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Evaluation of Laser Assisted and Conventional Open Flap Debridement Procedure in Patients with Chronic Periodontitis - A Clinical and Microbiological study

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ABSTRACT

Introduction: Research has shown that diode lasers are one of the most commonly used soft tissue laser due to its additional advantages and are nowadays the choice for many periodontal surgeries.

Aim: The aim of our study is to assess and compare clinically and microbiologically the treatment outcomes of diode laser alone, open flap debridement (OFD) alone and OFD with diode laser as an adjunct in patients with chronic periodontitis.

Settings and Design: This is a prospective, comparative and split mouth study in which lottery method was used for allocation of samples into groups.

Methods and Material: Patients were randomly selected and divided into three groups with 15 patients in each group. SRP was performed followed by diode laser pocket decontamination alone, OFD alone and combination of diode laser and OFD in group A, B and C respectively. Assessment of plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and healing index (HI) and was done at baseline and after 3 and 6 months. Microbiological assessment of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* was done by culture technique at baseline, immediate, 3 and 6 months postoperatively.

Statistical analysis: The statistical analysis was performed using ANOVA, Kruskal Wallis-H test, and Bonferroni test.

Results: The differences in clinical parameters in all the groups reached a statistical significance except PI. Significant reduction was observed in colony forming units (CFU) in all the groups but greatest reduction was observed in the combination group.

Conclusion: All the treatment modalities were effective in improving clinical and microbiological parameters, although the combination group was more effective.

Key words - chronic periodontitis, diode laser, laser pocket decontamination, open flap debridement, periodontal pathogens, split mouth study

Introduction:

As per AAP, chronic periodontitis is defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment and bone loss and is characterized by pocket formation and/or gingival recession [1]. Plaque is the primary etiology for chronic periodontitis, removal of which depends upon its location and other plaque retentive factors. The standard phase 2 periodontal therapy i.e., open flap debridement has few limitations like bleeding, post operative oedema and discomfort, need for sutures and incomplete removal of biofilm.

Diode laser is one of the most reliable and popular choices for various clinical procedures especially in dental field because it is economical, portable and convenient. In addition, it has good tissue penetration [2], haemostasis and coagulation [3]. Diode laser with wavelengths ranging from 810 to 980 nm in a continuous or pulsed mode are used for soft tissue surgeries in the oral cavity. Based on the photothermal effect of the diode laser, the lesions of the oral mucosa are removed with an excision technique, or by ablation/vaporization procedures [4-6].

Many periodontopathogens are susceptible to this thermal range. Laser transmits energy to the cells causing warming, welding, coagulation, protein denaturation, drying, vaporization and carbonization [7,8]. Benefits of diode laser over traditional surgery with scalpel comprises convenient mucosa removal, high precision in tissue destruction [9,10], easy ablation of soft tissue, immediate sterilization, decreased mechanical trauma, increased patients acceptance, no or few sutures [11], homeostasis, decreased bacteremia, decreased edema, less operative and postoperative discomfort little wound contraction and minimal scar [10,11]. Laser application on soft tissue can cause stimulation of fibroblast proliferation, collagen synthesis and vessels proliferation leading to faster wound healing [12]. Pain is lesser in laser application since no or little need for anesthesia is required in procedures such as pocket decontamination which results in higher patient acceptance.

Materials and Methods :

The present study was a prospective, experimental, comparative and split mouth randomized clinical trial and was conducted after clearance from institutional ethical committee from 2016-2017 in department of periodontology at sri aurobindo college of dentistry, indore (M.P). Patients diagnosed with chronic periodontitis were selected.

Out of 60 quadrants, 45 quadrants were selected for the study by Lottery method. The selected patients (28 females, 21 males) were randomly divided into three groups comprising 15 patients in each group; Group A (diode laser pocket decontamination), Group B (Open Flap Debridement alone) and Group C (diode laser used as an adjunct with Open Flap Debridement). The comparison of all clinical and microbiological parameters was carried out.

Systemically healthy patients with probing pocket depth ≥ 5 mm after phase I therapy in at least three quadrants, minimum of 4 teeth in a quadrant, presence of good oral hygiene after phase I therapy and patients with age ≥ 18 years were included in the study. Exclusion criteria were pregnant and lactating women, patients on antibiotics within 3 months prior to the study, patients with history of smoke or smokeless tobacco, patients with poor compliance and history of periodontal surgery in same areas within 6 months.

