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ORIGINAL RESEARCH

Antibacterial Effects of Photodynamic and Diode Laser Therapies as Adjunctive Treatments in Periodontitis

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Abstract

Background: Besides standard periodontal procedures, additional approaches, including medication, laser therapy, and photodynamic therapy are employed to manage excessive inflammation and pathological reactions. The present study aimed to evaluate laser and photodynamic therapy (PDT) as complementary treatments for periodontitis.

Materials and Methods: This clinical trial enrolled 12 patients (30-60 yrs.)With periodontitis stages I and II. Following initial treatment, one quadrant served as the control, one treated with a diode laser, and the other one with Photosan wavelength power with methylene blue dilution. Clinical variables including bleeding on probing (BOP), pocket depth (PD), and clinical attachment level (CAL) were measured and surveyed. Also, samples from gingival crevicular fluid were taken at 2 and 6 weeks for evaluation. The data collected were analyzed using the Friedman test, ANOVA, Kruskal-Wallis, and LSD post-test.

Results: The mean bacteria levels in all three treatment groups were significantly reduced ($P < 0.001$). There were no significant differences in P.g and P.i bacteria ($P > 0.05$) in all three treatment methods. A.as was notably reduced at 6 weeks ($P = 0.037$). CAL and PD significantly decreased in all three treatment groups ($P < 0.001$), with no statistically significant difference between the treatment methods ($P > 0.05$). The mean BOP significantly decreased in the laser therapy ($P < 0.001$) and PDT groups ($P < 0.002$).

Conclusion: This study found that diode laser therapy and PDT can effectively reduce periopathogens, particularly A.a, and improve clinical signs in patients with periodontitis stages I and II.

Keywords: Chronic periodontitis; Laser therapy; Photodynamic Therapy

Introduction

Gingivitis and periodontitis, primarily, are caused by complex bacterial biofilms that contain periopathogenic microorganisms on tooth surfaces. Certain bacterial species are more strongly associated with the active phase of the disease, so controlling and suppressing these species can lead to better clinical outcomes following treatment (1). While mechanical removal of these biofilms is the foundation of periodontal treatments, it is often insufficient. Thus, Host modulation treatments (HMT) are used to modulate excessive inflammatory and pathological responses without disrupting the body's natural defence mechanisms of inflammation (2). The most common HMT is medications like Doxycycline (3). Laser and photodynamic therapy (PDT) are other beneficial adjunctive treatments.

These treatments use a range of laser beams with various wavelengths and mechanisms to destroy periopathogenic bacteria, including those that invade tissues inaccessible by usual methods of periodontal therapy (4).

PDT action is established on dye adsorption to the surface of the bacterial cell membrane based on electrical charge, and activation of this agent by electromagnetic radiation destroys the bacterial cell membrane and lipid peroxidation of the tissue by generating single oxygen (5).

PDT is an alternative treatment for antimicrobial chemicals in eliminating sub-gingival bacteria during periodontal treatment (6). Studies have examined periodontal adjunctive therapies and compared them to mechanical treatments (7). Analyzing gingival

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sulcus fluid (GCF) can be a reasonable method to evaluate the effect of these treatment modalities on periopathogenic bacteria (8). Among the main periopathogenic bacteria in periodontal diseases, Aggregatibacter actinomycetemcomitans bacteria (A.a), Porphyromonas gingivalis (P.g), and Prevotella intermedia (P.I) have essential roles in the progression of periodontal disease(9). Destroying periopathogens by mechanical and adjuvant treatments seems necessary, although previous research on the antimicrobial properties of laser and photodynamic therapies has yielded conflicting results. Thus, the present study aimed to compare the effects of these complementary periodontal treatments on the clinical status and number of main periopathogens.

Materials and Methods

This clinical trial study with registration code IRCT2014040617143N1 was performed on patients referred to the Periodontics ward, School of Dentistry, Islamic Azad University, Isfahan (khorasgan) Branch in 2014.12 patients aged 30-60 years old with moderate to severe generalized chronic periodontitis and at least having four sites with probing depth of 4-mm in each quadrant were selected. (10) Three quadrants were selected as the studied quadrants in all samples. Systemic diseases, medications, pregnancy, lactation, consumption of tobacco and alcohol, broad-spectrum antibiotics during the last six months, periodontal treatments in the previous 12 months, and plaque index above 40% were exclusion criteria.

