

Comparative Clinical And Microbiological Evaluation In Periodontal Flap Surgery With Diode Laser As An Adjunct To Mechanical Debridement Versus Conventional Mechanical Debridement: A Split Mouth Study

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Abstract: The purpose of this study was to evaluate the adjunctive effects of the diode laser in open flap debridement as compared with the conventional mechanical debridement evaluated by clinical and microbiological parameters and also limited literatures were available on such studies. Twenty patients with bilateral periodontal pockets in the quadrant of either maxilla or mandible at least 6 teeth in quadrant diagnosed with chronic periodontitis with probing pocket depths ≥ 5 mm after phase I therapy. Group-A (Control Group) Open flap Debridement (OFD) were done and Group-B (Test Group) Open flap Debridement (OFD) with Diode Laser were done under local anesthesia. In the present study there were a statistically significant difference in Plaque index, Gingival index, pocket probing depth and relative attachment level at baseline and at 3 months in the control and the test group and also there was a statistically significant reduction in the number of CFUs of anaerobes in the laser-treated group as compared with the control group. Colony-forming units were statistically insignificant at baseline and 3 months as the P-value was 0.06 and 0.7 respectively and statistically significant at 3 months as the p-value was <0.001 . Colony-forming units were statistically significant in Group B (Test group) Laser treated group at 3 months as the p-value was <0.001 . Therefore, lasers can be used as an adjunctive treatment with open flap debridement as a part of periodontal therapy in the future.

Keywords: Diode Laser, Open Flap Debridement, Periodontal pocket, Anaerobic bacteria, Scaling and root planing

Introduction

Periodontitis is a chronic inflammatory disease of long-term resulting in tooth loss. Main causative factor for periodontal disease are the microorganisms that resides in the gingival sulcus/periodontal pocket¹. Only some species of microorganisms resident in bacterial plaque are sensitive to a given antibiotic which suggests that selective organisms have a role in periodontal disease. As described by Socransky, the microbial flora in healthy periodontal tissues of humans is scanty and located almost entirely supragingivally on tooth surface and are mainly comprised of gram positive coccal forms. The main objective of periodontal therapy is to eliminate the microbial causative factors that causes periodontal disease either by mechanical debridement consisting of meticulous scaling and root planing using manual and/or power driven instruments combined with adequate oral hygiene measures to arrest the disease progression².

Non-surgical periodontal procedures like scaling and root planing followed by surgical periodontal procedures carried out to promote the periodontal health and function³. The surgical procedure involving treatment of periodontal pockets mainly aims at reattachment and readaptation of the pocket walls rather than the surgical eradication of the outer walls of the pockets⁴.

Recent advances like microsurgery, lasers and other treatment modalities that have set a breakthrough in the periodontology. Laser technology, specifically the diode laser is gaining popularity in general dental practice with potential benefits in a wide range of applications⁵. In addition, it has good tissue penetration⁶ and is well absorbed in pigmented tissues, it can specifically target the pigmented bacteria and granulation tissue⁷. In vitro evaluation has shown the diode laser to achieve a more complete elimination of the epithelial lining of the periodontal pocket⁸. Soft tissue surgical procedures using lasers were found to

have good hemostasis, sterilization and minimal postoperative pain when compared to conventional surgical procedures⁹. The thermal effect of laser energy on soft tissue primarily revolves around the water content of the tissue and the temperature rise of the tissue. When the tissue temperature reaches approximately 60°C, proteins begin to denature without any vaporization of the underlying tissues. This phenomenon is useful in surgically removing diseased granulomatous tissue.

The purpose of this study was to evaluate the adjunctive effects of the diode laser in open flap debridement as compared with the conventional mechanical debridement using clinical and microbiological parameters and also there was a lack of literature available on such studies.

