

Use of diode laser 980 nm as adjunctive therapy in the treatment of chronic periodontitis. A randomized controlled clinical trial

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SUMMARY

The primary goal of periodontal therapy is the removal of supra and subgingival bacterial deposits by mechanical debridement consisting in scaling and root-planing (SRP) using manual or power-driven instruments. The complete removal of bacteria and their toxins from periodontal pockets is not always achieved with conventional mechanical treatment. The use of lasers as an adjunctive therapy for periodontal disease may improve tissue healing by bactericidal and detoxification effects.

The aim of this study was to compare the effectiveness of Diode laser used as adjunctive therapy of SRP to that of SRP alone for non surgical periodontal treatment in patients with chronic periodontitis.

Nineteen pairs of teeth with untreated chronic periodontitis were selected in 13 patients and randomly treated by SRP alone (control group) or by SRP + laser irradiation (test group). Clinical measurements (PPD, CAL, BOP, GI, PI) were performed before treatment at baseline (T0) and at T1 (after 4 weeks), T2 (8 weeks), T3 (12 weeks), T4 (6 months). Subgingival plaque samples were taken at baseline and after treatment and examined for 8 periopathogens bacteria using PCR technique.

The present study showed that the additional treatment with diode laser may lead to a slightly improvement of clinical parameters, whereas no significant differences between test and control group in reduction of periodontopathogens were found.

KEY WORDS: Chronic periodontitis, Laser, Non surgical periodontal therapy

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INTRODUCTION

The aim of periodontal therapy is to eliminate the microbial causative factors of periodontal disease by mechanical debridement consisting in meticulous scaling and root planing using manual and/or power driven instruments combined with adequate oral hygiene measures. The complete removal of supra and subgingival bacterial

deposits consisting of adherent plaque, calculus and infected root cementum is not always achieved by conventional mechanical debridement.

In search of a more efficient and atraumatic technique which improves periodontal healing, researchers proposed the use of lasers for periodontal treatment.

Several laser systems, with different characteristics and wavelengths, have been used for the treatment of periodontal disease. Nd: YAG (neodymium-doped: yttrium, aluminium and garnet) is a high power laser capable of excellent soft tissue ablation, with strong hemostatic and bactericidal effects. However, this laser is not useful for the treatment of dental hard tissues, due to

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thermal side effects on the pulp and carbonization of alveolar bone (Aoki *et al.*, 2004).

Er: YAG (erbium-doped: yttrium, aluminium and garnet) is indicated for both treatment of soft and hard tissues, and it seem to be the laser of choice for removal of calculus without producing thermal damage to the pulp and side effects on root cementum (Aoki *et al.*, 2004, Cobb 2006, Schwarz *et al.*, 2003).

Diode laser is indicated for the treatment of soft tissues and has a bactericidal effect, but does not ablate calculus on the root surface, therefore it may be useful as an adjunctive means for scaling and root planing due to its bactericidal and detoxification effects (Moritz A. *et al.*, 1998; Harris D.M., Yessik M. 2004).

The number of subgingival microorganisms for three months after scaling and root planing is dramatically reduced (Sbordone L. *et al.*, 1990). Furthermore the composition of subgingival plaque shifts from one with high numbers of gram-negative anaerobes to one dominated by gram-positive facultative anaerobes compatible with periodontal health (Magnusson I. *et al.*, 1984; Mousques T. *et al.*, 1980; Sbordone L. *et al.*, 1990). This positive microbial change must be sustained by the periodic scaling and root planing performed during supportive periodontal therapy. The addition of laser irradiation during this crucial stage of therapy may improve periodontal tissue healing, achieving a deeper bacterial inhibition and hopefully prolonging intervals between maintenance visits.

The aim of the present study was to compare the effectiveness of Diode laser (980 nm) used as an adjunctive therapy to SRP to that of SRP alone for non surgical periodontal treatment in patients with chronic periodontitis.

MATERIALS AND METHODS

Patients selection

Patients were recruited during the first visit to the Department of Odontostomatologic, Orthodontic and Surgical Disciplines (Periodontal Division) of the Second University of Naples, according to the following selection criteria:

- systemically healthy (with special regard to disease affecting tissue repair);
- no medications required for periodontal thera-

py and no use of any antibiotic and/or anti-inflammatory medication in the preceding month;

- no periodontal therapy in the preceding three months;
- at least 10 teeth per dental arch.

