Estimation of Red-complex Bacteria in Diode Laser Treated Chronic Periodontitis Patients: A Clinical and Microbiological Study

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Context: Laser has been widely accepted as a substitute to traditional periodontal treatment. Only a finite number of studies are available based on the use of diode laser as a supplement to scaling and root planing (SRP) in the reduction of red-complex bacteria. Aim: This split-mouth study was aimed to determine the clinical and microbiological effects of diode laser as a supplement to SRP. Materials and Methods: For this split-mouth study, systemically healthy 34 patients with chronic periodontitis were selected. In the test quadrant, SRP + laser therapy was carried out, whereas in control quadrants, SRP alone was performed. Clinical and microbiological data were acquired at baseline and 3 months postoperative, and statistical analysis was carried out on the findings. Results: The results showed that both the treatment modalities were impelling. Considerable reduction in the mean probing depth and a notable improvement in the attachment level were observed in both groups in comparison to baseline, with a statistically significant reduction in the laser group. Microbiological analysis results showed more reduction in red-complex bacteria in the laser group compared to the SRP group, but they were statistically insignificant. Conclusion: Within the limitation of this study, it is recommended that both the SRP and SRP + laser are effective in chronic periodontitis management, but using laser with SRP has propitious results. Thus, in the forthcoming years, clinical experiments with a greater sample size may be chosen to further analyze the fringe benefits of laser as a supplement to SRP.

Keywords: Chronic periodontitis, diode laser, polymerase chain reaction, red-complex pathogens

INTRODUCTION

Periodontitis is a chronic inflammatory disorder of multifactorial etiology characterized by site-specific degradation of the supporting tissues of the tooth, resulting in periodontal pockets, gingival recession, and ultimately leading to the loss of the tooth.\(^1\)

The main aim of periodontal treatment is to make the hard tissue and soft tissue free from pathogenic bacteria by removing supragingival and subgingival plaque and calculus.\(^2\) Numerous treatment approaches are available in the field of periodontics to achieve these goals, and they can be broadly classified into either nonsurgical or surgical procedures. Though conventional mechanical therapy has been widely used, they carry certain drawbacks. One of which is producing a smear layer, preventing a complete eradication of harmful bacteria and its toxins. It was also found that such procedures are also likely to produce roughness, deep grooves in the root surfaces, and restricted access

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to areas such as furcations, concavities, grooves, and distal sites of molars.[3,4]

Hence, laser therapy has been widely accepted as a substitute for traditional periodontal therapy and has the potential to improve healing. Laser irradiation shows bactericidal and detoxification effects without creating a smear layer, therefore providing favorable conditions for periodontal tissue attachment. It is expected that the exemplary ablation of tissue with a laser would facilitate the recuperation of periodontal tissues. The diode laser is the most popular choice in dentistry as it is inexpensive, compact, and easy to use. It has strong penetration power, which helps in penetrating into the tissue, and is well absorbed in pigmented tissues. It can also target pigmented bacteria and granulation tissue.[5]

Because of the flexible nature of the laser fiber, the diode laser is currently used as suitable pocket insertion.[6] However, limited data are available on clinical research, providing scientific support for the specific use of a 980-nm diode laser as a supplement to phase-I periodontal therapy in the reduction of red-complex bacteria. Hence, this study involved a comparative analysis between the efficiency of traditional Phase I therapy and laser therapy in reducing the bacterial population, namely the bacteria belonging to the red complex—Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia.

**Materials and Methods**

Thirty-four systemically healthy individuals visiting the department of periodontics were screened, and they underwent a comprehensive periodontal examination that included probing pocket depth (PPD) and clinical attachment level (CAL). Chronic periodontitis patients were selected based on the American Academy of Periodontology 1999 Classification. Institutional ethics committee (IEC) and review board (1084/IEC/2016) reviewed and accepted the research design. Before enrollment in the study, the written, informed consent was acquired from each subject. All clinical approaches were performed in conformity with the Helsinki Declaration and the Principles of Good Clinical Practice. The following exclusion requisites were examined for medical and dental records: (1) Use of antimicrobials during the last 6 months, (2) persons with immune deficiency, (3) patients with severe periodontitis, (4) patients who are pregnant and breastfeeding mothers, and (5) patients who indulge in the consumption of tobacco in any form. The requisites for inclusion in the research were as follows: (1) Patients aged 30–50 years, (2) patients diagnosed with generalized chronic periodontitis, (3) minimum of 10 teeth in each arch with at least two teeth with a PPD of ≥5 mm and CAL 3–4 mm, (4) free of systemic ailments that impede with periodontal recuperation, and (5) did not undergo periodontal treatment within 6 months. The subjects were arbitrarily chosen and split into two groups by using a coin flip method. The control group (SRP group) received SRP alone whereas the test group (SRP + laser group) received diode laser application along with SRP.

