ORIGINAL RESEARCH

Effectiveness of Diode (810 nm) Laser in Periodontal Parameters and Reduction of Subgingival Bacterial Load in Periodontitis Patients

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Abstract

Aim: This split-mouth randomized trial (RCT) aimed to assess the effect of diode laser on the clinical parameters in patients with periodontitis, compare the results with scaling and root planing (SRP) alone, and assess the implications of diode laser (DL) on plaque bacteria.

Materials and methods: Seventeen periodontitis patients were randomly assigned into two equal groups based on the therapy delivered. Group I (control site) received just SRP at baseline, while group II (test site) received both SRP and DL irradiation. For both groups, the clinical periodontal parameters probing pocket depth (PPD), and clinical attachment level (CAL) were measured at baseline, 30 days, and 90 days.

Microbiological amount was also measured at baseline, 30, and 90 days after periodontal treatment. The amounts of *Aggregatibacter actinomycetemcomitans* (*A.a*), *Prevotella intermedia* (*Pr. intermedia*), and *Porphyromonas gingivalis* (*P. gingivalis*) were determined using real-time PCR probing with specific bacterial primers.

Results: In both groups, PPD and CAL showed statistically significant reductions at different time intervals ($p < 0.05$). No significant difference were observed in CAL values after 1 and 3 months in both test and control groups ($p > 0.05$).

The mean values of the concentration of *A.a, Pr. intermedia* and *P. gingivalis* were lower in the case group as compared to the control group and the difference was statistically significant after 1 month ($^*p = 0.001$).

Clinical significance: According to this study, non-invasive laser treatment has the potential to improve clinical outcomes by lowering the quantity of *A.a, Pr. intermedia* and *P. gingivalis*.

Conclusion: In both groups, a considerable decrease in the periodontal pathogens *A.a, Pr. intermedia* and *P. gingivalis* were discovered; however, the intergroup comparison was insignificant in relation to PD and CAL. The adjunctive treatment with diode laser showed better efficacy in ensuring a better periodontal treatment than SRP alone.

Keywords: *Aggregatibacter actinomycetemcomitans*, Diode laser, Non-surgical periodontal therapy, Periodontitis, *Porphyromonas gingivalis*, *Prevotella intermedia*.

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Introduction

Periodontitis is a complex multifactorial disease marked by inflammation mediated by the host and related microbes that gradually destroy the structure supporting the teeth. Periodontitis is a disease that is initiated and progressed by dysbiotic ecological changes in the oral microbiome, which are linked to the destruction of periodontal tissue, the host immuno-inflammatory response, and other variables among them smoking and diabetes.[1–3]

Periodontitis is the most common form of periodontal disease, and it is one of the seven most destructive diseases, it is prevalent in adults but can occur in children.[4] Microbial products generally provoke host responses, which are also determined by genetic and environmental factors. Perpetuation of the host response by a persistent bacterial challenge disrupts homeostatic mechanisms and results in the release of biologic mediators such as interleukin-1, interleukin-6, tumor necrosis factor-α, matrix metalloprotease, and prostaglandin E2. These mediators of inflammation lead to extracellular matrix destruction of the periodontium and stimulate bone resorption.[5] Periodontitis lesions are associated with subgingival microflora which consists mainly of gram-negative bacterial species, of which the dark-pigmented organism *Porphyromonas gingivalis (P. gingivalis)* is considered a major pathogen.[4–6] *Prevotella intermedia (Pr. intermedia),* and *Aggregatibacter actinomycetemcomitans* (*A.a*) are associated with periodontitis. *Aggregatibacter actinomycetemcomitans* is

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infrequently found in periodontally healthy individuals, whereas *Pr. intermedia* was found in healthy subjects and is more frequent in patients with chronic periodontitis. *Porphyromonas gingivalis* has not been found in either healthy subjects or patients with gingivitis. Consequently, increased levels of these putative pathogens may be useful indicators of both active periodontitis and increased risk of gingival attachment loss.[7] *Porphyromonas gingivalis, Pr. intermedia* and *A.a* are considered principal putative periodontal pathogens.[8]

