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The Effect of Diode Laser (980 nm) Treatment on Aggressive Periodontitis: Evaluation of Microbial and Clinical Parameters

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Abstract

Objective: The aim was to compare the effect of scaling and root planing (SRP) alone, diode laser treatment (LAS) alone, and SRP combined with LAS (SRP + LAS) on clinical and microbial parameters in patients with aggressive periodontitis. ***Materials and Methods:*** Thirty patients with aggressive periodontitis were assessed for plaque, bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). Four plaque samples were randomly obtained, one from each quadrant that was randomly assigned to SRP alone, SRP + LAS, LAS alone, and control (CRL). A 980-nm diode laser was used in continuous mode at 2 W power. Plaque samples were collected 2 wk, 12 wk, and 6 mo post-treatment. The levels of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, and total bacterial load (TBL) were evaluated using ssrRNA probes. ***Results:*** Bacterial counts were decreased with all three treatment modalities and they did not reach baseline levels at 6 mo post-treatment. The SRP + LAS group showed statistically significantly lower TBL and bacterial levels of *P. gingivalis* and *T. denticola* at 6 mo post-treatment compared to SRP or LAS treatments alone. At the end of the observation period significant differences were observed for PPD and CAL between the SRP + LAS group and both the SRP alone and LAS alone groups. No differences were detected for percentage of plaque and percentage of BOP between any of the treatment groups at 6 mo post-treatment. ***Conclusions:*** Within the limits of this study, diode laser–assisted treatment with SRP showed a superior effect over SRP or LAS alone for certain microbial and clinical parameters in patients with aggressive periodontitis over the 6-mo monitoring period.

Introduction

MECHANICAL SUBGINGIVAL INSTRUMENTATION consisting of scaling and root planing (SRP) is a widely used procedure for the treatment of inflammatory periodontal diseases and it is known as the gold standard therapy. A number of studies based on site analysis have shown beneficial results for both microbial and clinical parameters.[1] The clinical benefits of SRP are derived from the disruption of the subgingival biofilm, which reduces the bacterial load and results in a delay in repopulation by pathogenic microbes.[1,2] The effects of SRP on selected bacterial species have been evaluated for both the short and the long term.[3–7]

Bacteria associated with more aggressive forms of periodontitis include *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Campylobacter rectus*, and some species of spirochetes.[8–13] Mechanical therapy alone may fail to eliminate these pathogenic bacteria because of their ability to invade within periodontal tissues.[14]

Recently, the bactericidal and detoxifying effects of lasers have been proposed as an alternative adjunctive treatment modality to facilitate non-surgical periodontal treatment.[15,16] In this context good results have been obtained with the 810-nm diode laser.[15]

Also, varying degrees of improvement in clinical parameters, namely probing pocket depth (PPD), clinical attachment level (CAL), and bleeding upon probing (BOP), have been achieved with the application of diode laser irradiation (809 and 980 nm) used adjunctively with SRP.[17,18]

The aim of the present study was to evaluate the effects of laser-assisted treatment on microbial and clinical parameters in patients with aggressive periodontitis.

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Materials and Methods

Experimental design

This study was a prospective randomized controlled quadruple split-mouth, single-blind clinical trial with 6 mo of follow-up.

Sample size calculation

A sample size of 13 subjects achieves 91% power to detect a difference of 0.9 mm in PPD (15% reduction) between the null hypothesis mean of 5.9 mm in SRP, laser (LAS), and control (CRL) treatment groups, and the alternative hypothesis mean of 5 mm in the SRP + LAS treatment group, with a known standard deviation of 1.0 and with a significance level (alpha) of 0.05 using a two-sided one-sample *t*-test. Also, sample size calculation determined that 17 subjects would provide 90% power to detect CAL differences, and 11 subjects to detect differences in *P. gingivalis*, *T. forsythia*, and *Treponema denticola* levels. The number of subjects required to assess the differences in all clinical and microbiological parameters between baseline and post-treatment periods or among treatment groups at 90% power varied from 11 to 17.