After obtaining written informed consent, an initial periodontal examination was performed, during which clinical parameters including bleeding on probing (BOP), pocket depth (PD), and clinical attachment level (CAL) were recorded for each patient.

Subsequently, three quadrants for each patient were allocated randomly to quadrant 1 (control), quadrant 2 (diode laser therapy), and quadrant 3 (PDT). In all these quadrants only phase I of periodontal treatment was conducted. In the first phase of laser treatment (initial laser curettage) an 810 nm laser diode set (Dr. Smile, Italy) was used to reduce the number of periodontal bacteria. The power setting of the machine was 1 W with a fibre tip diameter of 300 μm with rotational movements to the base of the periodontal pocket. A week after the initial treatment, the second phase of laser-assisted periodontal therapy was performed. In this step, to delay epithelial re-growth and eliminate A.a bacteria invasion in tissues, the sulcus de-epithelization procedure was performed on the outer surface of the gingival margin and the inside of the sulcus with cautions for minimal injury to the underlying lamina propria. The fibres were cleaved before each irradiation session for the maintenance of their initial physical characteristics (11).

In the quadrant treated by PDT, the treatment procedure was performed using the photosan system (DK-2800; CMS Dental, Copenhagen, Denmark) and the light-sensitive high-viscosity material of toluidine blue. The material was placed inside dental pockets and irradiated by periodontal head No. 15 for 10 sec. Then, the first phase of the SRP was performed using an ultrasonic device (12).

GCF sampling procedure

After isolation of the area in each quadrant, with paper cone No 25, GCF samples were taken from four regions of the deepest pockets. Afterward, they were placed in sterile screw pipes with a 5-mm autoclaved intermediate *thioglycolate broth medium*. All samples were transferred to the laboratory in less than 30 min in anaerobic jars for culturing in an absolute anaerobic environment.

A 5% sheep blood brucella agar fibrinated culture was used for anaerobic bacteria. Cultures were performed according to bacteria-specific needs. Vitamin K1 and 5% horse serum have been added to improve the culture environment.

The sampling procedure was done once at baseline before any of the treatments, then at recalls 2 and 6 weeks after the first treatment session. Clinical parameters, including PD, BOP, CAL, and plaque index, were recorded, and the GCF samples were collected again according to the procedure described above.

Sample culturing technique

The Colistin antibiotic, vancomycin, and kanamycin with vancomycin antibiotics were used to culture P.g., P.I, and A.a, respectively. All plates were placed in anaerobic jars and evaluated after 48-72 h at 37 °C. Samples were cultured in two stages and underwent mass and differential analysis with positive and negative controls.

The data were analyzed in SPSS software (version 24) using the Kolmogorov–Smirnov test for the normality of the distribution, The Friedman test was performed to compare the means of the groups (laser, PDT, control). ANOVA was used for multiple group comparisons at different times (first, 2 weeks, and 6 weeks), and LSD post-test was performed to compare the two groups. Kruskal-Wallis's test was used to compare the difference in the mean of gingival bleeding index in comparison with groups at various times ($\alpha = 0.05$).

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Results

This study was conducted on three groups: laser treatment, photodynamic therapy, and the control group. The results of the Friedman test revealed the mean level of A.a, P.g, and P.i bacteria at two and six weeks after laser treatment (P<0.001), PDT (P<0.001), and control group (P<0.022) underwent a significant reduction (Table 1).

Table 1: Comparisons of the mean value of A.a, P.g and P.I in treatment groups at various times

Groups	Times	P.I	P.g	A.a	value P
		Mean± SD	Mean± SD	Mean± SD	
Laser	Initial	527 ± 398	1384 ± 1378	6818 ± 6041	< 0.001
	2 weeks	280 ± 205	670 ± 568	3573 ± 3009	
	6 weeks	178 ± 131	310 ± 280	1492 ± 1326	
PDT	Initial	432 ± 252	919 ± 878	4027 ± 2557	< 0.001
	2 weeks	319 ± 192	607 ± 517	2063 ± 1485	
	6 weeks	146 ± 89	340 ± 365	945 ± 556	
Control	Initial	352 ± 302	355 ± 109	601 ± 328	0.022
	2 weeks	195 ± 142	254 ± 66	442 ± 231	
	6 weeks	235 ± 181	252 ± 102	471 ± 279	

The results of the ANOVA test revealed that the mean of A.a bacteria in all three groups was not significantly different at baseline to 2 weeks (P = 0.084). However, it significantly decreased at baseline to 6 weeks (P = 0.036) and from 2 weeks to 6 weeks (P = 0.037) (Table 2).