All the patients were subjected to treatment after phase I therapy. For all the selected patients, routine radiographic and blood investigations were done. Acrylic stent was made and measurements were recorded at baseline and at 3 and 6 months postoperatively, at the deepest pocket site with the pressure sensitive UNC-15 probe. The parameters recorded were plaque index (PI) [13], gingival index (GI) [14], probing pocket depth (PPD), clinical attachment level (CAL) and healing index (HI) [15]. On the day of surgery, microbiological sampling of sites was carried out by subgingival plaque sample collection from the deepest pocket using sterilized absorbent paper points at baseline (preoperatively and immediate postoperatively), 3 months and 6 months postoperatively. The paper points were transported to laboratory in reduced transport medium (RTF). (Figure 1 and 2)

For group A, pocket decontamination was done using diode laser 980 nm (doctor smile®, wiser) (Figure 3) under topical anaesthesia. The settings for the diode laser were “perio” settings. The optic fiber of 400 μ m was activated and inserted into the depth of periodontal pocket in the parallel direction to the long axis of the tooth to be treated. The tip was retracted 1mm from the base of pocket. The irradiation consisted of 3 cycles, at 0.8 watts, continuous wave mode, each lasting 30 seconds, with a 60 seconds interval. The tip was moved from the base of the pocket towards the gingival margin in a sweeping motion [16]. (Figure 4)

For group B, open flap debridement was performed. (Figure 5)

For group C the procedure involved using diode laser as an adjunct to mechanical debridement (Figure 6). In this group diode laser with power setting of 2.5 watts was used in continuous, contact mode with a flexible fiber optic delivery system. The fiber was used in a “brush stroke” motion on the undersurface of the flap to remove the pocket lining [17].

Microbiological techniques : The samples were first vortexed then it was diluted in RTF 1:10 proportion and inoculated in the culture medium. *Porphyromonas gingivalis* and *Prevotella*

intermedia were grown and incubation was done at 37 °C for 3 - 4 days in anaerobic jar. *Aggregatibacter actinomycetemcomitans*, was incubated at 37 °C in 5-10% CO₂ jar for 48-72 hrs. After completion of incubation. After completion of incubation identification of the selected bacterial species was confirmed by gram staining and key biochemicals i.e., glucose, sucrose, cellibiose and arabinose. Colony count was done for quantification and total colony forming units were estimated and expressed as the number of viable counts per milliliter of transport medium.

Statistics :

The data were analyzed using SPSS for Windows, version 17.0 Trial. Assuming that PI, GI, PPD, CAL and bacterial colonies followed normality, ANOVA was used to identify the significance of mean differences among groups and among sampling stages. For non-continuous variables Healing index (HI) Kruskal Wallis-H test was used. The inter-group differences were evaluated by Post-Hoc test which was carried out by using Bonferroni test. P value <0.05 was considered statistically significant.

Results :

Table 1 projects that no statistically significant changes were observed with PI, PPD and CAL, at baseline, 3 months and 6 months. Although, GI showed statistically significant changes at 3 and 6 months post treatment ($p < 0.001$).

Table 2 reports that intragroup comparison of PI showed insignificant differences at all points of time. Although GI, PPD and CAL showed highly statistically significant differences at baseline, 3 months and 6 months ($p < 0.001$).

Table 3 highlights that intergroup comparison of PI did not show statistical significance at any point of time. Although at 3 and 6 months GI showed statistically significant differences.

Table 4 demonstrates that intergroup comparison of PPD and CAL did not show statistical significance at any point of time.

Table 5 shows that the intrasampling stage comparison of mean CFUs of Pg, Pi and Aa in all the groups was statistically insignificant at baseline. While at other sampling stages, the mean CFUs of Pg showed highly statistical significance. Mean CFUs of Pi showed highly statistically significant differences at 3 months postoperatively. Mean CFUs of Aa showed statistically significant difference at immediate postoperative stage.

Table 6 depicts that the intragroup comparison of mean CFUs of Pg, Pi and Aa showed highly statistically significant differences at all the sampling stages.

Intergroup differences of microbial load are highlighted in table 7 which shows that the mean CFUs of Pg showed statistically significant differences at all points of time. The intergroup comparison of CFUs of Pi showed statistically significant differences only at 3 months. The intergroup comparison of CFUs of Aa showed statistically significant difference only among group B and C at 3 months postoperatively.