Study population

The study group consisted of 30 subjects with aggressive periodontitis, 14 men and 16 women aged 41.8 y ± 6.2 y, and 18 were smokers and 12 were nonsmokers. All subjects were selected from a private dental clinic limited to periodontic treatment in Piraeus, Greece. The subjects were consecutively entered into the study and were treated in the private periodontal clinic between March 2004 and April 2006. All participants gave their consent to take part in the study. The study was conducted according to the principles outlined in the Helsinki Declaration of 1975 and revised in 1983, on experimentation involving human subjects.

Patients were diagnosed as having aggressive periodontitis if they were <35 y old and exhibited severe periodontal destruction, consisting of clinical attachment loss exceeding 5 mm at 2–3 sites in more than 14 permanent teeth (at least three of which were not first molars or incisors), along with radiographic evidence of advanced alveolar bone loss at the time of initial diagnosis.[19,20] The diagnostic criteria took into consideration only clinical, and not laboratory, evidence. Efforts were made to ascertain familial aggregation by means of a questionnaire, and when possible, by examining first-degree relatives. However, patients showing clear clinical signs of aggressive periodontitis, but who were without a positive family history, were still included.[21] Smoking was measured by self-report.

Periodontal examination

Clinical examination included measurements of plaque[22] and BOP,[23] which were recorded as dichotomous values. PPD and CAL[24] were measured to the nearest millimeter using a Goldman/Fox-Williams periodontal probe at six sites per tooth for all affected teeth except third molars. The total number of teeth present was also recorded. One site with PPD >5 mm was then randomly selected in each quadrant for microbial sampling (Table 1).

Clinical recordings and selection of sampling sites were performed 1 wk before microbial sampling. Clinical parameters at sampling sites were re-examined immediately after microbial sampling and these values were used in the analysis. Clinical recordings were performed again at the sampling sites 12 wk and 6 mo post-treatment.

Investigator calibration

A total of 10 patients with aggressive periodontitis who were not included in the study group were used for the calibration evaluation. The single designated examiner performed full-mouth PPD and CAL measurements for all 10 patients and repeated the measurements after 15 min. The intra-examiner standard deviation for repeated measures was 0.1 mm, and the examiner's reproducibility was 99.8%.

Clinical procedures

After baseline clinical and microbiological evaluation, the subjects received oral hygiene instruction and supragingival scaling. This resulted in resolution of clinical inflammation. At the next session 2 wk later, each quadrant was randomly assigned to one of the following treatment groups: SRP alone, diode laser (980 nm) (LAS) treatment alone, and SRP combined with LAS (SRP + LAS). One quadrant was not treated and served as a control (CRL).

A computer program was used for randomization of the quadrants. There are 24 different permutations of the four treatments corresponding to each quadrant within any given mouth. The discrete uniform random number generator MATLAB was used to generate a number between 1 and 24, and this was used to choose the permutation applied to each patient.

Table 1. Demographic, Behavioral and Periodontal Variables

Characteristic	*Aggressive periodontitis patients* (n = *30*)
Male/female	14/16
Age	41.8 ± 6.2 y
Smokers	18 (31.1 ± 8.7 cigarettes/d)
Number of teeth	25.5 ± 1.9
Mean PPD (mm)/sampling site	6.3 ± 0.3
Mean CAL (mm)/sampling site	6.9 ± 0.1
Plaque[a]	53.3
BOP[a]	86.9

[a]Mean percentage of positive sites.

SRP under local anesthesia was performed using primarily Gracey curettes. SRP + LAS or LAS treatment alone were also performed under local anesthesia. A 980-nm diode laser (SmilePro980™; Biolitec, Jena, Germany) was used for the laser treatment in continuous focused mode at 2 W of power with a flexible glass fiberoptic guide with a 300-μm spot diameter and power density of 2830 W/cm^2. The total energy per unit area (fluency) was 94.3 J/cm^2. The end of the fiberoptic guide was calibrated to the PPD approximately 1 mm less than the measured pocket depth. This shortening by 1 mm allowed absorption of laser energy around the tip, and irradiation of the pathogenic periodontal tissues without thermal damage to healthy tissues.