Table 2. The mean of A.a bacteria at various times after treatment in 3 groups

Groups	Times	Mean ± SD (Colony)	value P
Initial- 2 weeks	Laser	3244 ± 3560	0.084
	PDT	1964 ± 1516	
	control	158 ± 103	
Initial- 6 weeks	Laser	5326 ± 5045	0.036
	PDT	3082 ± 2297	
	control	130 ± 67	
2weeks- 6weeks	Laser	2081 ± 1849	0.037
	PDT	1118 ± 1194	
	control	61 ± 45	

The LSD test showed that there was a significant difference only between the laser group and the time between the first and two weeks (P=0.030), and there was no significant difference in the other groups (p>0.05).

The mean level of P.g bacteria in all three treatment groups was not significantly different at baseline to 2 weeks (P = 0.21), at baseline to 6 weeks (P = 0.149), and from 2 weeks to 6 weeks (P = 0.187) (Table 3).

The mean of P.I bacteria in all three treatment groups was not significantly different at baseline to 2 weeks (P = 0.457), baseline to 6 weeks (P = 0.286), and from 2 weeks to 6 weeks (P = 0.148) (Table 4).

The results of the Friedman test revealed that the mean clinical attachment loss (CAL) significantly reduced at baseline (P < 0.001), two (P < 0.001), and six weeks (P = 0.037) after treatment with laser, PDT, and in the control group. The mean pocket depth (PD) significantly reduced at baseline (P < 0.001), two (P < 0.001), and six weeks (P = 0.009) in the laser, PDT, and control group (Table 5).

Table 3. The mean of P.g bacteria at various times after treatment in 3 groups

Groups	Times	Mean ± SD (Colony)	value P
Initial- 2 weeks	Laser	714 ± 912	0.210
	PDT	312 ± 596	
	control	101 ± 65	
Initial- 6 weeks	Laser	1074 ± 1245	0.149
	PDT	580 ± 738	
	control	103 ± 35	
2weeks- 6weeks	Laser	360 ± 377	0.187
	PDT	267 ± 264	
	control	58 ± 35	

Table 4. The mean of P.I bacteria at various times after treatment in 3 groups

Groups	Times	Mean ± SD (Colony)	value P
Initial- 2 weeks	Laser	251 ± 281	0.457
	PDT	142 ± 158	
	control	157 ± 160	
Initial- 6 weeks	Laser	349 ± 320	0.286
	PDT	285 ± 248	
	control	117 ± 123	
2weeks- 6weeks	Laser	112 ± 80	0.148
	PDT	174 ± 164	
	control	50 ± 31	

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Table 5. Comparison of mean values of CAL and Pd in 3 groups at various times

Groups	Times	PD	CAL	Pvalue	value P
		Mean± SD	Mean± SD	CAL	PD
Laser	Initial	3.3 ± 0.19	4.9 ± 0.99	< 0.001	< 0.001
	2 weeks	2.8 ± 0.15	3.5 ± 0.51		
	6 weeks	2.4 ± 0.12	2.7 ± 0.45		
PDT	Initial	3.5 ± 0.22	4.9 ± 0.99	< 0.001	< 0.001
	2 weeks	3.2 ± 0.16	3.7 ± 0.62		
	6 weeks	2.8 ± 0.18	2.8 ± 0.38		
Control	Initial	3.4 ± 0.18	4.8 ± 0.83	0.037	0.009
	2 weeks	3.2 ± 0.12	4 ± 0.0		
	6 weeks	2.9 ± 0.18	3 ± 0.0		

The results of the ANOVA test revealed that the mean CAL in all three treatment groups was not significantly different at baseline to 2 weeks (P = 0.225), baseline to 6 weeks (P = 0.295), and from 2 weeks to 6 weeks (P = 0.891) (Figure 1).

