiazine -5-ium, 3,7-bis (diethylamino) chloride, buffered with citrate buffer of pH 3.5, isotonized and its viscosity modified with 1% hydroxypropyl methylcellulose (HELBO, Walldorf, Germany). The dosage was 10 mg/ml. Each site was filled up to its top with the photosensitizer using a blunt cannula as instructed by the manufacturer. The dye stayed for one minute and then it was rinsed using NaCl 0.9% solution. The laser light application had the total duration of one minute (Figure 2).

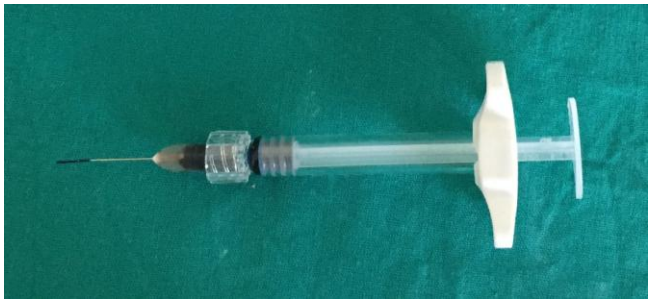

Figure 2. The photosensitizer.

The LLLT group was treated Laser HF® (Hager & Werken, Duisburg, Germany) with the same characteristics of the laser light, but without the photosensitizing agent (Figure 3).

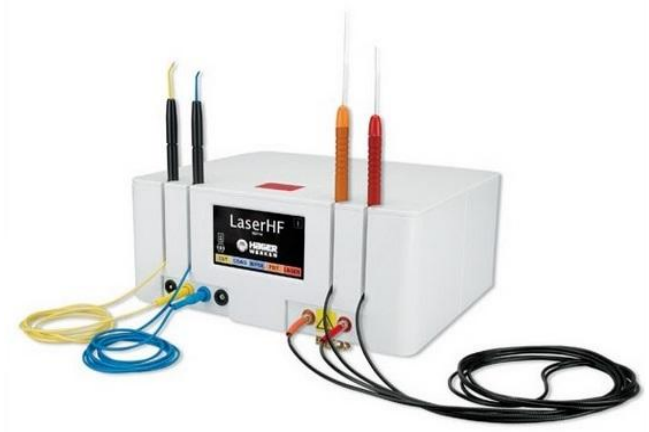

Figure 3. LaserHF for the LLLT group.

The microbiological examination determined the presence of periopathogenic bacteria in the periodontal lesions in all working groups before and after the treatment. The microbiological analysis of the samples was performed in the Microbiology Department of the National Institute of Public Health of Kosova. The sample was taken using sterile paper point #50 inserted in the periodontal pocket for ≈ 10 seconds and then transferred on the cultivation terrain of Schaedler agar plate enriched with 5% sheep blood (Biomérieux, France). A plastic bag hermetically closed the plate, creating favorable conditions with an enclosed anaerobic generator and anaerobic indicator. The bag was immediately transferred to the Microbiology Department and incubated for 48 hours in 35-37^{0}C. The reading from the terrain was performed on Vitek® 2 compact (Biomérieux, France) using Vitek® 2 ANC ID Card (Biomérieux, France). This card detects nearly 90 types of anaerobic and coryneform bacteria, including some periopathogenic bacteria, such as *P. gingivalis*, *P. intermedia*, *P. melaninogenica*, *F. nucleatum*. The presence at least one of periopathogenic bacteria was recorded as a positive finding.

Computer statistical software Statistica was used for analysis. The data were analyzed using T-test and One Way ANOVA. The posthoc Tukey test was used to evaluate pairs of study groups and also pairs of same working groups in different examinations.

Results

Mean and median age of the examined patients was 34, with a range from 20 to 55. A slightly higher percentage of the patients was male (52%) than female (48%). More than ¾ (78%) of the patients were residents of urban areas, while less than ¼ (22%) of them being from the countryside. Four out of five subjects of this study were with secondary school. (Table 1)

Age (years)			Gender N (%)		Residing N (%)		Education N (%)		
Mean	Median	Range	Female	Male	Urban	Rural	Primary	Secondary	High
34	34	20-55	24 (48)	26 (52)	39 (78)	11 (22)	6 (12)	40 (80)	4 (8)

Table 1. Sociodemographic data.

The periodontal parameters recorded at the baseline examination showed no significant difference between groups, presuming that the periodontal condition at starting point of the treatment was identical for all quadrants. (Table 2)

Parameter	Group	Mean	Standard deviation	ANOVA *P*
Periodontal pocket depth (mm)	Control	2.27	0.61	0.73
	SRP	2.30	0.58	
	LLLT	2.28	0.58	
	PDT	2.35	0.57	
Clinical attachment level (mm)	Control	2.35	0.70	0.8188
	SRP	2.38	0.66	
	LLLT	2.37	0.67	
	PDT	2.43	0.65	
Gingival recession (mm)	Control	0.09	0.22	0.5221
	SRP	0.10	0.20	
	LLLT	0.10	0.20	
	PDT	0.09	0.21	
Gingival index (0-3)	Control	1.55	0.32	0.5319
	SRP	1.58	0.37	
	LLLT	1.54	0.28	
	PDT	1.59	0.26	
Bleeding on probing (%)	Control	77.8	16.5	0.8463
	SRP	76.4	14.9	
	LLLT	76.7	13.7	
	PDT	77.6	13.6	

Table 2. Periodontal parameters at the baseline.