**Assessment of clinical parameters**

The clinical variables were measured at baseline and at 3 months post-surgically. Plaque index (PI) and gingival bleeding index (GBI) were recorded for all sites. Using custom-made acrylic stents with guiding grooves and the cementoenamel junction as a reference point, the following clinical variables were measured: PPD and CAL.

**Conventional scaling and root planing group**

At the time of the initial visit (baseline), clinical plaque samples were collected bilaterally followed by comprehensive ultrasonic scaling in the test and control quadrants. All the subjects were given instructions on oral hygiene. During the second visit, root planing was carried out using area-specific curettes. The test quadrants were given an additional application of diode laser after root planing had been completed.

**Laser decontamination**

Laser application was carried out using a diode laser unit (Zolar Technology, Mississauga, Ontario, Canada). Protective eyeglasses had been worn by the patient, operator, and assistant. Highly reflective instruments have been avoided. After applying a topical anesthetic agent, the activated fiber was inserted parallel to the long axis of the root surface into the periodontal pocket and moved in an apicocoronal direction in a sweeping motion. After each session, the periodontal pocket was irrigated with saline solution. If the patient had sensitivity, they were advised to use desensitizing toothpaste. After 3 months, the patients were recalled for assessing the clinical parameters, and plaque samples were collected bilaterally and stored in the transporting medium (TE buffer solution) at -20°C.

**Microbiological analysis**

Plaque samples were collected at baseline and after 3 months postoperatively, by inserting sterile paper points in the deepest pocket bilaterally and left undisturbed for 15 s. After that, they were stored in an Eppendorf tube containing 1 mL of TE buffer solution at 20°C for the analysis of red-complex bacteria by real-time polymerase chain reaction (RT-PCR) method.
directed by the manufacturer, the deoxyribonucleic acid (DNA) was retrieved from the subgingival plaque sample using a genomic DNA extraction kit (QIAamp DNA mini kit, Germantown, MD, USA). The samples were checked for the presence of *P. gingivalis, T. forsythia, T. denticola*, by standard PCR methods. The primers for the microorganisms were based on the 16S ribosomal ribonucleic acid (rRNA) gene\(^{[7-9]}\) [Table 1].

The DNA quantification was done by RT-PCR or quantitative PCR (qPCR) reaction through QuantStudio 5. The amplification plot and the melt curve were generated by QuantStudio Design and Analysis Software, version 1.4.2 (Waltham, MA, USA) [Figure 1].

### Table 1: Primers for polymerase chain reaction analysis

| Target organism | Sequence (5’→3’)                  |
|-----------------|-----------------------------------|
| *T. forsythia*  | GCGTATGTAACCTGCCCGCA              |
| (Forward)       |                                   |
| *T. forsythia*  | CCGTTACCACCAACTACCTAATG           |
| (Reverse)       |                                   |
| *T. denticola*  | TAATACCGAATGTGCTCATTTACAT         |
| (Forward)       |                                   |
| *T. denticola*  | TCAAAGAAGCATCCCTCTTCTTCTTTTA      |
| (Reverse)       |                                   |
| *P. gingivalis* | CTTGACCTTCAGTGCCGGCAG             |
| (Forward)       |                                   |
| *P. gingivalis* | AGGGAAGACGTTTTCACCA               |
| (Reverse)       |                                   |

**Statistical analysis**

The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) software package, version 19.0 (Chicago, IL, USA). Qualitative data were expressed as frequencies or percentages, and quantitative data as the mean values and standard deviation (SD). Microbiological parameters were expressed in terms of \(C_i\) value, and chi-square test was used to correlate the proportions. The comparison of mean values for the clinical parameters was executed using paired \(t\) test for within a group (intragroup analysis) and unpaired \(t\) test for intergroup comparisons. In all the aforementioned statistical tools, the probability value \(P \leq 0.05\) was chosen as significant.

**Results**

**Clinical parameters**

This study evaluated the impact of diode laser as a supplement to SRP in chronic periodontitis patients. The study included a total of 34 subjects (134 samples), and three patients failed to complete the study and were omitted from the analysis. These results assess the effectiveness of diode laser on the basis of clinical and microbiological parameters. The mean reduction in PI from baseline (2.79 ± 0.78) to 3 months was 0.72 ± 0.45, which was statistically significant \((P = 0.001)\), thus showing good compliance. The GBI value was statistically significant \((P = 0.001)\) at 3 months (0.56 ± 0.52) compared with the baseline (2.18 ± 0.84). Table 2 shows the mean clinical measurements of SRP and SRP.
+ laser groups at baseline and at 3 months. The mean reduction in PPD from baseline to 3 months is 2.77 ± 0.76 and 3.41 ± 0.91 for SRP and SRP + laser group, respectively, with no statistically significant difference between the groups (P = 0.523). At baseline, the mean CAL in the SRP group was 4.48 ± 1.18, and in laser group, it was 4.44 ± 1.07. At 3 months, the mean CAL in the SRP group was 2.61 ± 0.55, and in the laser group, it was 2.27 ± 0.51; the difference between the test group and control group was statistically significant (P = 0.001).