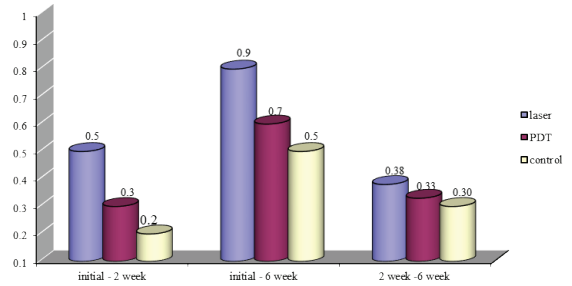


Figure 1. The mean of CAL at different times after treatment in 3 groups

The mean pocket depth (PD) in all three treatment groups was not significantly different at various times (Figure 2).

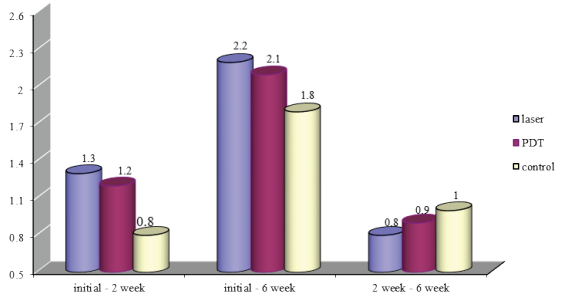


Figure 2. The mean of PD at different times after treatment in 3 groups

The mean value of bleeding on probing significantly decreased after 2 weeks and 6 weeks from baseline in the laser (P<0.001) and PDT treatment (P=0.002) groups; however, it did not differ significantly in the control group (P=0.223). According to the results of Kruskal-Wallis's test, the mean value of bleeding on probing did not show a difference between the studied groups in three different time intervals (Figure 3).

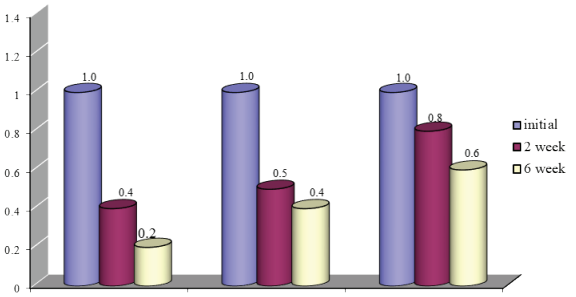


Figure 3. The mean of BOP at different times after treatment in 3 groups

Discussion

Traditional therapies, like SRP in periodontitis with deep pockets, will not result in complete bacterial biofilm removal. Furthermore, antibiotic resistance is increasing in microorganisms involved with residual deep pockets, presenting challenges to periodontal health. Thus, alternative treatment modalities, such as laser therapy or PDT, are necessary to improve treatment outcomes (13).

After analyzing all three groups (laser, photodynamic, and control), this study found that the number of A.a, P.g, and P.i bacteria decreased significantly. This was followed by improvement in clinical parameters such as CAL, PD, and BOP. previous studies have shown that soft tissue laser therapy is effective in controlling periodontal diseases. laser irradiation has bactericidal effect that improves gingival indices and reduces the number of bacteria, including P.g and P.i,for up to six months after treatment (14, 15). Other Studies have indicated that PDT helps reduce bacterial pocket contamination, promote soft tissue, and bone healing, and resolve inflammation. (16).

According to the findings of our study, PDT can significantly reduce the number of A.a. A reduction in P.g and P.i was seen, although not significant. These results are consistent with those of previous studies, including the research conducted by Voos et al. (7) and Silva et al. (17). However, Sigusch et al. (3) reported that 4 and 12 weeks after PDT, periopathogenic bacteria

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can significantly reduce, which is inconsistent with the results of the present study. This inconsistency may be due to the differences in the experimental design since Sigusch et al. (3) studied a mixture of bacteria instead of a specific bacterium; moreover, their study had a longer duration. In AlAhmari's (18) review of 36 studies on the effects of photodynamic therapy in periodontal diseases, 149 articles appeared from various sources, and 36 were related to the research objective. They showed that adjunctive treatment with PDT may promote additional clinical and microbiological outcomes, although PDT photosensitizers, wavelength, number of sessions, and treatment durations are not understood well. Therefore, large, randomized control trials with longer follow-ups are needed to assess the potential of PDT in the treatment of periodontal disease. According to previous studies, dyes can reduce the levels of certain bacteria since they specifically react with bacterial membranes and make them more exposed and susceptible to applied radiation. Therefore, the type of reagent used in the PDT as the light-sensitive dye is one of the factors that influence the effect of PDT on periopathogenic bacteria (19).