The fiberoptic guide was inserted into the pocket parallel to the long axis of the root surface and aimed at the diseased soft tissue lining the pocket, and not toward the root surface, and was moved around the tooth. The laser was activated after the fiber had reached the calibrated depth. The fiber was moved towards the top of the pocket with overlapping horizontal and vertical strokes, maintaining contact with the soft tissue at all times. This procedure was repeated until the full circumference of the root was irradiated. Lasing was complete when signs of a new wound site (fresh bleeding) appeared. The total irradiation period for the entire procedure was approximately 30 sec per pocket. This allowed laser-assisted soft-tissue curettage. In cases of bleeding during laser irradiation, thorough rinsing with saline solution was performed to prevent thermal damage to the root surface.[25,18]

The fiber was cleaned and disinfected by soaking it for 5 sec in ethanol at 95°F, followed by 5 sec of wiping, between treatments of each tooth.[26] The end of the tip was cut and tested prior to and between successive treatments to ensure good beam emission.

Bacterial sampling and analysis

Four plaque samples were randomly obtained from each patient at one site with PPD >5 mm in each quadrant. One paper point was inserted into each selected periodontal pocket for 10 sec and used for DNA probe analysis. Subgingival plaque samples were collected from the same sites in each quadrant at 2 wk, 12 wk, and 6 mo post-treatment. The level of *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, and *T. denticola*, as well as total bacterial load (TBL) was evaluated using the IAI Pado Test 4.5 (Institut für Angewandte Immunologie, Zuchwil, Switzerland).

Samples were sent to and analyzed blindly by the Institut für Angewandte Immunologie, Zuchwil, Switzerland. Samples were processed by standard procedures and hybridized with 32P-labeled specific probes for the small subunit ribosomal RNAs (ssrRNAs) of *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans, T. denticola*, and a universal bacterial probe.[27] The values for each bacterial species were computed by comparison with an homologous standard of each bacterium. The total bacterial count was determined using the universal probe. The results were translated by the Institut für Angewandte Immunologie into millions of bacteria by arbitrarily deciding that one bacterium was equivalent to 10^4 copies of ssrRNA.

Statistical analysis

Differences in demographic data between genders were tested using a *t*-test. Friedman tests were used for testing mean differences between treatment groups at baseline. Repeated measures ANOVA with Greenhouse-Geisser adjustment was used for an initial analysis of all bacteria. Wilcoxon tests were used to test pairwise differences of treatment modalities at each time point, and of time points for each treatment modality for all bacterial species. In all pairwise comparisons for differences between groups a Bonferroni adjustment to the significance level was made to compensate for the multiple testing. It was decided to run three comparisons between the treatment groups. The SRP + LAS group was compared to all other treatment groups. Therefore the significance level for these tests was set equal to $p = 5\%/3 = 1.7\%$ or $p = 0.05/3 = 0.017$. This makes the tests for differences between treatments very strict, leading to difficulty in rejecting the null hypothesis of no difference. McNemar tests were used for testing equality of plaque and BOP presence at different time points and between treatments. All computations were made by SPSS v.13.0 statistical software (SPSS, Inc., Chicago, IL, USA).

Results

Demographic patient data are shown in Table 1. Mean age was no different between genders ($t = 0.85$, $p = 0.411$), nor was mean PPD ($t = 1.25$, $p = 0.235$). However, CAL was greater for males ($t = 3.51$, $p = 0.004$). A series of Friedman tests showed that there were no statistically significant differences in bacterial species among the treatment groups at baseline: *A. actinomycetemcomitans* ($p = 0.954$), *T. forsythia* ($p = 0.056$), *P. gingivalis* ($p = 0.695$), *T. denticola* ($p = 0.567$), or for TBL ($p = 0.313$).

Table 2 presents mean counts of bacterial species for each treatment modality at baseline and post-therapy. The improvement following treatment is evident for all bacterial species. SRP + LAS seems to be the most effective treatment, since levels of bacterial species were decreased to minimal levels in the majority of cases. In general, the formal analysis showed significant post-treatment effects on bacterial counts, which were decreased following the three treatment modalities in all quadrants, and they did not return to baseline levels at 6 mo post-therapy. The majority of differences appeared between the treatment and the control groups, while the SRP + LAS group was clearly better than the other treatments at all time points.