Site selection and pair matching

Patients were considered recruitable if they presented at least two sites affected by periodontal lesions suitable to work as test and control, exhibiting the following features:

- being localized in interproximal sites;
- showing a probing depth of 5 or more mm;
- showing a difference in probing depth ≤ 2 mm.

Moreover, the two sites had to be localized in two contralateral hemiarches belonging to the same tooth typology (incisor or cuspid or premolar or molar).

Each patient considered recruitable was informed about the nature and aim of the study and signed an informed consent form.

All patients were then instructed with oral hygiene instructions.

Study design

The study was designed as a split-mouth, case-control, randomized clinical trial.

Each tooth of the selected pair of sites was randomly allocated to the control group (scaling and root planing periodontal therapy) or in the test group (scaling and root planing with adjunctive laser therapy) by tossing a coin.

Thirteen patients and 38 teeth were finally recruited for the experimental design.

Clinical measurements

At *baseline (T0)*, an expert periodontal examiner measured the following parameters at the experimental sites:

- PPD (Probing Pocket Depth) using a customized resin stent and a Williams probe of 15 mm;
- CAL (Clinical Attachment Level) was also recorded in the same way referring to the enamel-cementum junction;
- BOP judged as positive if appearing within 20 seconds after probing;
- GI (gingival index Löe and Sillness);
- PI (plaque index Silness and Loe).

At T0 a plaque sample was also obtained from the selected sites.

At four weeks after completing periodontal therapy (T1), partial clinical records were performed: GI and PI and the plaque sample repeated.

At eight weeks (T2) clinical measurements were performed: PPD, BOP, GI and PI.

At twelve weeks (T3) the records of PPD, BOP, GI and PI were newly performed.

At six months (T4) the clinical measurements (PPD, CAL, BOP, GI, PI) were performed and the final plaque sample taken.

Microbiological sampling

Plaque sampling was performed subgingivally after carefully removing the supragingival plaque at the experimental sites, test and control.

Sampling was obtained inserting in the pockets three sterile blistered paper points per site, leaving them undisturbed for 15 seconds each and then inserting them in the same sterile eppendorf.

The sample was then sent to the laboratory for PCR analyses.

PCR analyses

Plaque samples were stored at 4°C in saline solution until DNA extraction (24-48 h later). Vortexed plaque samples were centrifuged for 15 min at 14000 rpm. Pellet was suspended in 300 µl lysis solution (50 mM Tris, 10 mM EDTA, 10% SDS), treated with lysozyme (5 mg/ml) and incubated for 1 h at 37°C. Proteinase K was added and incubated for 1h at 65°C, DNA was extracted according to the method phenol/chloroform-isoamyl alcohol. Nucleic acids were precipitated in alcohol, washed with 70% (vol/vol) alcohol and suspended in bidistilled water to create the DNA template.

Each plaque was analysed using a multiplex PCR, to reveal the following species: *Aggregatibacter* (formerly known as *Actinobacillus*) *actinomycetemcomitans*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Tannerella forsythia*, *Eikenella corrodens*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*.

PCR was performed using ubiquitous primers with modified 18S rRNA to determine the bacterial count.

Negative controls (no DNA) and Positive controls with DNA coming from pure bacterial cultures were tested. Amplification was performed in 100 µl reaction, containing 10 mM Tris-HCl, pH 8.0,

50 mM KCl (1 x PCR Buffer), 1.5 mM MgCl, 200 µM of each nucleotide, 30 pml of each primer, 2.5 U Hot Start Taq DNA Polymerase (Quiagen S.P.A. Milan Italy) and 5 µl of DNA Template.

The solution was amplified in a first cycle at 98°C for 15' to activate the polymerase, 40 cycles at the following temperatures: denaturation for 30" at 95°C, annealing for 1' at 60°C, extension for 1' at 72°C.

Final extension step 10' at 72°C. The reactions were conducted in a thermocycler iCycler System (Bio-Rad Laboratories srl, Segrate, Italy). Amplification of ubiquitous primers was conducted in the same way.