Material and Method

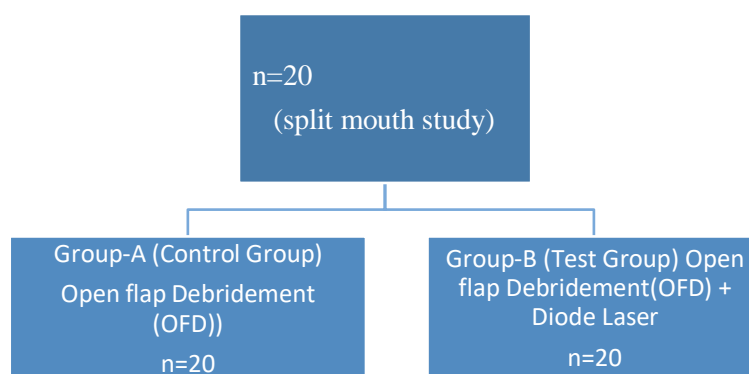
This study involved 20 patients mean age of 40 years (30 to 50 years). The sample size was calculated using this formula

$$N = \frac{(Z_{\alpha})^2 \cdot (S)^2}{(\bar{d})^2}$$

The study protocol was explained to each potential subject and written informed consent was obtained prior to beginning any data collection, each potential subject was briefed about study methodology & provided with written consent form and this study was approved by the research review board committee of Karnavati School of Dentistry (KSDEC/19-20/Apr/024).

Study Design and Subject Selection

After approval from the ethical committee, data was collected from the patients who came to the Department of Periodontology, Karnavati School of Dentistry according to inclusion and exclusion criteria. For division in the control group and test group, baseline clinical parameters were recorded with the help of stent and the subjects were categorized into two groups of 20 patients each as follows: -



The subjects were selected randomly divided into two groups by coin toss method with no discrimination of sex, caste and religion or socio-economic status. Prior to surgery patient who complied with requirements after giving informed consent had initial blood investigations like blood pressure, Random blood sugar, hemoglobin, complete blood count, bleeding time, clotting time was done prior to surgery.

Inclusion criteria

- Patients 30–50 years of age diagnosed with chronic periodontitis with probing pocket depths ≥ 5 mm after phase I therapy.
- Two or more interproximal sites with attachment loss ≥ 4 mm or Two or more interproximal sites with probing depth ≥ 5 mm, not on the same tooth¹⁰
- As this was a split-mouth study, patients with bilateral periodontal pockets in the quadrant of either maxilla or mandible at least 6 teeth in quadrant were selected for the study.
- Patients who have not undergone periodontal therapy in the past 6 months.

Exclusion criteria

- Smokers, pregnant women and lactating mothers, diabetic patients were excluded from the study.
- Patients with a history of administration of antibiotics 3 months

prior to their first visit were excluded from the study.

- Patients with bony defects where regenerative or resective surgeries needed were not included in this study.
- Patients with Grade III tooth mobility.

Measurements (Clinical parameters)

Plaque index (PI) (silness and loe), gingival index (GI) (Loe and Silness), probing depth (PD) (Using an acrylic stents), relative clinical attachment level (CAL) (Using an acrylic stents) The clinical parameters were recorded with the help of a UNC-15 probe at the baseline and at 3 months post operatively.

Microbial Procedure

After diagnosis, prior to the start of the treatment, subgingival plaque samples were collected from the deepest pocket areas in both the groups with the help of paper points at baseline before treatment, 1 month and 3 months after treatment. A sterile paper point (no 30) was introduced in the sulcus as far apically as possible and left undisturbed for 10 s (Figure 1 a). The plaque samples were transported in Robertson cooked meat media to Qualitech Pathology Laboratory, Ahmedabad for bacterial culture analysis (Figure 1 b). There the paper points were inoculated in duplicates on 5% sheep blood with Columbia agar as base. One plate was incubated at 37°C under strict anaerobic conditions 5-10% CO₂ jar by anaerobic gas pack (Himedia) for the growth of anaerobic (Figure 1 c). The culture plates were incubated for 48-72 hrs (Figure 1 d). After 72 hrs of incubation, subculture of anaerobic plate was done again on anaerobic blood agar for

pure isolation. After that colony count was also done for quantification. Colony forming units per ml were calculated for total anaerobes (Figure e & f).