Table 8 implies that the intrasampling stage comparison of mean HI showed statistical significance only at 6 months postoperatively reached a level of significance irrespective of the pocket depth in all the groups.

Table 9 depicts that the intragroup comparison of mean HI showed highly statistically significance at all the sampling stages.

Discussion:

On observation in our study it was found that there were no significant differences observed with PI, which can be attributed to the maintenance of oral hygiene by the patients as per instructions given to them during the study period which resulted in minimal plaque scores. The results of our study were consistent with Gokhale, et al. [17] and Lobo and Pol [18] who in a three months randomized controlled trial found that PI did not differ at 3 months post operatively when compared to baseline in both OFD and OFD with diode laser group.

On the contrary, GI reduced consequently from baseline to 3 months and 6 months postoperatively in every group (Table 1). It was found that at all points of time the GI scores were significantly less in group C than in group A and group B which implies that there is greater reduction in gingival inflammation when site is treated by laser along with flap surgery. Moreover the intergroup comparison of PI and GI (Table 3) also implies that laser has an additional benefit of reducing gingival inflammation.

The GI changes observed in our study are in accordance with the observations of Zare [19], Lobo and Pol [18] and Qadri et al. [20] who concluded that there is additional benefit of laser along with mechanical debridement in reducing gingival inflammation. Conversely, Gokhale, et al. suggested that GI reduced consequently from baseline to 3 months post operatively in both OFD alone and OFD with laser group which shows the effectiveness of flap surgery in improving inflammation by complete removal of subgingival calculus along with granulation tissue [17].

Intrasampling stage comparison (Table 1), intragroup comparison (Table 2) and intergroup comparison (Table 4) signifies that all the groups were equally effective in improving PPD and CAL. Nguyen, et al. in a non surgical study reported that laser does not provide additional benefits in improving clinical parameters [21]. Gokhale, et al., Lobo and Pol and Jayachandran, et al. who performed surgical periodontal therapy and showed in their respective studies that OFD alone and OFD with laser treatment group showed equal improvement in PPD and CAL [17, 18, 22]. Conversely, Qadri, et al. in a systematic review concluded that SRP and laser combination showed better results in improving clinical parameters [20].

Intrasampling stage comparison of CFUs (Table 5) shows that although reduction in CFUs were observed at every stage, CFUs of Pg of group C showed maximum reduction which can be contributed to the fact that laser exhibits bactericidal properties which led to killing of pathogens at the operating site [20]. The results of our study are in accordance with the non surgical studies by Moritz, et al. who stated that diode laser favoured reduction of Aa [23]; Chan and Lai who concluded that diode laser resulted in Aa, Pg and Pi reduction [24]; Harris who showed that diode laser reduced colony count of Pg [25]; Vukelic, et al. who concluded that diode laser led to reduction of Aa and Pg [26] and Gupta, et al. who demonstrated that when diode laser was used additionally it reduced Aa and Pi [27]. Moreover, Gokhale, et al. performed a surgical periodontal therapy and concluded that OFD in combination with laser resulted in statistically significant reduction in CFUs of obligate anaerobes [17]. The results of our study contradicted with the results of non surgical studies by Caruso, et al. and Alves, et al. who concluded that the additional treatment with diode laser led to a slight improvement in clinical parameters, whereas

no significant differences were observed between test and control group in reduction of periodontopathogens [21, 28].

Intragroup comparison of CFUs (Table 6) implies that significant reduction of periopathogens was observed in all the groups which implies that all the treatment modalities have equal potential in elimination of periodontal pathogens.

In our study, there was a statistically significant reduction in the number of CFUs within the laser treated groups in comparison with OFD group (Table 7). The wavelength of the diode laser (980 nm) is very well absorbed by the pigments of the pigmented anaerobic periodontal pathogens, since it uses haemoglobin and melanin as chromophores which lead to vaporization of water causing bacterial cell wall lysis showing its bactericidal effect [17].

The healing index was found to be improved after treatment at 3 and 6 months in all the groups (Table 8). Intragroup comparison of HI shows that all the groups are equally effective in improving healing of the operated site (Table 9).