An initial analysis showed that *A. actinomycetemcomitans* counts after treatment were no different than those at baseline ($F = 0.362$, $p = 0.617$). The same results were obtained for the comparisons between treatment modalities ($F = 1.73$, $p = 0.215$). Therefore, this bacterium was not analyzed further.

Mean levels of *T. forsythia* were significantly decreased at 2 wk post-treatment for all treatment modalities ($p = 0.002$, $p = 0.005$, and $p = 0.001$, for the LAS, SRP, and SRP + LAS groups, respectively). After that time, the deterioration seen (increases in bacterial counts) were not statistically significant (Table 3). The same trend was seen for *P. gingivalis*, *T. denticola*, and TBL. Regarding TBL, in the SRP group the deterioration seen from 2 wk to 6 mo ($\delta_{2,4}$) post-treatment was statistically significant ($p = 0.002$).

Table 4 presents mean differences between SRP + LAS and the other treatments at 2 wk, 12 wk, and 6 mo post-treatment. The mean differences with the control group were all

Table 2. Means per Treatment Group for All Bacterial Species Post-Therapy [Mean (SD)]

Bacterial species	Treatment group	Time point			
		Baseline	*2 wk*	*12 wk*	*6 mo*
A. actinomycetemcomitans	SRP	0.023 (0.044)	0.008 (0.026)	0.022 (0.077)	0.040 (0.087)
	SRP + LAS	0.025 (0.069)	0.000 (0.0)	0.016 (0.058)	0.034 (0.0)
	LAS	0.093 (0.278)	0.007 (0.026)	0.040 (0.094)	0.021 (0.036)
	CRL	0.063 (0.175)	0.065 (0.190)	0.040 (0.094)	0.124 (0.023)
T. forsythia	SRP	1.852 (1.636)	0.369 (0.685)	0.553 (0.662)	0.549 (0.791)
	SRP + LAS	2.134 (1.471)	0.153 (0.387)	0.178 (0.345)	0.346 (0.464)
	LAS	2.002 (1.803)	0.360 (0.499)	0.378 (0.596)	0.837 (1.052)
	CRL	1.207 (1.359)	1.436 (1.425)	0.905 (0.640)	1.729 (1.406)
P. gingivalis	SRP	2.695 (2.987)	0.548 (1.115)	0.672 (1.166)	1.192 (2.046)
	SRP + LAS	2.740 (2.792)	0.073 (0.279)	0.094 (0.296)	0.266 (0.613)
	LAS	2.629 (2.357)	0.288 (0.433)	0.311 (0.584)	0.628 (1.030)
	CRL	2.461 (3.199)	2.370 (3.042)	2.128 (1.792)	2.093 (2.637)
T. denticola	SRP	0.483 (0.423)	0.211 (0.277)	0.313 (0.452)	0.567 (0.684)
	SRP + LAS	0.900 (0.525)	0.126 (0.346)	0.098 (0.223)	0.155 (0.234)
	LAS	0.678 (0.573)	0.201 (0.247)	0.235 (0.333)	0.519 (0.492)
	CRL	0.828 (0.952)	0.399 (0.349)	0.560 (0.519)	0.718 (0.523)
Total bacterial load	SRP	36.706 (27.513)	6.798 (6.247)	10.337 (9.710)	10.172 (6.396)
	SRP + LAS	35.835 (17.119)	3.034 (1.881)	2.763 (1.910)	3.580 (2.594)
	LAS	35.236 (28.473)	5.989 (4.781)	6.704 (4.568)	7.492 (4.631)
	CRL	32.734 (21.932)	27.511 (19.696)	27.277 (16.774)	28.293 (18.505)

All figures $\times 10^6$.

statistically significant. SRP + LAS was statistically significantly different from SRP ($p = 0.016$) at 12 wk post-treatment for *T. forsythia*. *P. gingivalis* counts for the LAS and SRP groups, however, were statistically significantly different from those of the SRP + LAS group at 2 wk post-treatment ($p = 0.010$ and $p = 0.011$ for the LAS and the SRP group, respectively). Later on, these differences disappeared with the exception of the SRP group, which was again statistically significantly different from the SRP + LAS group at 6 mo post-treatment ($p = 0.010$). *T. denticola* counts for the SRP + LAS group were statistically significantly different from those of the LAS group ($p = 0.011$) and the SRP group ($p = 0.011$) at 6 mo post-treatment. Finally, the TBL counts were notably lower and statistically significantly different from those of all treatments at every time point studied (Table 4).