The main objective of periodontal therapy is to eliminate bacterial deposits and their niches by eliminating supragingival and subgingival biofilms.[9] Scaling and root planing (SRP) is a common approach to the control of inflammation in non-surgical treatment modalities, but conventional treatment fails in many situations, especially in severe cases. It has been speculated that using antibiotics, chlorhexidine, or lasers in addition to SRP can aid in the elimination of microbial plaque and lower the amount of periodontal pathogenic bacteria. However, complete eradication of subgingival plaque biofilm and calculus is not achievable while instrumenting deeper periodontal pockets and furcations since it is uncertain whether they can reach the surfaces of the tooth roots. Furthermore, it will be extremely difficult to keep antibiotics at a concentration that provides therapeutic advantages in terms of periodontal pocket reduction, and there is much concern about the development of antibiotic resistance[10] Lasers have been proposed as an adjuvant to traditional periodontal therapy (SRP) due to their demonstrated advantages such as antibacterial, hemostatic, and anti-inflammatory properties, and their capacity to expedite wound healing. The diode laser is the most popular of the several lasers created for dental applications since it is a compact, light, durable, and relatively affordable device.[11] Additionally, the diode laser rapidly absorbs pigmented molecules like melanin and hemoglobin. Given that pigmented bacteria represent the majority of those responsible for periodontal disease, this characteristic is crucial to the treatment process. Other non-pigmented bacteria, such as *A.a*, are often heat-sensitive and eliminated after touching the hot laser optical fiber.[12,13]

To promote periodontal health and individual quality of life, adjuvant treatment to routine mechanical periodontal therapy is essential.

Given the advantages of using a diode laser to disinfect the gingival sulcus and improve periodontal health, as well as the fact that periodontitis is a common oral condition that results in tooth loss and lowers people's quality of life globally, using a diode laser in conjunction with SRP may be the most effective strategy for treating patients with periodontitis.[14]

Hence, this study aims to evaluate the clinical effects of incorporating the 810 nm diode laser alongside SRP compared to the effects of SRP alone in treating patients with periodontitis.

Materials and Methods

Participants and Study Design

This study was a randomized clinical trial (RCT) using split-mouth, conducted at Khartoum Teaching Dental Hospital (KDTH) and Amina Specialization Dental Center (ASDC). Sudanese adult patients with periodontitis who satisfied the eligibility criteria, during the period of data collection from June 2017 to March 2018 were included in the study. Among 17 participants, there were 50 affected sites, divided equally into test and control groups, with 25 cases and 25 controls, respectively. The sample size was calculated using openepi.com, assuming a two-sided confidence interval of 95%, power of 80%, and the ratio of exposed to unexposed in the sample of 1:1. Two groups were tested for a statistically significant difference between the means using the two-tailed paired *t*-test. Z (power) = power of the study, Z (1–α) = standard normal distribution, ($p = 0.8416$, Z1 = 1.96, Z2 = 0.8, Gamma1 = 1.3, Gamma2 = 0.9, SD = 1). According to these, the sample size was 20 patients (40 sites).

Patients with periodontitis who met the following requirements were eligible for inclusion: they had to be above eighteen years old, exhibit at least four anterior or posterior teeth in each of the upper and lower jaw's right and left quadrants, and had interdental CAL ≥2 and pocket depth >3 mm. Excluded from the trial were patients with systemic disorders that could impact the outcome and prognosis of periodontal treatment, such as diabetes mellitus, patients who had received periodontal treatment during the 12 months before the study, and patients who had taken antibiotics or anti-inflammatory drugs within the last three months. Additionally, patients with partial dentures, those who smoked or drank alcohol, and those with periodontitis stage IV were excluded as well.

The study protocol was approved by the National Research Ethics Review Committee of the Sudanese Federal Ministry of Health (No. 3-8-17). The CONSORT guidelines were followed (Flowchart 1). The treatment protocol was discussed with the patients, and they signed consent forms before participating in the trial. The experiments were carried out in conformity with the Helsinki Declaration.