Well in our study significant improvement was observed clinically at 3rd and 6th month post operative stage in all three groups (Figure 7, 8, 9). Moreover improvement was observed in reduction of CFUs of Aa, Pg and Pi in all the groups at all stages post operatively with significant reduction in group C. (Figure 10 and 11)

Overall, research showed that patients of group C showed significantly improved periodontal status as compared to group B and A which reflected that open flap debridement with laser as an adjunct can be successfully used as an effective modality in treating periodontitis.

Conclusion

The results of the present study indicate that all three treatment modalities prove to be effective in terms of improvement in clinical as well as microbiological parameters. Small sample size and prolonged transport time were limitations to the study. Long term clinical trials and researches are necessary to confirm the aforesaid observations.

In this modern era, non-surgical treatment modalities like pocket decontamination by LASER have been effective in reducing periodontal pathogens. Our present study has open doors to use of LASER in reducing periodontal pathogens instead of just focusing on mechanical debridement alone which is the main aim of conventional flap therapy.

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TABLES

Table 1 - COMPARISON OF CLINICAL PARAMETERS OF PATIENTS AMONG THREE SAMPLING STAGES (BASELINE AND POST TREATMENTS) IN GROUPS (Intrasampling Stage Comparison)

Parameter	Sampling stage	Spread (Mean ± SD)			p value (LOS)
		Group A	Group B	Group C	
PI	Baseline	0.72±0.12	0.72±0.13	0.74±0.11	p>0.05 [⊗]
	At 3 month	0.67±0.09	0.67±0.12	0.71±0.13	p>0.05 [⊗]
	At 6 month	0.67±0.09	0.67±0.12	0.71±0.13	p>0.05 [⊗]
GI	Baseline	1.44±.11	1.44±0.11	1.45±0.13	p>0.05 [⊗]
	At 3 month	0.52±0.11	0.44±0.11	0.29±0.12	p<0.001 [#]
	At 6 month	0.52±0.11	0.32±0.07	0.22±0.09	p<0.001 [#]

	Baseline	6.93±1.09	7.33±1.17	7.00±1.19	p>0.05 [⊗]
PPD	At 3 month	3.33±0.81	3.47±0.51	3.20±0.56	p>0.05 [⊗]
	At 6 month	3.33±0.81	3.47±0.51	3.20±0.56	p>0.05 [⊗]
	Baseline	7.33±1.11	7.60±0.98	7.33±1.11	p>0.05 [⊗]
CAL	At 3 month	3.60±0.73	3.67±0.61	3.53±0.51	p>0.05 [⊗]
	At 6 month	3.60±0.73	3.67±0.61	3.53±0.51	p>0.05 [⊗]

[⊗] The differences among groups are not significant insignificant at the 0.05 level of significance.

[#] The differences among groups were **highly significant at the 0.001 level of significance.** [p value-Probability value; SD-Standard Deviation; LOS-Level of Significance, PI- Plaque Index, GI- Gingival index, PPD- Probing Pocket Depth, CAL- Clinical Attachment Level, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

**Table 2 -
COMPARISON OF CLINICAL PARAMETERS AMONG GROUPS AT BASELINE, 3RD
AND 6TH MONTH (Intragroup Comparison)**

Parameter	Sampling stage	Spread (Mean ± SD)			p value (LOS)
		Baseline	At 3 month	At 6 month	
PI	Group A	0.72±0.12	0.67±0.09	0.67±0.09	p>0.05 [⊗]
	Group B	0.72±0.13	0.67±0.12	0.67±0.12	p>0.05 [⊗]
	Group C	0.74±0.11	0.71±0.13	0.71±0.13	p>0.05 [⊗]
GI	Group A	1.44±0.12	0.53±0.12	0.53±0.12	p<0.001[#]
	Group B	1.44±0.11	0.44±0.11	0.32±0.07	p<0.001[#]
	Group C	1.45±0.13	0.29±0.12	0.22±0.09	p<0.001[#]
PPD	Group A	6.93±1.09	3.33±0.81	3.33±0.81	p<0.001[#]
	Group B	7.33±1.17	3.46±0.51	3.46±0.51	p<0.001[#]
	Group C	7.00±1.19	3.20±0.56	3.20±0.56	p<0.001[#]
CAL	Group A	7.33±1.11	3.60±0.53	3.60±0.53	p<0.001[#]