All treatments showed significant improvement in PPD values from baseline to 6 mo post-treatment. However, statistically significant differences were found between the SRP + LAS group and the other treatment groups (the mean difference with the LAS group = 0.67, z = 2.577, $p = 0.012$; and the mean difference with the SRP group = –0.46, z = –2.411, $p = 0.016$) at 12 wk post-treatment. This reduction was somewhat greater at the end of study. At 6 mo post-treatment, statistically significant changes were detected again between the SRP + LAS group and the other treatment groups (mean difference with the LAS group = –0.81, z = –1.265, $p = 0.016$; and mean difference with the SRP group = –0.66, z = –1.927, $p = 0.013$). CAL values showed similar performance, and statistically significant differences were seen between treatments at 12 wk post-treatment, with a mean difference between the SRP + LAS group and the LAS group of 0.43 (z = 2.587, $p = 0.012$), and with the SRP group it was

Table 3. Mean Differences Between Baseline and 2 Weeks ($\delta_{1,2}$), Between 2 Weeks and 6 Months ($\delta_{2,4}$), and Between 12 Weeks and 6 Months ($\delta_{3,4}$) for All Treatment Groups for *T. forsythia*, *P. gingivalis*, *T. denticola*, and TBL

	T. forsythia			
	CRL	*LAS*	*SRP*	*SRP + LAS*
$\delta_{1,2}$	−0.23 (0.778)	1.64 (**0.002**)	1.48 (**0.005**)	1.98 (**0.001**)
$\delta_{2,4}$	−0.29 (0.363)	−0.48 (0.136)	−0.18 (0.196)	−0.18 (0.086)
$\delta_{3,4}$	−0.82 (0.031)	−0.46 (0.249)	−0.01 (0.799)	−0.15 (0.875)
	P. gingivalis			
	CRL	*LAS*	*SRP*	*SRP + LAS*
$\delta_{1,2}$	0.09 (0.778)	2.34 (**0.002**)	2.15 (**0.007**)	2.67 (**0.002**)
$\delta_{2,4}$	0.277 (1.0)	−0.34 (0.074)	−0.64 (0.155)	−0.19 (0.028)
$\delta_{3,4}$	−0.03 (0.910)	−0.32 (0.480)	−0.52 (0.063)	−0.17 (0.374)
	T. denticola			
	CRL	*LAS*	*SRP*	*SRP + LAS*
$\delta_{1,2}$	0.43 (0.084)	0.48 (**0.005**)	0.27 (**0.011**)	0.77 (**0.001**)
$\delta_{2,4}$	−0.32 (0.017)	−0.32 (0.034)	−0.36 (0.053)	−0.03 (0.034)
$\delta_{3,4}$	−0.16 (0.078)	−0.28 (0.026)	−0.25 (0.237)	−0.06 (0.158)
	TBL			
	CRL	*LAS*	*SRP*	*SRP + LAS*
$\delta_{1,2}$	5.22 (0.012)	29.25 (**0.001**)	29.92 (**0.001**)	32.80 (**0.001**)
$\delta_{2,4}$	−0.78 (0.191)	−1.50 (0.156)	−3.37 (**0.002**)	−0.55 (0.140)
$\delta_{3,4}$	−1.02 (0.334)	−0.79 (0.017)	−0.17 (0.211)	−1.08 (0.281)

p Values (in parentheses) by Wilcoxon testing.
Boldface denotes statistically significant results.