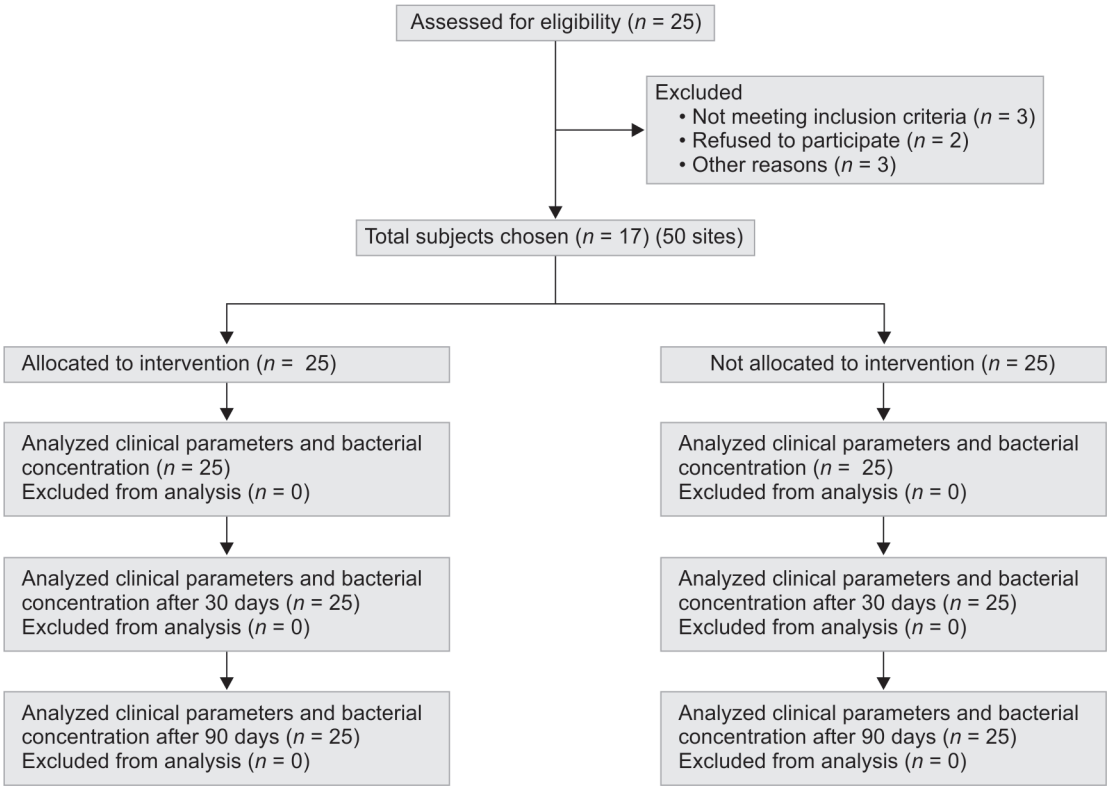
Clinical Procedure

Each participant was asked to rinse with water before the examination. Periodontal measurements were performed using the University of Michigan O probe, with William's marking. Periapical X-rays were taken for confirmation of periodontitis diagnosis and staging and grading of the case according to the new classification of periodontal diseases.[2]

Every patient was examined, and the teeth that fulfilled the criteria of periodontitis according to the new classification of periodontitis disease were included periodontitis were involved in the study. The criteria are as follows: Interdental CAL is detectable at ≥2 non-adjacent teeth, or buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth. Reduction of 1–2 mm CAL and up to 15% of root length or ≥2 mm and ≤3 mm bone loss was characterized as Stage I periodontitis, 3–4 mm CAL, and 16–33% or >3 mm and ≤5 mm bone loss as stage periodontitis and CAL ≥ 5 mm and bone loss >30% as periodontitis stage III or IV).[2] The split-mouth design was performed using tossing a coin as the first half (The right side of the mouth) received SRP in combination with laser therapy (test quadrant) and the second half (The left side of the mouth) received SRP alone (control quadrant).[15] Every patient received oral hygiene instructions and full mouth SRP under local anesthesia performed for each patient using hand instruments (Gracey curettes) and an ultrasonic device.

The laser therapy using an 810 nm diode laser (Germany, Oralia) was performed. The operating protocol of the treatment of periodontal pockets by 810 nm diode laser provided for the application of the following dosimetric values; Continuous mode of 1 W and 400 μm optical fiber. The fiber was placed on the tissue at the top of the sulcus that directs laser energy away from the tooth surface. Upon light beam activation, the tip was moved within the pocket in both horizontal and vertical directions, to draw a pattern in a rapid movement, leading the tip toward the inner wall of the pocket or to remove junctional epithelium migrated and to induce bleeding necessary for the formation of a clot. It was important during the treatment to maintain the

Flowchart 1: Flowchart of the participant



aspirator near the site, to avoid overheating. When simulating the tip in the pocket, it might have residues of sulcular epithelium and infected granulation tissue, which need to be cleaned with gauze soaked in disinfectant before proceeding to the next pocket. The periodontal pockets were irrigated with saline solution after each session of irradiation. To control for the same conditions, pockets were also rinsed with saline after SRP on the control side.[16] The clinical periodontal parameters were taken on the same day of bacterial sample collection. All clinical measurements, bacterial sampling, SRP treatments, and laser application were performed by the same experienced investigator. The clinical evaluation of the periodontal status of patients will be determined by; the gingival index (GI), plaque index (PI), probing pocket depth (PPD), and clinical attachment level (CAL).[17–20]

Calibration measures for probing measurements (Kappa test) were done, and inter-examiner agreement was considerable with a κ-value of 0.806.