Group B	7.60±0.98	3.67±0.67	3.67±0.67	p<0.001 #
Group C	7.73±1.12	3.53±0.51	3.53±0.51	p<0.001 #

⊗ The differences among sampling stages are not significant (insignificant) at the 0.05 level of significance. # The differences sampling stages were **highly significant at the 0.001 level of significance**. [p value-Probability value; SD-Standard Deviation; LOS-Level of Significance, PI-Plaque Index, GI- Gingival index, PPD- Probing Pocket Depth, CAL- Clinical Attachment Level, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

Table 3 - MULTIPLE COMPARISONS OF PLAQUE INDEX AND GINGIVAL INDEX BETWEEN TWO GROUPS USING POST HOC TEST

<i>Group</i>	<i>Groups</i>	Plaque Index			Gingival Index		
		MD	Std. Error	p-value	MD	Std. Error	p-value
Post-treatment (At 3 month)							
Group A	Group B	-0.00	0.03	>0.05 ⊗	0.08	0.04	>0.05 ⊗
	Group C	-0.02	0.03	>0.05 ⊗	0.23	0.04	<0.001 #
Group B	Group C	-0.02	0.03	>0.05 ⊗	0.14	0.04	<0.05 *
Post-treatment (At 6 month)							
Group A	Group B	0.01	0.04	>0.05 ⊗	0.20	0.03	<0.001 #
	Group C	0.11	0.04	>0.05 ⊗	0.30	0.03	<0.001 #
Group B	Group C	0.10	0.04	>0.05 ⊗	0.10	0.03	<0.05 *

⊗ The mean differences are not significant (insignificant) at the 0.05 level of significance. The mean differences are **significant at the 0.05* and 0.001# levels of significance**. [p value-Probability value, Std. Error-Standard Error of Difference; MD-Mean Difference, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

**Table 4:-
MULTIPLE COMPARISONS BY POST HOC TEST OF PROBING POCKET DEPTH
AND CLINICAL ATTACHMENT LEVEL BETWEEN TWO GROUPS**

Group	Groups	Probing Pocket Depth			Clinical Attachment Level		
		MD	Std. Error	p value	MD	Std. Error	p value
Post-treatment (At 3 month)							
Group A	Group B	-0.13	0.23	>0.05 [⊗]	-0.06	0.23	>0.05 [⊗]
	Group C	0.13	0.23	>0.05 [⊗]	0.06	0.23	>0.05 [⊗]
Group B	Group C	0.26	0.23	>0.05 [⊗]	0.13	0.23	>0.05 [⊗]
Post-treatment (At 6 month)							
Group A	Group B	-0.13	0.23	>0.05 [⊗]	-0.06	0.23	>0.05 [⊗]
	Group C	0.13	0.23	>0.05 [⊗]	0.06	0.23	>0.05 [⊗]
Group B	Group C	0.26	0.23	>0.05 [⊗]	0.13	0.23	>0.05 [⊗]

[⊗]The mean differences are insignificant at the 0.05 level of significance. The mean differences are **significant at the 0.05* and 0.001# levels** of significance. [p value-Probability value, Std. Error-Standard Error of Difference; MD-Mean Difference, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

Table 5 : COMPARISON OF MICROBIAL LOAD IN PATIENTS AMONG SAMPLING STAGES (BASELINE AND POST TREATMENTS: IMMEDIATE POST OPERATIVE , AT 3RD AND 6TH MONTH) IN GROUPS (Intrasampling Stage Comparison)

Variable	Sampling stage	Spread (Mean ± SD)			p value (LOS)
		Group A	Group B	Group C	
Pg	Baseline	123.33±17.5	128.67±17.26	128.67±18.07	p>0.05 [⊗]
	Immediate post-op	75.33±16.84	84.00±9.10	58.67±15.52	p<0.001 [#]
	At 3 month	48.67±11.87	56.00±10.45	32.67±11.62	p<0.001 [#]
	At 6 month	32.00±8.61	46.67±15.43	20.00±7.55	p<0.001 [#]
Pi	Baseline	89.33±10.33	85.33±13.08	93.33±12.91	p>0.05 [⊗]