Microbiological analysis

Intragroup comparison of mean Ct value showed that P. gingivalis, T. forsythia, and T. denticola were significantly decreased after 3 months as against the baseline in both the groups [Table 3]. The intergroup comparison showed that no statistically significant difference (P = 0.174) was observed in the mean Ct value among the SRP group (53.57 ± 5.62) and the laser group (56.31 ± 9.33) at baseline. Similarly, at 3 months, the mean Ct value between the SRP group (69.76 ± 11.59) and the laser group (74.53 ± 12.10) was statistically insignificant (P = 0.026) [Table 4].

Discussion

SRP is one of the most frequently used treatment methods for periodontal disease and is the gold standard therapy.[2] Most of the beneficial effects of SRP tend to occur within the first 3 months. Though SRP is considered to be fundamental to periodontal therapy, it does have drawbacks such as restricted access to deep periodontal pockets.[4,5] This study assessed the effect of diode laser as an additive to SRP in the treatment of chronic periodontitis using clinical and microbiological parameters.

On clinical evaluation, the PI showed similar clinical values in both groups at baseline and after 3 months, with no significant change, thus suggesting good hygiene maintenance by all patients during the course of the study. These results coincide with those from a study by Yukna et al.,[10] who observed that patients undergoing periodontal therapy try to maintain optimal oral hygiene. The mean value of CAL at baseline and at 3 months was 4.02 ± 0.09 and 1.88 ± 0.09, respectively. The mean reduction in CAL from baseline to 3 months was 2.14, which was statistically significant (P = 0.001). Studies conducted by Kamma et al.,[11] Kreisler et al.,[12] and Gokhale et al.[13] have obtained similar results. These results are in agreement with the studies done by Đukić et al.,[14] who also observed statistically significant reduction in CAL.

In our study, bleeding on probing (BOP) is expressed as present or absent at baseline and 3 months. At baseline, BOP was expressed in 27 subjects and absent in 4 subjects. At 3 months, the presence of BOP was expressed in 2 subjects, and the absence of BOP was expressed in 29 subjects. The result showed that there is a significant decrease in BOP at 3 months with a P-value, 0.001. These findings were identical to the results obtained by

### Table 2: Comparison of clinical parameters—Group I and Group II

| Parameters | Group I (SRP) | Group II (SRP + laser) | Group I vs. Group II |
|-----------|--------------|------------------------|---------------------|
|           | At baseline  | At 3 months | P value | At baseline  | At 3 months | P value | At baseline  | At 3 months | P value |
|           | Mean ± SD    | Mean ± SD    |         | Mean ± SD    | Mean ± SD    |         | Mean diff    | P value    | Mean diff    | P value |
| PI        | 2.79 ± 0.78  | 0.72 ± 0.45  | 0.001*  | 2.18 ± 0.84  | 0.56 ± 0.52  | 0.001*  |
| GBI       | 6.42 ± 1.36  | 3.65 ± 0.60  | 0.001*  | 4.48 ± 1.18  | 2.61 ± 0.55  | 0.001*  |
| PPD (mm)  | 18.51 ± 3.50 | 24.30 ± 6.67 | 0.001*  | 16.16 ± 2.04 | 24.26 ± 4.85 | 0.001*  |
| CAL (mm)  | 3.64 ± 1.40  | 3.23 ± 0.49  | 0.001*  | 2.27 ± 0.51  | 0.04 ± 0.11 | 0.012*  |

*pSignificant

### Table 3: Intragroup comparison of Treponema Forsythia (Tf), Porphyromonas gingivalis (Pg), and Tannerella denticola (Td) in scaling and root planing and laser group

| Ct value | Group I (SRP) | Group II (SRP + laser) | Group I vs. Group II |
|----------|---------------|------------------------|---------------------|
|          | At baseline   | At 3 months | P value | At baseline   | At 3 months | P value |
|          | Mean ± SD     | Mean ± SD |         | Mean ± SD     | Mean ± SD |         | Mean diff    | P value    | Mean diff    | P value |
| Tf       | 18.20 ± 1.69  | 21.62 ± 6.03 | 0.002*  | 18.51 ± 3.50 | 24.30 ± 6.67 | 0.001*  |
| Pg       | 15.82 ± 1.70  | 23.65 ± 4.13 | 0.001*  | 16.16 ± 2.04 | 24.26 ± 4.85 | 0.001*  |
| Td       | 19.44 ± 3.66  | 21.14 ± 5.55 | 0.001*  | 21.06 ± 5.55 | 26.08 ± 5.05 | 0.001*  |

*pSignificant

Higher the Ct value indicates less amount of bacteria identified
Lesser the Ct value indicates more amount of bacteria identified
Moritz et al.\textsuperscript{[13]} Contradictory findings were also found related to our study by Euzebio Alves et al.\textsuperscript{[16]} in 2013.