In 2005 Sigusch, et al. (19) concluded that chlorine 600 reduces the P.g bacteria. The dye used in his study was toluidine blue which reduced P.g and P.i, compared to the control group; however, this was not significant. The most effective light-sensitive agent and the best duration of time are not identified yet (20).

The PDT also inactivates the virulence factors of periopathogenic bacteria, which was not examined in the present study. Nevertheless, based on the clinical parameters, and especially the significant reduction of BOP, it can be mentioned as one of the effects of PDT (21).

As mentioned before, the significant decrease in the level of A.a bacteria versus the non-significant, while the significant decrease in the level of P.g and P. I bacteria might be due to a special dye which only reduced specific bacterium. Based on the review of the related literature, few studies used the same dye; nevertheless, they achieved the same results regarding the A.a bacteria, such as the research conducted by Mattiello et al. (22).

Based on the results of the present study, after the application of PDT (2 and 6 weeks), the clinical signs (CAL, PD, BOP) improved, and the BOP parameter underwent a significant reduction. These results are in line with the study performed by Azarpazhooh et al. (23). However, studies have emphasized the notable effects of PDT on clinical parameters, such as the research conducted by Andersen et al. (24) in 2007 and Lulic et al. (25) in 2009. That may be due to the longer duration of these studies.

Previous studies did not examine CAL as it requires a long time for its changes to become recordable, and it is among the last parameters that undergo reduction. In the present study, no significant reduction was observed in the CAL.

According to the findings, there was a marked reduction in periopathogenic bacteria rate with laser treatment, compared to PDT and control groups. A.a reduction was seen significantly only after 6 weeks. A.a species are overly sensitive to temperature changes compared to other periopathogenic species; therefore, increasing temperature killed the bacteria quickly even with low-intensity laser photons (9).

The gradual reduction observed in the level of P.g and P. I show that repeating mechanical and adjunctive treatments could reduce periopathogenic bacteria more significantly. These findings are consistent with the results of the study conducted by De Micheli et al. (26) and Jiang et al. (27); however, they are inconsistent with the results of other studies. Chan and Lai (28) have mentioned that wavelength and energy density influence the function and efficiency of the lasers. In addition, wavelength and optimal doses are considered practical variables in the bactericidal process. Sometimes, improper laser system setups can cause reversed results. (29).

In 2014, Porteous and Rowe (30) mentioned influential factors such as periopathogenic bacteria behavior in biofilm and differences in tissue response to laser therapy depending on the type and health status of the tissue could be involved in different results between studies. They also found that laser therapy and PDT are the most effective on target tissues, and other factors involved are the composition of the biofilm, laser wavelength, and energy. As these factors are uncontrollable, and there is no unanimous agreement on the preventable causes, further studies are suggested.

Based on the results, the clinical parameters (PD, CAL, and BOP) in laser and PDT groups achieved greater improvements, compared to the control group. This change was significant for the BOP parameter. This is consistent with the study conducted by Slot et al. (15) in 2014, which reviewed the effect of diode lasers on clinical parameters in 416 studies. They reported that the PD and CAL changes were moderate with significant differences in the BOP compared to the control group. The results reported by De Micheli et al. (26) indicated no significant differences between the study and control groups.

A comparison of microbiological and clinical parameters results between laser and PDT treatment methods revealed no significant difference concerning the studied items. However, in all cases, laser showed better results than PDT. The obtained results are consistent with previous studies (31, 32), including the research performed by Sigusch et al. (19).

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Conclusion

Adjunctive treatment with laser and PDT can reduce periopathogenic bacteria rates being less remarkable in the early weeks (2 weeks), while it became more profound in the final weeks of the study (6 weeks). Evaluation of the effects of treatments on clinical parameters indicated that the clinical signs improved, especially for the BOP parameter, in which the changes were more significant in the final weeks of the study (6 weeks).

Conflict of Interests: None

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