	Immediate post-op	44.67±9.90	54.67±14.07	44.67±13.02	p>0.05 [⊗]
	At 3 month	28.00±6.76	36.00±11.83	23.33±7.24	p<0.001 [#]
	At 6 month	14.67±5.16	18.67±9.15	17.33±5.93	p>0.05 [⊗]
	Baseline	115.33±9.90	116.67±9.75	115.33±9.90	p>0.05 [⊗]
Aa	Immediate post-op	66.67±10.46	71.33±10.60	58.67±10.57	p<0.05 [*]
	At 3 month	41.33±9.90	45.33±13.55	35.33±14.57	p>0.05 [⊗]
	At 6 month	20.67±4.57	22.67±8.83	17.33±5.93	p>0.05 [⊗]

⊗ The differences among groups are not significant (insignificant) at the 0.05 level of significance. The mean differences are **significant at the 0.05^{*} and 0.001[#] levels of significance**. [p value-Probability value; SD-Standard Deviation; LOS-Level of Significance, Pg- *Porphyromonas gingivalis*, Pi- *Prevotella intermedia*, Aa- *Aggregatibacter actinomycetemcomitans*, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

**Table 6 -
ASSESSMENT AND COMPARISON OF MICROBIAL LOAD AMONG GROUPS AT ALL SAMPLING STAGES (PRE-TREATMENT, IMMEDIATE POST OPERATIVELY, AND POST TREATMENT) [Intragroup Comparison]**

Specie	Group	Spread (Mean ± SD) of Microbial Load at				p value (LOS)
		Baseline	Immediate post-op	3 month	6 month	
Pg	Group A	123.33±17.59	75.33±16.84	48.67±11.87	32.00±8.67	p<0.001 [#]
	Group B	128.67±17.26	84.00±9.10	56.00±10.55	46.67±15.43	p<0.001 [#]
	Group C	128.67±18.07	58.67±15.52	32.67±11.63	20.00±7.56	p<0.001 [#]
Pi	Group A	89.33±10.32	44.67±9.90	28.00±6.76	14.67±5.16	p<0.001 [#]

	Group B	85.33±13.02	54.67±14.07	36.00±11.83	18.67±9.84	p<0.001 #
	Group C	93.33±12.91	44.67±13.02	23.33±7.24	17.33±5.94	p<0.001 #
Aa	Group A	115.33±9.90	66.67±10.46	41.33±9.91	20.67±4.57	p<0.001 #
	Group B	116.67±9.76	71.33±10.60	45.33±13.55	22.67±8.83	p<0.001 #
	Group C	115.33±9.90	58.67±14.57	35.33±14.57	17.33±5.93	p<0.001 #

The differences among sampling stages were **highly significant at the 0.001 level of significance**. [p value-Probability value; SD-Standard Deviation; LOS-Level of Significance, Pg- *Porphyromonas gingivalis*, Pi- *Prevotella intermedia*, Aa- *Aggregatibacter actinomycetemcomitans*, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

**Table 7 -
MULTIPLE COMPARISONS OF MICROBIAL LOAD BY POST HOC TEST
BETWEEN TWO GROUPS AT BASELINE, IMMEDIATE POST-OP, AT 3RD AND 6TH
MONTH POST TREATMENT**

Group	Groups	Load of Bacterial Colony					
		Pg		Pi		Aa	
		MD	p value	MD	p value	MD	p value
Immediate post-operatively							
Group A	Group B	-8.66	>0.05 [⊗]	-10.00	>0.05 [⊗]	-4.66	>0.05 [⊗]
	Group C	16.66	<0.05 [*]	0.00	>0.05 [⊗]	8.00	>0.05 [⊗]
Group B	Group C	23.73	<0.001 [#]	10.00	>0.05 [⊗]	12.66	<0.05 [*]
Post-treatment (At 3 month)							
Group A	Group B	-7.33	>0.05 [⊗]	-8.00	<0.05 [*]	-4.00	>0.05 [⊗]
	Group C	16.00	<0.05 [*]	4.66	<0.05 [*]	6.00	>0.05 [⊗]
Group B	Group C	23.33	<0.001 [#]	12.66	<0.05 [*]	10.00	>0.05 [⊗]
Post-treatment (At 6 month)							
Group A	Group B	-14.66	<0.05 [*]	-4.00	>0.05 [⊗]	-2.00	>0.05 [⊗]

Group C	12.00	<0.05 *	-2.66	>0.05 [⊗]	3.33	>0.05 [⊗]
Group B	Group C	26.66	<0.001 #	1.33	>0.05 [⊗]	5.33 >0.05 [⊗]