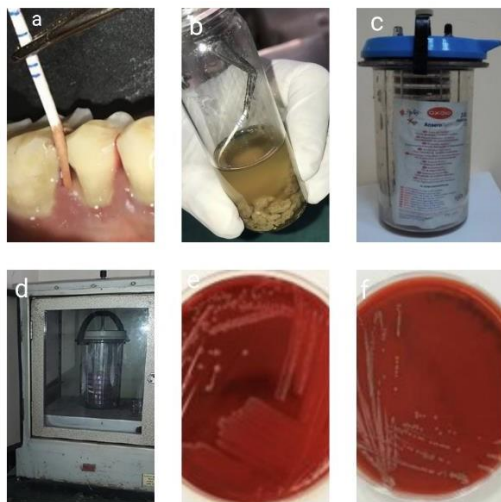


Figure 1

Surgical Procedure

All the patients coming at the outpatient department of the college hospital were examined and assessed for the suitability of the study. The patients were explained about the details of the study and nature of the treatment and a signed consent was obtained from each patient. Patients in group 1 served as control group and received conventional flap surgery. The patients allocated in this group were administered local anesthesia (2% lignocaine in the ratio of 1:80,000); and after achieving proper anesthesia, intrasulcular incisions were given using no.15 BP blade. Full thickness mucoperiosteal flap was elevated and root debridement and curettage was done using area specific Gracey curettes, without any chemical agents. The flap was then sutured with 3-0 silk sutures. Surgical site was covered by using periodontal pack.

Patients in group 2 received 940nm diode laser therapy as an adjunct to open flap debridement therapy which was performed under local anaesthesia. Full thickness mucoperiosteal flap was reflected after which 940 nm diode laser with power setting of 2.5 W in continuous wave

was used to inner surface of the flap in contact mode for 10 seconds (Figure 2c). The charred layer that formed on the pocket wall was removed using moist gauze (Figure 2d). The tip was directed towards the apical aspect of the flap. The site was irrigated with normal saline. Subgingival calculus that was present on the root surface removed with the help of ultrasonic scaler. Exposed bone and root surfaces were exempted from the radiation. The flap was then sutured with 3-0 silk sutures. Surgical site was covered by using periodontal pack (Figure 2e & f). Clinical and microbiological parameters were recorded periodically at regular intervals. Microbiological samples were collected from the deepest pockets. The samples were collected at baseline T0 (before scaling and root planning), T1 (04 weeks after scaling), T2 (03 month after surgery).



Figure 2

Statistical Analysis

The absolute change in each periodontal index at 3-month follow-up with respect to baseline was calculated using the formula: (postoperative index – preoperative Index). DIODE LASER assisted periodontal flap surgery. The statistical significance of several periodontal indices studied between two study groups was tested using the Anova test. In each study group, the intragroup analysis of baseline and 3-month follow-up indices was tested using Student's Paired t test, the statistical analysis tool for paired data. A p value < 0.01 was considered statistically significant. The entire data were analyzed using Statistical Package for

Social Sciences (SPSS version 11.5) for MS Windows.

Result

This split-mouth study was done to evaluate clinical and microbiological parameters between the test group in which OFD+ Laser flap surgery was done as compared to the control group in which OFD alone used in the treatment of Periodontal pockets at Karnavati School of Dentistry, Gujarat. The subjects were selected randomly and divided into test and control group. Microbiological sampling was taken using paper points from the deepest periodontal pockets. No significant complications were recorded at the end of the surgical period, and all the site showed uneventful healing.