PCR products were visualized using electrophoresis on agarose gels. Experimental design included a negative control with a DNA-free template and a positive control coming from pure cultures.

PCR products were tested for specificity with restriction enzymes: Eco RI, Spe I, Xba I, Hind III, Kpm I for *C.rectus*; Dra I for *T. forsythensis*; Apa I, Taq I, Sma I for *Treponema denticola*; Sma I for *A. actinomycetemcomitans*, *P. gingivalis*, *E. corrodens*; Dra I for *P. intermedia*.

Electrophoretic amplified products were identified by electrophoresis of 20 µl from each PCR tube, placed in agarose gel 2% buffered with TAE (Tris-Acetate-EDTA buffer) for 2 h at 80V.

Electrophoretic bands were visualized and photographed with a UV rays transilluminator (gel Doc 2000, Bio-Rad) after staining for 30' with ethidium bromide (1 µg/ml). Amplified fragments were compared to a DNA marker (number VIII, Roche Diagnostics SPA, Milan Italy).

Periodontal treatment

Each selected tooth was subjected to mechanical debridement by the same expert periodontist, using Gracey curettes until the operator achieved a hard, smooth and calculus-free root surface.

A diode laser (Valore S9- Lasering Medical Laser-Modena; Italy) 980 nm at a power output of 2.5 W in pulsed mode (30 Hz, pulse duration 10 ms) was used for the adjunctive therapy in the test group after conventional mechanical treatment. The optic fiber of 400 µm was moved from the coronal to the apical side of the pocket in parallel paths with an inclination of approximately 20°. Each pocket of the test group was lased for 30 s twice, with a 60 s interval.

RESULTS AND STATISTICAL ANALYSES

At baseline examination the plaque index (PI) was 1.2 (S.D. 0.45) in both groups. The plaque indexes were significantly reduced at 4 weeks and remained low until 8 weeks in both groups (Table 1). Gingival indices (GI) were similarly reduced with both treatment modalities (Table 2), where-

TABLE 1 - Plaque index (\pm SD).

	Test	Control	P-value
Baseline	1.263 (0.45)	1.263 (0.45)	1
T1	0.421 (0.50)	0.894 (0.56)	0.0029*
T2	0.421 (0.50)	0.894 (0.56)	0.0029*
T3	0.842 (0.37)	1.105 (0.56)	0.0271
T4	1.105 (0.56)	1.315 (0.58)	0.0487

*Wilcoxon signed-rank test.

TABLE 2 - Gingival Index (\pm SD).

	Test	Control	P-value
Baseline	2 (0)	1.947 (0.2)	0.3435
T1	0.421 (0.60)	0.842 (0.60)	0.0121*
T2	0.736 (0.56)	1 (0.66)	0.062
T3	1 (0.57)	1.578 (0.60)	0.0042*
T4	1.789 (0.53)	1.789 (0.53)	1

*Wilcoxon signed-rank test.

TABLE 3 - Probing Depth (\pm SD).

	Test	Control	P-value
Baseline	6.052 (0.70)	6.052 (0.91)	0.897
T1			
T2	4.052 (0.84)	4.315 (1)	0.3889
T3	4.05 (0.97)	4.263 (1.24)	0.4367
T4	4.631 (1.06)	4.947 (1.26)	0.1406

*Wilcoxon signed-rank test.

as the BOP indices were reduced more in the test group than in the control group until 12 weeks (Table 4). The mean PD and CAL values for baseline and T2, T3, T4 for both groups are presented in table 3 and 5. The microbiological parameters showed no significant differences between the test and control group in terms of reduction of periodontopathogens (Table 6).

DISCUSSION

The results of the present study showed that the additional treatment with diode laser in the treatment of chronic periodontitis may lead to a slight improvement of clinical parameters (PPD, CAL, GI, PI) after 4, 8 and 12 weeks compared with that of SRP alone.

In addition, to the laser bactericidal effect, the reduction of periodontal inflammation may also be related to the reduction of prostaglandin E₂ (PGE₂) levels, due to the effects of laser treatment. PGE₂ levels increase in the periodontal connective tissues of periodontal lesions being a potent

TABLE 4 - BOP.