[⊗]The mean differences are not significant (insignificant) at the 0.05 level of significance. The mean differences are **significant at the 0.05 * and 0.001 # levels of significance**. [p value-Probability value, MD-Mean Difference, Pg- *Porphyromonas gingivalis*, Pi- *Prevotella intermedia*, Aa- *Aggregatibacter actinomycetemcomitans*, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

Table 8 - COMPARISON OF HEALING INDEX (HI) AMONG SAMPLING STAGES AT PRE (BASELINE) AND POST TREATMENTS: AT 3RD AND 6TH MONTH IN GROUPS (Intrasampling Stage Comparison)

Parameter	Sampling stage	Spread (Mean ± SD)			p-value (LOS)
		Group A	Group B	Group C	
Healing Index	Baseline	2.00±0.00	2.00±0.00	2.00±0.00	p>0.05 [⊗]
	At 3 month	3.47±0.51	3.53±0.51	3.80±0.41	p>0.05 [⊗]
	At 6 month	4.53±0.51	4.60±0.50	5.00±0.00	p<0.05 *


[⊗]The differences based on ranks among groups were not significant at the 0.05 level of significance. * The differences based among ranks of groups were **significant at the 0.05 level of significance**. [HI- Healing index, p value-Probability value, SD-Standard Deviation, LOS- Level of Significance, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]

Table 9 - COMPARISON IN HEALING INDEX AMONG THREE GROUPS AT ALL SAMPLING STAGES (PRE AND POST TREATMENTS: AT 3RD AND 6TH MONTH) (Intragroup Comparison)

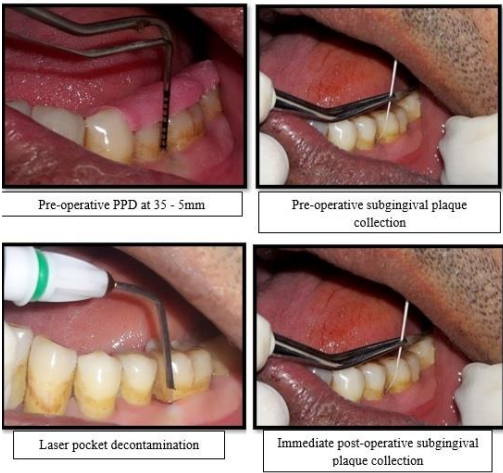
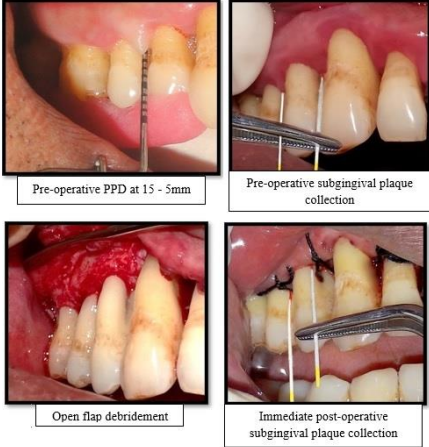
Group	Spread (Mean ± SD)			p-value
	Baseline	3 month	6 month	
Group A	2.00±0.00	3.47±0.51	4.53±0.51	p<0.001 #
Group B	2.00±0.00	3.53±0.51	4.60±0.50	p<0.001 #
Group C	2.00±0.00	3.80±0.41	5.00±0.00	p<0.001 #

® The differences based on ranks among groups were not significant at the 0.05 level of significance. * The differences based among ranks of groups were **significant at the 0.05 level of significance**. [p value-Probability value, SD-Standard Deviation, Group A- Diode laser pocket decontamination alone group, Group B- Open flap debridement alone group, Group C - Combination of diode laser and open flap debridement]



<p>FIGURE 1</p>	<p>FIGURE 2</p>
	
<p>FIGURE 3</p>	

FIGURES

 <p>Pre-operative PPD at 35 - 5mm</p> <p>Pre-operative subgingival plaque collection</p> <p>Laser pocket decontamination</p> <p>Immediate post-operative subgingival plaque collection</p>	 <p>Pre-operative PPD at 15 - 5mm</p> <p>Pre-operative subgingival plaque collection</p> <p>Open flap debridement</p> <p>Immediate post-operative subgingival plaque collection</p>
<p>FIGURE 4</p>	<p>FIGURE 5</p>