The Plaque index scores recorded at baseline and 3months were respectively 2.08 ± 0.35 and 1.91 ± 0.33 , showing a mean difference of 0.35 and 0.33 from baseline to 3 months with P values of <0.01 . (Table 1). The plaque score decreased significantly from baseline to 3 months.

The Gingival Index scores recorded at baseline and 3months were 1.85 ± 0.77 and 0.94 ± 0.61 , respectively, showing a mean difference of -0.90 from baseline to 3 months with P values of 0.000, <0.001 (Table 2). The gingival score decreased significantly from baseline to 3 months.

The pocket probing depth (in mm) for the test group was 7.15 ± 0.93 at baseline, which was decreased to 4.4 ± 0.63 at 3 months. Hence the intragroup comparison showed a significant decrease in probing depth from baseline to 3 months with a mean difference of 2.75 and 2.70, respectively, with P values of <0.001 and 0.001. In the control group, the pocket probing depth (in mm) recorded at baseline and 3months were 7.3 ± 0.86 and 4.6 ± 0.64 , respectively, showing a mean difference of 2.70 from baseline to 3 months with P values of <0.001 (Table 3).

On the intergroup comparison between the test and control group after the t-test, the probing

depth was statically insignificant at baseline and 3 months as the P-value was 0.6 and 0.5. (Table 4).

The relative attachment level (in mm) for the test group was 8.35 ± 0.87 at baseline, which was decreased to 6.2 ± 0.89 at 3 months. Hence the intragroup comparison showed significant improvement in attachment level from baseline to 3 months with a mean difference of 2.15 and 2.45, respectively, with P values of 0.001 and 0.001. In the control group, the relative attachment level (in mm) recorded at baseline and 3months were 8.50 ± 0.51 and 6.05 ± 0.68 , respectively, showing a mean difference of 2.45 from baseline to 3 months with P values of 0.001. (Table 5).

On the intergroup comparison between the test and control group after the t-test, the relative attachment level; was statically insignificant at baseline and 3 months as the P-value was 0.5 and 0.5, respectively. (Table 6).

The Colony-forming units recorded for the test group at baseline, 1 month and 3-month postoperatively were $1.4 \times 10^6 \pm 1.8 \times 10^7$, $1.3 \times 10^7 \pm 2.0 \times 10^4$ and $1.1 \times 10^4 \pm 2.0 \times 10^3$ respectively. Hence intragroup comparison shows a significant reduction in colony-forming counts from baseline to 1 month and 1 month to 3 months with a mean difference of 1.1×10^1 , 1.3×10^2 and 1.1×10^1 with the p values 0.001, 0.001 and 0.001, respectively. In the control group at baseline, 1 month and 3-month postoperatively were $1.4 \times 10^6 \pm 1.8 \times 10^7$, $1.3 \times 10^7 \pm 2.0 \times 10^4$ and $1.1 \times 10^4 \pm 2.0 \times 10^3$ respectively. Hence intragroup comparison shows a significant reduction in colony-forming counts from baseline to 1 month and 1 month to 3 months with a mean difference of 1.1×10^1 , 1.3×10^2 and 1.1×10^1 with the p values 0.001, 0.001 and 0.001, respectively.

On an intergroup comparison between the test and control group after the t-test, the Colony-forming units were statically insignificant at baseline and 3 months as the P-value was 0.06 and 0.7 respectively and statically significant at 3 months as the p-value was <0.001 (Table 7).

Table 1: Intragroup Comparison of plaque index values at baseline and 3 months

	N	Mean	SD	Mean Difference	P value	S or SN
Baseline	20	2.08	0.35	0.17	<0.001	S
3 Months	20	1.91	0.33			

Inference: Plaque Index scores significantly decreased from baseline to 3 months.

Table 2: Intragroup Comparison of gingival index values at base line, 3 months

Intragroup Comparison	N	Mean	Std. Deviation	Mean difference	P value	S orNS
Baseline	20	1.85	0.77	0.91	<0.001	S
3 months	20	0.94	0.61			

Inference: Gingival Index scores significantly decreased from baseline to 3 months.