	Test	Control	P-value
Baseline	100	94.7	0.895
T1	NA	NA	NA
T2	5.2	21	0.3711
T3	15.8	63.1	0.0159*
T4	84.2	84.2	1

*McNemar Test.

TABLE 5 - CAL (\pm SD).

	Test	Control
Baseline	7.123 (0.9)	6.912 (1.0)
T1	NA	NA
T2	NA	NA
T3	5.121 (0.7)	5.218 (0.7)
T4	5.089 (0.8)	5.123 (0.9)

TABLE 6 - Number of samples positive for the presence of periopathogens bacteria.

Bacterial species	T 0 (Baseline)		T1 (1 month)		T4 (6 months)	
	CG	TG	CG	TG	CG	TG
<i>Aggregatibacter actinomycetemcomitans</i>	5	5	3	2	5	5
<i>Tannerella forsythensis</i>	13	11	10	7	10	10
<i>Campylobacter rectus</i>	10	9	9	5	9	8
<i>Eikenella corrodens</i>	7	8	5	6	7	7
<i>Fusobacterium nucleatum</i>	10	8	10	6	10	8
<i>Porphyromonas gingivalis</i>	10	9	8	7	10	10
<i>Prevotella intermedia</i>	8	8	7	7	7	7
<i>Treponema denticola</i>	10	5	8	3	9	5

stimulator of inflammation and bone resorption (Salvi G.E., Beck J.D. 1998. Offenbacher S. *et al.* 1986).

An in vitro study by Sakurai *et al.* showed that low level laser irradiation may inhibit PGE₂ production by lipopolysaccharide (LPS) of periodontopathogens in human gingival fibroblast (hGF). The inhibitory effects on PGE₂ production was time and dose dependent.

The results showed that most of the clinical and microbiological changes occurred during the first 3 months after treatment in both groups. The following clinical parameters PD, CAL, GI, PI, BOP showed a marked improvement 3 months after treatment in both groups. The probing depth reduction was slightly greater in the test group. The decrease in plaque index was also greater in the laser group.

At baseline examination, 100% of the surfaces in the test group and 94 % of the surfaces in the control group demonstrated bleeding on probing. At T2 examination 5.2% of the surfaces in the test group and 21% of the control group demonstrated bleeding on probing. At T3 examination 15.8% of the surfaces in the test group and 63% of the control group demonstrated bleeding on probing. At T4 examination 84% of the surfaces in both groups demonstrated bleeding on probing. Several studies showed that BOP is an important clinical parameter used to determine the presence or absence of periodontal disease progres-

sion (Lang N.P. *et al.*, 1986; Joss A. *et al.*, 1994).

The microbiological evaluation showed that at baseline examination the percentage of positive samples for periopathogens bacteria were similar in both groups. At T1 examination the decrease in the percentage of positive samples for periopathogens was slightly larger in the test group. At T4 examination the microbial analyses showed no differences between the test and the control group. These findings are in agreement with results from previous studies which showed that the bacterial recolonization occurs 3 months after active periodontal treatment (Sbordone L. *et al.*, 1990).

CONCLUSION

The aim of the present study was to compare the effectiveness of a Diode laser (980 nm) used as an adjunctive therapy to SRP with that of SRP alone for non surgical periodontal treatment in patients with chronic periodontitis.

The basic approach to periodontal infections has always been and remains the removal of supra and subgingival bacterial deposits. Non surgical periodontal therapy is still considered the gold standard to which other methods are compared. Several clinical studies conducted in the past few decades confirmed the effectiveness of the non surgical approach in treating periodontal infec-

tion. Supportive periodontal treatment is an integral part of periodontal therapy.

Within the limits of this study, the present investigation showed that the additional treatment with diode laser may lead to a slight improvement of clinical parameters (PPD, CAL, GI, PI) after 4, 8 and 12 weeks, whereas the BOP indices were reduced more in the test group than in the control group. No significant differences between the test and control group in terms of reduction of periodontopathogens was found.

The use of diode laser as adjunctive therapy to scaling and root planing provided no additional clinical and microbiological benefit over conventional mechanical treatment during medium terms of observation. Therefore it was not useful to try to prolong intervals between supportive periodontal treatments.

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