Bacterial Sampling and Analysis

The microorganisms *Aggregatibacter actinomycetemcomitans (A.a.)*, *Porphyromonas gingivalis (P.g.)*, and *Prevotella intermedia (P.i.)* were among those analyzed from the gathered bacterial samples. Before the NPT and the assignment of test/control quadrants, the full-mouth periodontal examination and the bacterial collection were completed. Using size 30 sterile paper points, bacteria were extracted from the subgingival aspect (CFPM-France). The paper points were inserted in the crevice until mild resistance was felt and left for 10 seconds all paper points contaminated with blood were discarded.[21] For each tooth, two paper points were used in the deepest periodontal pocket. To prepare an extract for the two polymerase chain reaction tests (PCR tests) per appointment, the split-mouth design allowed the bacterial collection per side to be grouped (control quadrants and test quadrants sample for the PCR test).

The samples were collected into sterile labeled cryogenic vial tubes and were diluted in phosphate buffer saline up to 650 µL.[22] Samples were stored at –80°C until assayed.

Thirty days and 90 days following the first intervention, the bacterial collection was performed at the same baseline periodontally treated sites, where one randomly selected side received only NPT (control side) and the other side received NPT plus the diode laser application (test side).

DNA Extraction

The DNA extraction was performed using the guanidine chloride method as described by Gassoum et al. After overnight incubation of bacteria isolates in peptone water, cells were centrifuged at 6,000 RPM for 10 minutes, then washed with TBE buffer, and centrifuged again. To the pellet 2 mL of cell lysis buffer, 1 mL of guanidine chloride, 350 µL of ammonium acetate and 20 µL of proteinase K were added tubes were vortexed and then incubated at 37°C overnight. The tubes were vortexed and 2 mL of pre-chilled chloroform was added, the tubes were mixed by using a vortex mixer, after that the tubes were centrifuged at 6,000 RPM for 10 minutes. Then the supernatant was transferred into a new falcon tube (15 mL) and, 8 mL of pre-chilled ethanol was added to each tube with gentle mixing to precipitate the DNA, for completion of DNA precipitation the tubes were incubated at –80°C for 2 hours. After incubation the tubes were centrifuged at 6000 RPM for 10 minutes, then the ethanol was poured into a disposal bottle, after *t* mL of 70% alcohol was added and after that, the tubes were centrifuged at 6,000 RPM for 10 minutes, the 70% alcohol was

Table 1: Bacterial primers used in real-time PCR

Bacteria	*Primer*
P. gingivalis	Forward 5"-CTTGACTTCAGTGGCGGCGA-3' REVERSE 5'- AGGGAAGACGGTTTCACCA-3'
Pr. intermedia	Forward 5"AATACCCGATGTTGTCCACA 3' Reverse 5'- TTAGCCGGTCCTTATTCG-3'
A.a	Forward 5" – CTTACCTACTCTTGACATCCGAA–3' Reverse 5' ATGCAGCACCTGTCTCAAAGC-3'

poured into disposal bottle. The tubes were bottled on filter paper, and then left to air dry, after completion of drying 100 µL of distilled water was added, then after that the tubes were incubated at 4°C for completion of DNA elution. The concentration of extracted DNA was read using a Spectrophotometer (Bioeppendrof).

SYBR-green Real-time PCR

A real-time PCR assay was used to estimate the copy number of *P. gingivalis, Pr. intermedia* and *A.a* before and after treatment. The primers are listed in (Table 1). This was done by using a 2x Real Mod™ Green Real-Time PCR Master Mix kit (iNtRON biotechnology/ catalog No. 25344). That contains EvaGreen dye master mix for 500 reactions. This was prepared by adding,10 µL (2x) of EvaGreen dye master mix, 1µL of both reverse and forward primers diluted 1/10 from stock concentration 100 pmol/µL, 2µL of target cDNA, and the total volume was completed to 20 µL by nuclease-free water. Standard curves were from 1.2 kb kanamycin positive control RNA for all primers. A tenfold serial dilution was used to test the efficiency of *P. gingivalis, Pr. intermedia* and *A.a*. Two microliters from each fold were added to 18 µL real-time PCR master mix in each tube, five points were used to contrast the curve, and finally ran into the real-time PCR machine (Rotor-Gene Q). The cycle condition was 95°C for 15 minutes per incubation, followed by 40 cycles of 95°C for 20 seconds, 60°C for 20 seconds, and 72°C for 20 seconds for *P. gingivalis, Pr. intermedia* and *A.a*. Dissociation curve was run followed as 95°C for 1min then 55°C to 95°C generating the melting curve. No template control (NTC) was amplified in each run.

Follow-up Intervals

The patients were recalled at 30 and 90 days after the intervention and the clinical indices were measured again.