Table 4. Mean Differences Between the SRP + LAS Group and the Other Treatment Groups at 2 Weeks, 12 Weeks, and 6 Months Post-Treatment for *T. forsythia*, *P. gingivalis*, *T. denticola*, and TBL

	T. forsythia		
Difference with SRP + LAS	*CRL*	*LAS*	*SRP*
2 wk	1.28 (**0.001**)	−0.21 (0.260)	−0.22 (0.169)
12 wk	−0.73 (**0.013**)	−0.2 (0.173)	−0.36 (**0.016**)
6 mo	1.38 (**0.004**)	−0.49 (0.022)	−0.20 (0.133)
	P. gingivalis		
2 wk	2.30 (**0.003**)	−0.22 (**0.010**)	−0.48 (**0.011**)
12 wk	−2.03 (**0.001**)	−0.22 (0.066)	−0.58 (0.026)
6 mo	1.83 (**0.005**)	−0.36 (0.110)	−0.93 (**0.010**)
	T. denticola		
2 wk	−0.273 (**0.009**)	−0.08 (0.214)	−0.07 (0.099)
12 wk	−0.46 (**0.001**)	−0.14 (0.017)	−0.22 (0.091)
6 mo	−0.563 (**0.001**)	−0.36 (**0.011**)	−0.41 (**0.011**)
	TBL		
2 wk	−24.48 (**0.001**)	−2.96 (**0.005**)	−3.76 (**0.001**)
12 wk	−24.51 (**0.001**)	−3.94 (**0.001**)	−7.57 (**0.001**)
6 mo	−24.71 (**0.001**)	−3.91 (**0.001**)	−6.59 (**0.001**)

p Values (in parentheses) by Wilcoxon testing.
Boldface denotes statistically significant results.

–0.40 (z = –2.828, $p = 0.017$). At the end of the observation period significant differences were seen between the SRP + LAS group and the other treatment groups. The mean difference between the SRP + LAS group and the LAS group was 0.47 (z = –0.087, $p = 0.019$), and with the SRP group was –0.32 (z = –0.066, $p = 0.014$) (Table 5).

Table 6 displays the proportions of plaque and BOP presence at baseline and at 6 mo post-treatment for all treatment groups. Substantial decreases in both plaque and BOP levels were seen in all treatment groups (McNemar test p values were all <0.001). However, a comparison of plaque and BOP levels at 6 mo post-treatment between the SRP + LAS group and the other treatment groups did not show statistically significant results (for BOP $p = 0.317$ and $p = 0.317$ for the LAS group and the SRP group, respectively, and for plaque $p = 0.467$ and $p = 0.552$ for the LAS group and the SRP group, respectively).

Discussion

Microbial evaluation

The randomized controlled clinical trial reported here evaluated the effectiveness of diode laser treatment on clinical and microbiological parameters of patients with aggressive periodontitis.

A potential weakness of this study is the possible carry-over effects due to the quadruple split-mouth design. This may be a potential disadvantage when comparisons are made on a within-subject basis. The carry-over effects of the split-mouth design may mask differences that do exist. If, however, these differences are statistically significant, as was the case in this study, it is less likely that carry-over effects masked them. On the contrary, these differences would have been more pronounced in the absence of carry-over effects.

This is the first study to our knowledge which has confirmed the *in vivo* antibacterial effectiveness of a 980-nm diode laser. Moritz et al.[15] earlier reported considerable bacterial elimination from periodontal pockets using irradiation with an 810-nm diode laser at 2.5 W power used in pulse mode (50 Hz, pulse duration 10 msec) following scaling as compared to scaling alone.

Scaling and root planing is one of the most common procedures for the treatment of periodontal disease, and has long been considered the gold standard of such treatments. Mechanical treatment alone has been shown to be clinically effective.[28] Numerous studies have reported beneficial results from this treatment for both clinical and microbial parameters. The clinical benefits are derived from the removal of subgingival plaque and disruption of the subgingival biofilm, which leads to a decrease in bacterial counts.

In the present study SRP alone was found to markedly decrease the counts of the bacterial species studied, namely *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, and *T. denticola*, as well as TBL. These data confirm the favorable effects of SRP in decreasing levels of *P. gingivalis* and *T. denticola* that were previously reported.[4,6,29,30]

The laser-assisted treatment (SRP + LAS) showed significantly lower TBL levels compared to SRP or LAS alone,

Table 5. Means in Millimeters by Treatment Group for PPD and CAL Values Post-Therapy