Table 3: Intragroup comparison of pocket probing depth values (in mm) of test and control sites at baseline and 3 months

Group	Intra group	N	Mean	Std. Deviation	Mean Difference	P	S/NS
Test	Baseline	20	7.15	0.93	-2.75	<0.001	S
	3 months	20	4.4	0.63			
Control	Baseline	20	7.3	0.86	-2.70	<0.001	S
	3 months	20	4.6	0.64			

Inference: Both test and control result showed significant pocket probing depth reduction from baseline to 3 months.

Table 4: Intergroup comparison of pocket probing depth values (in mm) of test and control sites at baseline and 3 months

Time period	Group	N	Mean	SD	Mean Diff	P Value	N or NS
Base line	Test	20	7.15	0.93	-0.15	0.6	NS
	Control	20	7.3	0.86			
3 months	Test	20	4.4	0.63	-0.2	0.5	NS
	Control	20	4.6	0.64			

Table 5: Intragroup comparison of Relative attachment level values (in mm) of test and control sites at baseline and 3 months

Group	Intra group	N	Mean	Std. Deviation	Mean Difference	P	S/NS
Test	Baseline	20	8.35	0.87	-2.150	<0.001	S
	3 months	20	6.2	0.89			
Control	Baseline	20	8.5	0.51	-2.45	<0.001	S
	3 months	20	6.05	0.68			

Inference: Both test and control result showed significant improvement in relative attachment levels at all-time intervals.

Table 6: Intergroup comparison of Relative attachment level values (in mm) of test and control sites at baseline and 3 months

Time period	Group	N	Mean	SD	Mean Diff	P Value	N or NS
Base line	Test	20	8.35	0.87	-0.15	0.5	NS
	Control	20	8.5	0.51			
3 months	Test	20	6.2	0.89	0.15	0.5	NS
	Control	20	6.05	0.68			

Table 7: Intergroup comparison of CFU of test and control sites, baseline, 1 months, 3 months.

Timeperiod	Group	N	Mean	SD	Mean Diff	P Value	N or NS
Base line	Test	20	1.4x10 ⁶	1.8x10 ⁵	-1.21x10 ⁶	0.06	NS
	Control	20	1.5x10 ⁶	2.1x10 ⁵			
1 months	Test	20	1.3x10 ⁵	2.0x10 ⁴	-0.14x10 ⁷	0.7	NS
	Control	20	1.37x10 ⁵	1.86x10 ⁴			
3 months	Test	20	1.1x10 ⁴	2.0x10 ³	-0.18x10 ⁴	<0.001	S
	Control	20	1.28x10 ⁴	1.3x10 ³			

Inference: Intergroup comparison shows significant reduction at 3 months follow-up in colony forming counts in test group as compared to control group.

Discussion

The periodontium can be defined as those tissues supporting and investing the tooth, comprising of root cementum, periodontal ligament, bone lining the tooth socket (alveolar bone), and that part of the gingiva facing the tooth (dentogingival junction). Periodontitis is

a disease of the supporting structures of the teeth. It causes alterations of the periodontium like loss of connective tissue attachment to the tooth, loss of alveolar bone, apical migration of the junctional epithelium. Statistics present the grim reality that 95% of the population in India suffer from periodontitis and only 50% of them use a toothbrush, and just 2% visit the dentist¹¹. Shah et al. observed that for periodontal diseases, the projection is alarming in India, with prevalence at present being 45% for the 15+ years age group and the actual prevalence

in lakhs at 3413.8 (the year 2010) and 3624.8 (the year 2015)¹².