In our present research, the average PPD depths at baseline were 6.42±1.36 in the SRP group and 6.64±1.4 in the laser group, showing that cases with similar severity of defects were selected for both the groups. Significant reductions in pocket depth were observed in both the groups at the end of 3 months (2.78 ± 0.18 mm in SRP and 2.21 ± 1.05 mm in SRP + laser), but no statistically significant difference was observed between the groups (\(P = 0.523\)). Such findings were similar to those found by Gokhale et al.\textsuperscript{[13]} who observed a reduction in PPD from 6.03 to 2.97 at 3 months in the laser group.

A study was conducted by Gupta et al.\textsuperscript{[17]} on the assessment of diode laser as a supplement to SRP in nonsurgical treatment of chronic periodontitis. They concluded that the adjunctive treatment of diode laser has shown better efficacy in ensuring better periodontal health as compared to SRP alone. Identical results were obtained in our study, by achieving a significant reduction in CAL in both groups.

According to our study, baseline mean \(C_t\) value in SRP group was 53.57 ± 5.62 and mean \(C_t\) value in laser group was 56.31 ±9.33 with a \(P\) value of 0.174; the discrepancy between the test group and control group showed a statistically insignificant value. At 3 months, mean \(C_t\) value in SRP group was 69.76 ± 11.59 and mean \(C_t\) value in laser group was 74.53 ± 12.10 with a \(P\) value of 0.122, which was statistically insignificant. Such findings mimic those reported by Caruso et al.\textsuperscript{[19]}

The decision of the high-intensity diode laser was in reference to studies that showed that its wavelength has a stronger penetration and affinity for the pigments present in some bacteria, which would serve as an absorbing chromophore. This would intensify its activity and allow it to enter black pigmented anaerobes such as \(P.\) gingivalis.\textsuperscript{[10]}

Antibacterial studies by Moritz et al.\textsuperscript{[13]} utilizing laser along with SRP identified that laser energy can pervade the underlying connective tissue up to 1–2 mm depth, which aids in the elimination of pigment-containing bacteria, particularly \(P.\) gingivalis. The actual effect of the diode laser on cellular and molecular level periodontal pathogens has not been confirmed.

We speculate that RT-PCR assay for the identification of microbes was found to be sensitive, accurate, and rapid. However, regular use of an RT-PCR assay remains limited because it is expensive and lacks standardization. RT-PCR is highly technique sensitive. A wide range of PCR assays are more liable to contamination than a species-specific PCR assay. Contaminations may ensue from the environment, labware, or enzymes. There is no threshold cycle (\(C_t\)) cutoff value to differentiate real infections from baseline contamination levels in negative samples.\textsuperscript{[21]}

Hence, we suggest that the conflicting results of our study may be due to absence of standardization of reports on irradiation parameters and incorrect dosimetry specifications such as power, beam area, time, dose, and contact mode.\textsuperscript{[22,23]} The study limitations include small sample size and lack of established protocol adjunctive laser treatment with SRP. Additional well-defined randomized, blinded, controlled longitudinal studies are needed to determine the long-term efficacy of chronic periodontitis treatment.

**Conclusion**

In periodontal practice, the advent of laser technology and the unearthing of its antimicrobial effects have contributed to its wide utility as an auxiliary treatment modality. We believe that using this diode laser technology, as an adjunctive tool for the treatment of periodontal disease, has proved to be extremely efficient, and can be used as a part of standard clinical practice.

In view of the good clinical and microbiological results procured in cases with application of the laser diode, it can be efficiently used in the treatment of periodontal pockets in patients with moderate to severe chronic

| Group              | \(N\) | \(C_t\) value | SD    | \(P\) value |
|--------------------|------|---------------|-------|-------------|
| Baseline           | 30   | 53.57         | ±5.62 | 0.174       |
| SRP group          |      |               |       |             |
| Laser group        | 31   | 56.31         | ±9.33 |             |
| 3 Months           | 30   | 69.76         | ±11.59| 0.122       |
| SRP group          |      |               |       |             |
| Laser group        | 30   | 74.53         | ±12.10|             |
periodontitis on a routine basis with nonsurgical therapy.

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Conflicts of interest
There are no conflicts of interest.

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