	Treatment group	*Time point*			
		Baseline	*2 wk*	*12 wk*	*6 mo*
PPD	SRP	6.47 (1.356)	4.13 (0.990)	4.13 (1.060)	4.13 (1.060)
	SRP + LAS	6.67 (1.291)	4.07 (0.884)	3.80 (0.941)	3.87 (0.915)
	LAS	5.93 (1.163)	4.00 (1.309)	3.93 (1.387)	3.93 (1.387)
	CRL	6.20 (1.740)	6.07 (1.624)	6.07 (1.624)	6.07 (1.624)
Comparison of mean differences for PPD					
		Mean difference at 6 mo			
SRP + LAS	LAS	−0.81	$z = -1.265$	$p = 0.016$	
	SRP	−0.66	$z = -1.927$	$p = 0.013$	
CAL	SRP	7.07 (1.580)	5.33 (1.676)	5.27 (1.751)	5.20 (1.656)
	SRP + LAS	7.07 (1.710)	5.13 (1.642)	4.93 (1.624)	4.93 (1.624)
	LAS	6.87 (1.598)	5.00 (1.773)	4.93 (1.668)	4.93 (1.668)
	CRL	7.00 (2.104)	6.73 (1.710)	6.73 (1.710)	6.73 (1.710)
Comparison of mean differences for CAL					
		Mean difference at 6 mo			
SRP + LAS	LAS	−0.47	$z = -0.087$	$p = 0.019$	
	SRP	−0.32	$z = -0.066$	$p = 0.014$	

Table 6. Plaque and BOP Percentages at Baseline and at 6 Months Post-Treatment in All Treatment Groups

	Treatment group	Time		
		Baseline	6 mo	p[a]
Plaque	SRP	54.1	32.6	$p < 0.001$
	SRP + LAS	52.7	29.2	$p < 0.001$
	LAS	50.7	31.6	$p < 0.001$
	CRL	55.9	36.5	$p < 0.001$
Comparison of plaque percentages at 6 mo				
SRP + LAS	SRP			$p = 0.467$
	LAS			$p = 0.552$
BOP	SRP	81.6	25.8	$p < 0.001$
	SRP + LAS	82.4	24.3	$p < 0.001$
	LAS	83.7	23.2	$p < 0.001$
	CRL	90.8	35.4	$p < 0.001$
Comparison of BOP percentages at 6 mo				
SRP + LAS	SRP			$p = 0.317$
	LAS			$p = 0.317$

[a]By McNemar testing.

at every time point post-treatment studied. This was a very effective treatment modality, that kept the levels of all bacterial species significantly lower than baseline, even at 6 mo post-therapy. The most significant bacterial reductions seen with this combined treatment were achieved at 2 wk post-therapy for all bacterial species tested, as well as for TBL.

While SRP is the most commonly used periodontal therapy for the cause-related phase of treatment, it has limitations, including an inability to adequately clean deep periodontal pockets, and a lack of removal of microorganisms from the tissues lining the periodontal pockets. Darby et al.[6] showed that SRP resulted in clinical improvement and significant reductions in the levels of *P. intermedia*, *T. forsythia*, and *T. denticola*. The study by Renvert et al.[3] had similar results. However, levels of *A. actinomycetemcomitans* remained high after therapy, probably due to its ability to invade deeply into periodontal tissues. It is well known that some periodontal pathogens are capable of invading periodontal tissues. *A. actinomycetemcomitans* was found in the connective tissue of active as well as inactive sites.[14] Also, *P. gingivalis* can adhere to and enter oral epithelial cells.[31] Our results indicate that SRP alone was unable to eliminate *A. actinomycetemcomitans*, while laser-assisted curettage showed better performance, and drastically reduced levels of this organism 2 wk after the combined therapy. Also, *P. gingivalis* and *T. denticola* were significantly reduced at 6 mo post-treatment in the SRP + LAS group compared to the SRP-alone group. As mentioned above, both are organisms having high pathogenicity due to their ability to invade tissues. The laser-assisted treatment showed strong antibacterial effects on the counts of *T. forsythia* at 12 wk post-treatment, much more than the effect seen with SRP alone. *T. forsythia* is a periodontal pathogen that according to clinical studies is difficult to eliminate with SRP alone.[4,6,32] The laser has been introduced as an adjunctive tool to mechanical therapy, due to its bactericidal and detoxifying effects.