Statistical Analysis

The data were analyzed using Statistical Package for the Social Science (SPSS) version 21.

Each pair of quadrants (control or test) was assessed for the bacterial collection and the clinical parameters to evaluate statistical significance. The means and standard deviations were calculated from each clinical parameter that was recorded including the bacterial assessment from the PCR results, at the test and control sides and statistically analyzed. The statistical analysis performed was based on a one-sample *t*-test. The differences in the periodontal clinical parameters and bacterial concentration before and after treatment were analyzed using a paired *t*-test of the mean values.

The differences in the mean values of the periodontal clinical parameters and bacterial concentration after 30 and 90 days were analyzed using one-way ANOVA.

$p < 0.05$ was considered statistically significant.

Table 2: Demographic characteristics of the study participants

	Frequency	*Percent*
Gender		
Male	6	30
Female	14	70
Age		
Mean ± SD	26.4 ± 6.8	

Results

The study started with 20 patients, however, 17 of them completed the study regularly with 50 sites (25 cases and 25 controls). Three patients were excluded from the study; one female who became pregnant and two males who travelled after the baseline visit. Among the 17 patients who completed the study, 13 were females and 4 were males, with a mean age of (26.4 ± 6.8) years (Table 2).

Clinical Assessments

The patients were all followed up on till the completion of the trial. provide the means and standard deviations (SD) of the clinical parameters (PD and CAL) in the "SRP alone" and "SRP + laser" groups at baseline, 30 and 90 days later. PD and CAL decreased significantly in both groups ($p < 0.05$).

The results of the site-specific PPD and CAL (mean ± SD) between baseline and time points in test and control groups are displayed in Figure 1. In both groups, PPD and CAL showed statistically significant reductions at different time intervals ($p < 0.05$).

The mean PPD at baseline was 4.72 ± 0.84 in the laser group and 4.64 ± 0.49 in the control group. After treatment, these values became 3.48 ± 1.08 and 3.84 ± 0.75 at 1 month and 2.63 ± 1.46 and 2.74 ± 0.99 at 3 months, respectively. The mean CAL at baseline was 2.72 ± 0.84 in the laser group and 2.64 ± 0.49 in the control group. After treatment, these values decreased to 1.48 ± 1.08 and 1.84 ± 0.75 at 1 month and 0.64 ± 1.11 and 0.64 ± 0.81 at 3 months, respectively.

No significant difference was observed in CAL values after 1 and 3 months in both test and control groups ($p > 0.05$).

Bacterial Profile

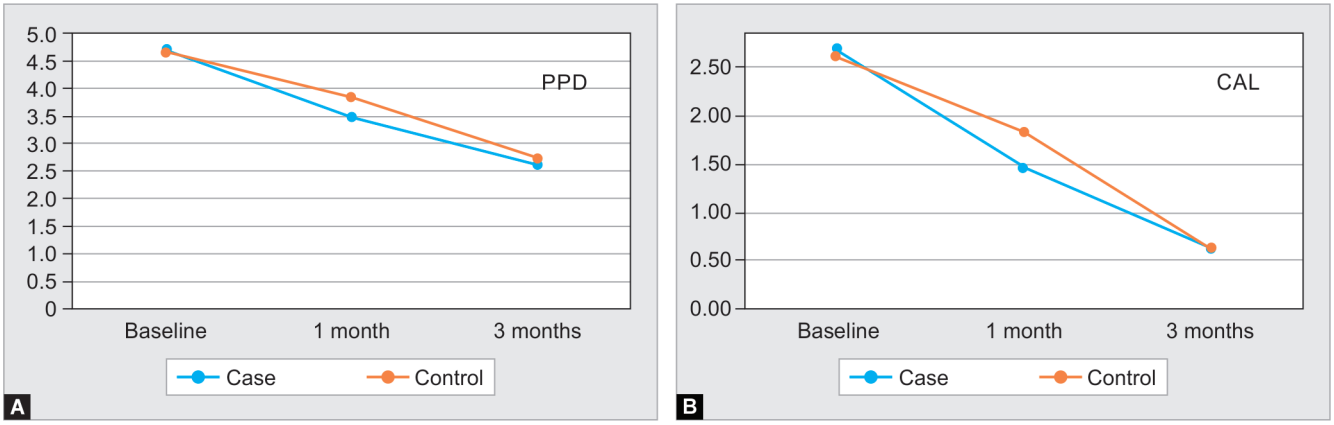
The maximum bacterial concentration enumerated by quantitative PCR was the *A.a bacteria*, followed by *Pr. intermedia* and the least concentration was *P. gingivalis* (Fig. 2).