One-way ANOVA for PPD at the second examination has *P*=.0004, with the lowest values in the PDT-treated group, with posthoc Tukey the differences are significant (*P*=.005) only between the control group and PDT group. The posthoc Tukey test for CAL has the same results, with one-way ANOVA *P*=.0036. GI and BOP had very high ANOVA *P*-values, presenting highly significant improvement in these parameters. The only parameter that didn't show a significant difference is GR. (Table 3)

Parameter	Group	Second examination		Third examination	
		Mean (SD)	ANOVA *P*	Mean (SD)	ANOVA *P*
Periodontal pocket depth (mm)	Control	2.34 (0.61) *	.0004	2.33 (0.61) ‡	.00004
	SRP	2.21 (0.58)		2.18 (0.58) §	
	LLLT	2.17 (0.58)		2.14 (0.58) *	
	PDT	2.02 (0.57) *		1.90 (0.57) ‡ § *	
Clinical attachment level (mm)	Control	2.42 (0.70) †	.0036	2.41 (0.70) ‡	.0005
	SRP	2.29 (0.66)		2.27 (0.66) §	
	LLLT	2.26 (0.67)		2.23 (0.67) *	
	PDT	2.10 (0.65) †		1.98 (0.65) ‡ § *	
Gingival recession (mm)	Control	0.09 (0.22)	.9614	0.09 (0.22)	.8527
	SRP	0.08 (0.20)		0.09 (0.20)	
	LLLT	0.09 (0.20)		0.09 (0.20)	
	PDT	0.08 (0.20)		0.08 (0.20)	
Gingival index (0-3)	Control	1.49 (0.32)	<.0001	1.40 (0.32)	.0004
	SRP	1.28 (0.37)		1.08 (0.36)	
	LLLT	1.14 (0.27)		0.84 (0.28)	
	PDT	1.05 (0.27)		0.64 (0.26)	
Bleeding on probing (%)	Control	72.9 (16.6)	<.0001	69.3 (16.6)	.0002
	SRP	65.7 (15.1)		55.7 (14.9)	
	LLLT	65.6 (13.6)		57.8 (13.5)	
	PDT	61.3 (13.5)		52.1 (13.4)	

Table 3. Periodontal parameters at the second and third examination.
SD: standard deviation. Tukey test: * *P*=.005, † *P*=.006, ‡ *P*=.004, § *P*=.003.

At the third examination, all parameters, except the GR, showed a significant difference. PPD, CAL, GI, and BOP have lowest values in PDT group, and the one-way ANOVA for all these parameters showed a highly significant difference, with *P*<.0001. Post-hoc Tukey test for PPD indicates that difference exists between control vs. PDT, SRP vs. PDT and LLLT vs. PDT (*P*=.004, *P*=.003, and *P*=.005, respectively). This test for CAL has same differences as for PPD. As for comparisons of GI and BOP between groups, the differences are significant for all groups. (Table 3)

The least presence of the periopathogenic bacteria was recorded in the PDT group at the second examination, showing 25% reduction from the baseline with *P*=.008. But, after eight weeks, the presence of these bacteria showed a slight increase, with PDT group least colonized and presuming that recolonization of the sites has occurred, regardless of the treatment option. (Table 4)

Group	Baseline (%)	Second examination (%)	Third examination (%)
Control	100.0	91.7*	93.3
SRP	100.0	81.7	85.0
LLLT	100.0	79.2	80.8
PDT	100.0	75.0*	79.2
P	NS	*.008	NS

Table 4. Percentage of positive findings of periopathogenic bacteria.
NS: non-significant.

Discussion

The results have shown that the application of PDT as an adjunctive treatment regime, besides the standard scaling and root planning, has a beneficial effect on the clinical periodontal parameters, and some improvement of the microbiological composition of the treated sites. Conventional mechanical treatment, with ultrasonic debridement, of persistent pockets in a clinical study, produced identical clinical results like PDT in patients with chronic periodontitis.[16] It also reported that microbial counts showed significant reduction of about 30% to 40% immediately after treatment, but returned to baseline values after three months in both treatment groups.

In a study comparing the treatment of aggressive periodontitis with PDT and systemic administration of amoxicillin and metronidazole, showed that both methods resulted in significant reduction of all periodontal parameters, and could not conclude that application of PDT after SRP is a better alternative to systemic antibiotics.[17] Out of 141 pockets with depth ≥7 mm in the antibiotics group, only three sites showed no improvement, and in the PDT group, the number reduced from 137 to 45. A randomized clinical trial using SRP with PDT or SRP alone for patients with chronic periodontitis reported short-term improvement of periodontal parameters.[18] The results of this study are somewhat identical to those of our study, showing that photodynamic treatment may enhance traditional periodontal therapy for better clinical results.

Other studies have shown that PDT is not superior to conventional periodontal therapy when treating aggressive periodontitis lesions.[19] In a study of treatment either with SRP or PDT, the three-month evaluation of ten pairs of contralateral maxillary single-rooted teeth with probing depth ≥5 mm, showed significant improvement of all clinical parameters, but

without statistically significant difference between the groups. In our study, mean PPD and CAL, as well as GI and BOP, showed major improvements at four and eight weeks compared to baseline examination, except for GR. This difference in the outcomes shows the contradictions arising from the variety of study designs used and conditions investigated.

One of the limitations of our study was that detection of periopathogenic bacteria is recorded as positive after identifying at least one of the red-complex bacterium. Thus, it could not determine the quantitative microbial load in the (un-)treated sites with periodontal lesions.

Conclusions

From the results of our study, we may conclude that photodynamic therapy might contribute to the overall success of conventional periodontal therapy showing significant improvement of periodontal condition. On the other hand, the microbiological composition of the treated sites did not significantly improve from the baseline. Long-term clinical and more complex microbiological studies are needed to elucidate the mechanism of action of this novel therapeutic approach.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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