These findings are consistent with results from previous studies, which have shown that short-term reductions in levels of bacteria occurred during the first 3 mo post-therapy,[29,33] and that bacterial re-colonization occurred after 70 d to 3 mo.[34,35]

In this study in the SRP + LAS group, mean levels for all bacteria at final follow-up (6 mo) were never higher than the corresponding levels seen with the other treatment groups at 2 wk post-therapy, and levels of *P. gingivalis* and *T. denticola* were significantly reduced at 6 mo post-treatment compared to SRP alone.

Within the limits of this study, we conclude that the combined treatment offered a very favorable microbial environment for the healing of periodontal tissues by keeping levels of periodontal pathogens quite low for up to 6 mo post-treatment.

Clinical evaluation

A considerable body of evidence indicates that mechanical instrumentation is effective in suppressing periodontal pathogens and promoting clinical improvement. It is the first necessary step in the treatment of all forms of periodontal disease, and its limitations have been described above. The need of a more powerful periodontal therapy for those with aggressive periodontitis, which is the most severe and rapidly destructive form of periodontal disease, is clear.

The advantages of diode laser-assisted periodontal treatment as presented in the literature include hemostasis and bacterial reductions,[36] reductions in the risk of bacteremia, and the fact that it is simpler than mechanical débridement.[37] Since the diode laser does not interact with hard dental tissues, it is an excellent instrument for treating oral soft tis-

sues. More complete epithelial removal has been shown with the use of a 980-nm diode laser, compared to the effects of mechanical débridement with curettes.[38]

Controversy about the necessity for gingival curettage in the mechanical treatment of periodontitis was reported in the reviews of the 1989[39] and 1996[40] World Workshops on Periodontology. Periodontal pathogens (*P. gingivalis, F. nucleatum*, and *T. denticola)*, as determined by *in situ* immunocytochemistry, appear to coexist and are present deep in the pockets in the biofilm lining the periodontal pocket wall and so-called "plaque-free zone."[41–43] The presence of periodontal pathogens near the pocket epithelium, and their ability to invade tissues in deeper parts of dentinal tubules, suggests that a solely mechanical therapy such as SRP is unable to eliminate these bacteria, especially in those with aggressive periodontitis. Also, this bacterial invasion of root structures may represent a reservoir of periodontopathic bacteria for re-colonization and re-infection.[44,45]

A possible disadvantage that should be underscored is the potential morphological alterations seen in root surfaces when they are covered by a thin coating of blood following improper use of diode laser irradiation, as has been seen *in vitro* by Kreisler et al.[25] However, a more recent *in vivo* study by Castro et al.[46] reported that 980-nm laser irradiation at 2 W of power and a pulse repetition rate of 100 msec used adjunctively with SRP induced no detectable root surface alterations.

From a clinical standpoint, attention must also be paid to the orientation of the optical fiber, which must always remain parallel to the long axis of the root surface and in contact with the soft lining of the pocket epithelium.

Considering the advantages of laser irradiation, its use in combination with mechanical instrumentation has the potential to speed periodontal healing. Also, given the evidence of bacterial invasion of the soft tissues of the periodontal pockets, not only débridement of the root surface, but also the removal of the pocket epithelium and granulation tissue may be important factors in promoting attachment of the connective tissues to the root surface.

The ultimate utility of this novel treatment modality must be more closely evaluated with continued scientific study, as the results found in the literature appear to be conflicting. Recently, in a literature review commissioned by the American Academy of Peridontology,[47] it was reported that there is limited evidence suggesting that laser-assisted SRP may provide additional benefit, if gains in CAL are considered to be an end-point of non-surgical therapy.

Conclusions

Within the limits of the present study, diode laser-assisted periodontal treatment with SRP was found to have a superior effect over that of SRP or LAS alone in reducing PPD and increasing CAL over the 6-mo study period.

Laser-assisted treatment combined with subgingival débridement not only showed substantial clinical improvements, it also caused favorable alterations in the subgingival biofilm, indicating that it may be effective in treating those with aggressive periodontitis, in whom anaerobic bacteria predominate. Thus, this adjunctive therapy does not replace mechanical instrumentation, but rather complements it.

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