The efficacy of SRP + laser (test side) as an adjuvant on the bacterial load at the initial intervention was compared to the bacterial load at the follow-up visits (1 month and 3 months later). A significant reduction in the mean number of *A.a., P. gingivalis,* and *Pr. intermedia* bacterial spp. was observed ($p = 0.001$) (Table 3).

The effectiveness of SRP alone (control side) on the bacterial load at the initial intervention was compared to the bacterial load at the control sides' follow-up visits (one month and three months later). There was a significant reduction in the bacterial colonies of *A.a, P. gingivalis,* and *Pr. intermedia*, with *p*-values of 0.001 for *A.a, P. gingivalis*, and 0.002 for *Pr. intermedia* (Table 4).

Pr. intermedia exhibited a drop in the number of colonies after 1 month, but an increase in the number of bacterial colonies after 3 months with no statistically significant difference was observed (Table 4).

A comparison in the mean number of copies of *A.a, P. gingivalis,* and *Pr. intermedia* between the two groups is illustrated in Figures 3 to 5 respectively.



Figs 1A and B: Site-specific PPD and CAL

No significant difference between the two treatment groups *$p > 0.05$

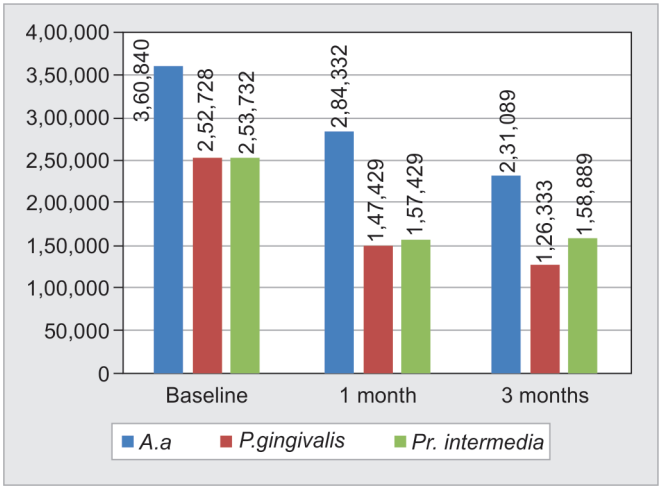


Fig. 2: Bacterial concentration enumerated by quantitative PCR for *A.a*, *P. gingivalis*, and *Pr. intermedia*

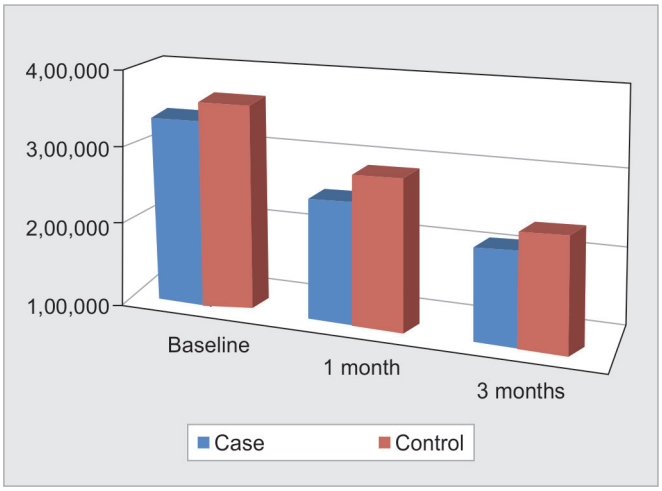


Fig. 3: Comparison of *A.a* between the case and control groups

Using paired samples *t*-test, at baseline the mean copy number of *A.a* was higher in the control group, however, at all the subsequent time intervals, the mean copy number was lower in the

Table 3: Case bacterial profile throughout the study period

	Baseline	*1 month*	*3 months*	*p-value*
A.a	3,38,480	2,46,128	2,05,944	0.001**
	93,504	96,415	1,03,728	
P. gingivalis	2,49,464	1,39,033	1,26,883	0.001**
	68,843	44,389	41,608	
Pr. intermedia	2,48,784	1,42,075	1,21,200	0.001**
	66,317	32,669	42,631	

**p-value is significant

Table 4: Control bacterial profile throughout the study period

	Baseline	*1 month*	*3 months*	*p-value*
A.a	3,60,840	2,84,332	2,31,089	0.001**
	71,839	101,849	103,566	
P. gingivalis	2,52,728	1,47,429	1,26,333	0.001**
	69,509	48,994	53,817	
Pr. intermedia	2,53,732	1,57,429	1,58,889	0.002**
	68,749	38,635	95,646	