Pathogenic plaque microflora, host immune responses, and environmental factors play a major etiologic role and cause both direct as well as host-mediated tissue injury¹³. As time passes, oral flora changes from predominantly gram-positive to gram-negative and from facultative aerobes to strictly anaerobic species. This mature biofilm/plaque takes around 12 weeks to develop¹⁴. The biofilm forms a reservoir for periodontal bacteria, and as the biofilm matures, the concentration and virulence of the periodontal bacteria change. Socransky and Haffajee have categorized bacteria by their periodontal pathogenicity, using a colour classification to identify the virulence of various oral bacteria, with the orange and red complexes denoting the most pathogenic bacteria¹⁵.

The conversion of “aerobic plaque” to “anaerobic plaque” is accompanied by a shift in oral health to tissue inflammation (gingivitis) and, eventually, periodontal disease (Periodontitis). In the studies in relay from health to gingivitis to Periodontitis a remarkable observation was made, i.e. Of the hundreds of different species that eventually occupied the periodontal environment, only a few were the members of “periodontopathic microbiota”. These include *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *Prevotella nigrescens*, *B. forsythus*, *C. rectus*, *F. nucleatum*, *Peptostreptococcus micros*, *E. corrodens*, *T. denticola* and *Treponema spp*¹⁷. Hence, the aetiology of periodontal disease is multifactorial (viz; oral hygiene, gender, race, socioeconomic status, age, systemic health status, genetics, use of medications, smoking, and alcohol and drug abuse), and periodontopathogens play a distinctive role in the development and progression of the disease.

Periodontal therapy is directed at arresting the progression of inflammatory periodontal disease with the goal of stabilizing the long-term prognosis of the periodontium. Disruption of the plaque biofilm serves as a

basis for the success of periodontal therapy, and mechanical debridement stands as a gold standard. Surgical periodontal procedures are, in general, used for treating periodontal disease, and the fact is that diseased root surfaces can be debrided more efficiently due to greater accessibility and visual instrumentation¹⁸. Periodontal treatment not only aims at reattachment but also at regeneration. The regeneration of periodontal tissues stands as an ultimate goal of successful periodontal therapy¹⁹. The bacteria present in the diseased pocket epithelium produce greater damage to the underlying connective tissue. The removal of this infected epithelium will promote healing of the connective tissue²⁰.

Commonly, dental lasers have been used for soft tissue surgical procedures. In recent years, many studies have been conducted to support the effectiveness of dental lasers as an adjunct to regenerative surgical procedures. The photothermal effect of diode laser on the tooth surface helps to remove the bacteria present in the necrotic cementum and underlying dentinal tubules. Through these photothermal and photodisruptive effects, diode laser application can produce a complete removal of pathogens within the periodontal pocket, which results in the healing of periodontal tissue and new attachment²¹.

Diodes lasers proved to have a stimulative/regulative effect on tissue that encompasses pain relief and wound healing, and also Gallium-aluminum-arsenide (GaAlAs) diode have shown faster wound healing and bone formation after tooth extraction compared with unlasers cases. Diode lasers have been shown to have a bactericidal effect, reduce inflammation and support the healing of periodontal pockets through the elimination of bacteria. Diode laser was proved to improve the gingival index, decrease probing pocket depth, bleeding on probing, bacterial content of periodontal pockets and improve the overall health of the periodontium²². The keyword in the American Academy of Periodontology report is alternative, and the laser is used as an adjunct to standard treatments rather than as a

replacement for standard treatments²³. Each of the materials and techniques addresses specific aspects of the regeneration process. The combination of one or more techniques currently available for periodontal regeneration has, therefore, the potential to enhance clinical results as compared to any of the techniques used alone.

During healing after flap surgery, the new junctional epithelium will be completely formed by the end of second week. The healing during the third week features the first histological evidence of new connective tissue attachment of the flap. From 4th week till the end of third month the healing will feature less proliferative activity while connective tissue maturation and osseous remodelling will become more dominant elements²⁴. The gingival tissues have a healing period of upto 8 weeks post phase I therapy²⁵. For this reason, follow up of 3 months was selected to compare and evaluate the healing of the periodontal tissues by measuring the clinical parameters and also evaluating the microbial levels during that interval.