**p-value is significant

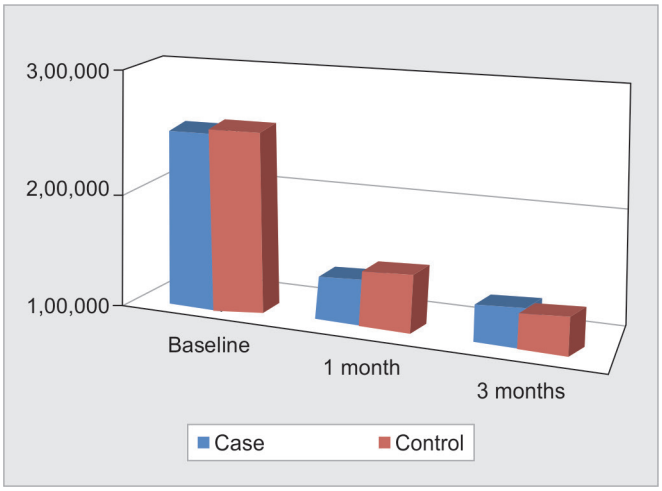


Fig. 4: Comparison of *P. gingivalis* between the case and control groups

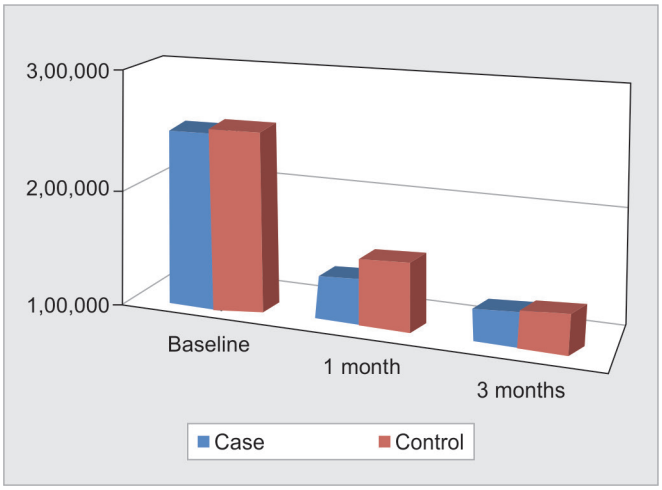


Fig. 5: Comparison of *Pr. intermedia* between the case and control groups

case group as compared to the control group and the difference was statistically highly significant after one month (*p = 0.001).

The mean copy number of *P. gingivalis* at baseline was much higher in the control group, however, at all the subsequent time intervals, the mean copy number was lower in the study group as compared to the control group and the difference was statistically significant after one month (*p = 0.006). Finally, the mean copy number of *Pr. intermedia* at baseline was higher in the control group, however, at all the subsequent time intervals, the mean copy number was lower in the case group as compared to the control group and the difference was statistically significant after one month (*p = 0.001).

Discussion

Diode laser has less clinical implications with dental hard tissues making it convenient for soft tissue operations, as lasers can achieve excellent tissue ablation with strong bactericidal and detoxification effects. Another advantage is that they can reach sites that conventional mechanical instrumentation cannot.[23] The adjunctive use of lasers with conventional mechanical instrumentation may facilitate treatment and has the potential to improve healing.[21] A diode laser with a wavelength of 655–980 nm can accelerate wound healing, promote angiogenesis, augment growth factor release, and prevent root surface ablation.[23]

This study looked at the effectiveness of an 810 nm diode laser, in addition to standard SRP, in improving periodontal parameters in individuals with periodontitis. Furthermore, this study assessed the efficacy of diode laser 810 nm in reducing the population of pathogenic bacteria (*A.a, P.gingivalis, and Pr.intermedia*).

There was no statistically significant difference between the two groups in PPD and CAL at different time intervals ($p > 0.05$). In the case group, there was a statistically significant decrease in PPD, and CAL at different time intervals ($p = 0.001$). Similarly, in the control group, there was a statistically significant decrease in PPD, and CAL at different time intervals ($p = 0.001$).

In contrast to Sharaf H,[15] discovered that on the right side of the mouth that received SRP plus laser therapy (SRP + Laser), the decrease in CAL was statistically significant after 2 weeks and 6 weeks, but there was no statistically significant difference from the baseline after 12 weeks. In the left side of the mouth, where SRP was performed, the decrease in CAL was statistically significant after 2 weeks, but there was no statistically significant change from the baseline after 6 weeks or 12 weeks.

Mechanical treatment alone is clinically and microbiologically effective. Sbordone et al. reported that diseased sites treated with a single episode of SRP showed a microflora similar to that in healthy sites 7 days after treatment. However, the treated sites were repopulating with potentially pathogenic microbes 21 days after treatment.[24]

The *A. a bacterium* had the highest bacterial concentration detected by quantitative PCR, followed by *Pr. intermedia*, and *P. gingivalis* had the lowest concentration. Between baseline and day 30, the highest decline in copy number was found in both groups. Furthermore, there was an intriguing drop in the copy number of *A. a, P. gingivalis*, and *Pr. intermedia* in both the case and control groups during the whole period of the study.

At baseline mean copy number of *A. a* was higher in the control group, however, at all the subsequent time intervals, the mean copy number was lower in the case group as compared to the control group and the difference was statistically highly significant in day number 30. The reduction of *A. a* by laser therapy can be attributed to the destruction of critical virulence factors (lipopolysaccharides and proteases) present in the bacteria.[25]

At baseline mean copy number of *P. gingivalis* was much higher in the control group, however, at all the subsequent time intervals, the mean copy number was lower in the case group as compared to the control group and the difference was statistically highly significant in day number 30 as well. The reduction of the bacterial copy number in this case might be explained by the fact that the wavelength of the diode laser has better penetration and affinity for the chromophores or pigments present in *P. gingivalis* which may result in lysis of the cell wall of these bacteria.[26]

The mean copy number of *Pr. intermedia* at the baseline was much higher in the control group, however, at all the subsequent time intervals, the copy number count was lower in the case group as compared to the control group and the difference was statistically highly significant in day number 30.

These results denote that the impact of laser therapy in combination with SRP extended to the 12th week of therapy. This was in agreement with Qadri et al. who found that the effect of laser therapy extended to the 12th week.[27] On the other hand, Coluzzi found that the effect of laser therapy extended to the 6th week.[28] This result could be attributed to the lower energy 810 nm diode laser of dosimetric values; continuous mode and 400 µm optical fiber. Some of the effects of laser therapy on periodontium may be due to an increase in microcirculation in the irradiated area.[29] Kreisler et al. showed that 1W power has no or little effects on the root surface and attachment level of periodontal tissue among different power of 1, 1.5, 2, and 2.5W diode laser, while 1.5w and higher power cause thermal damage and attachment loss[30] The favorable effect of diode laser might be due to the ability of radiation to eliminate bacteria in the dentinal tubules where they can act as a reservoir for recolonization and re-infection of pockets.[31] However, *A.a* remained in a high proportion of sites after therapy, probably due to its ability to invade periodontal tissues. In addition, *P. gingivalis* can adhere to and enter oral epithelial cells. Our results indicated that laser therapy succeeded in eliminating pathogenic bacteria only after the use of the combined therapy.

Regarding the maximum bacterial concentration, this study agrees with other studies in the literature that concluded the antibacterial effectiveness of low-energy diode laser irradiation on the management of periodontitis.[15,32] In contrast, a study by

Gupta et al. revealed a total bacterial amount in samples measured by culturing varied as *Pr. intermedia* was greater than *A.a*. The difference in the results between the current studies and the study by Gupta may attributed to the difference in methods of the detection of the bacteria in the studies.[33]

The current study revealed, an increased copy number of *Pr. intermedia* after three months in scaling and root planing group. This can be explained by the fact that most of the population in the study were females (70%) in which the hormonal variation acts as a selective factor for the bacterial growth in the subgingival environment resulting in increased subgingival counts of *Pr. intermedia* due to the interactions between female sex hormones with the fumarate reductase system.[34] As shown in the present study, SRP + Laser was the most effective treatment modality that keeps levels of all bacterial species low level up to 3 months after therapy. The most favorable bacterial reduction was achieved one-month post-therapy for all the bacterial species tested. This favorable effect might be due to the ability of laser irradiation to eliminate bacteria in dentine tubules where they can act as a reservoir for recolonization and re-infection of pocket.[15]

The short follow-up period—which can be extended to 12 months—was one of the study's shortcomings. More long-term, randomized clinical trials are needed to fully evaluate any potential advantages of combining laser therapy with traditional non-surgical periodontal therapy. These trials should also include bigger sample numbers. Additionally advised are modifications to the laser's parameters and application modes.

Conclusion

The overall findings of this investigation suggested that the standard mechanical approach of SRP, either alone or in combination with diode laser radiation, significantly improved pocket depth and clinical attachment loss over the experiment's observation period. As a result, while laser radiation of periodontal pockets may improve clinical parameters in patients with periodontitis, the effect is limited, and laser treatment should be regarded solely as an adjuvant to non-surgical periodontal therapy.

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