In the present study, PI was used to monitor the oral hygiene status of the patients. The results demonstrate that there was a statistically significant difference in the PI at baseline and at 3 months in the control and the test groups. These results are in accordance with the study done by Lobo et al. in 2015²⁶.

Also noted that GI decreased significantly from baseline to 3 months in control and the test groups. This suggests the effectiveness of access flap surgery in reducing the signs of inflammation caused by the effective removal of calculus and infected granulation tissue²⁷. These results are in accordance with the study done by Aena et al. 2015².

PPD is the evidence of periodontal health, or it is a disease indicator. The measurement of this parameter defines the periodontal status and the outcome of the treatment. In both the groups, from baseline to 3 months, it was found to be a significant reduction in pocket probing depth. But on the

other hand, in the comparison between intergroup the test and control group after the t-test, the probing depth was statically insignificant at baseline and 3 months as the P-value was 0.6 and 0.5. A similar observational study was done by Tawfig A, Abdullah A, et al. (2017)²⁸.

The relative attachment level for intragroup comparison in test and control sites showed significant improvement in attachment level from baseline to 3 months the relative attachment level; was statistically insignificant at baseline and 3 months a similar observational study was done by Aena et al. 2015².

In the present study, there was a statistically significant reduction in the number of CFUs of anaerobes in the laser-treated group as compared with the control group. Colony-forming units were statically insignificant at baseline and 3 months and statistically significant at 3 months as the p-value was <0.001. The wavelength of the diode laser was absorbed by protohemin and protoporphyrin IX pigments of the pigmented anaerobic periopathogens. This led to vaporization of water and caused lysis of the cell wall of the bacteria, leading to cell death. It was effective against the invasive tissue periopathogens caused by absorption of laser energy up to 2 – 1mm in the deeper tissues²⁹. A similar observational study was done by Gokhale et al. 2012⁵, Tawfig A, Abdullah A, et al. (2017)²⁸.

On the basis of the results achieved in the present research, it must be emphasized that the present study shows there is a significant correlation between patient treated with only Open flap debridement and Open flap debridement + Laser in that OFD+Laser group shows a significant reduction in the anaerobic count (bactericidal effect) at 3-month follow-up. Therefore, lasers can form an integral part of periodontal therapy in the future.

Conclusion

This clinical and microbiological study was done to evaluate the efficacy of diode laser used as an adjunct to conventional open flap debridement in comparison with conventional

open flap debridement alone and there was statistically significant difference at baseline and at 3 months in the control and the test group in Plaque index, Gingival index, pocket probing depth and relative attachment level. In the present study, there was a statistically significant reduction in the number of CFUs of anaerobes in the laser-treated test group as compared with the control group. Colony-forming units were statistically insignificant at baseline and 3 months as the P-value was 0.06 and 0.7 respectively and statistically significant at 3 months as the p-value was <0.001. Therefore, lasers can be used as an adjunctive treatment with open flap debridement as a part of periodontal therapy in the future.

Limitations


1. In the present study, clinical parameters and treatment procedure were performed by a single investigator. Since this was not a double-blinded experiment, bias with the data collections may have occurred. Therefore, it may be beneficial for future studies to be double-blinded to prevent the introduction of bias.
2. Further longitudinal studies are required to evaluate the long-term effects of diode laser on clinical as well as microbiological parameters. The bactericidal effect of diode laser on specific microorganisms and viruses and the time taken for microbial recolonization needs to be determined by further studies.
3. Scaling and root planning brought about positive changes in the clinical and microbial parameters when compared to the baseline.
4. Clinical parameters such as bleeding index should be included in the study as that is a classical sign for improvement after any periodontal therapy.

Conflict of interest: All listed authors have contributed to the preparation of this

manuscript and have permitted their names to be included as co-authors. We declare a lack of conflict of interest between the authors of this paper or other entities.

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