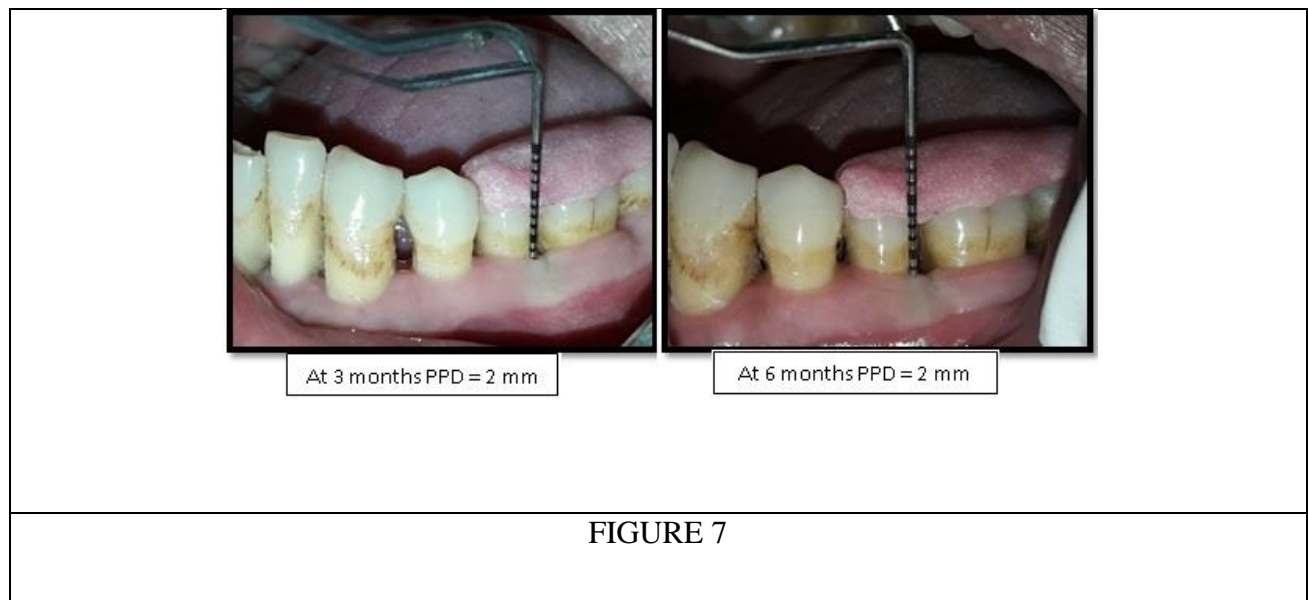
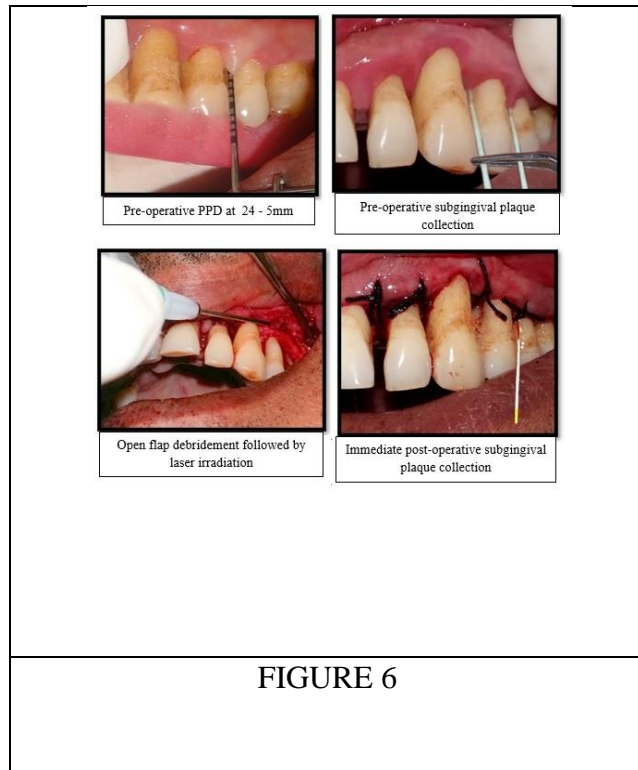




FIGURE 8



FIGURE 9

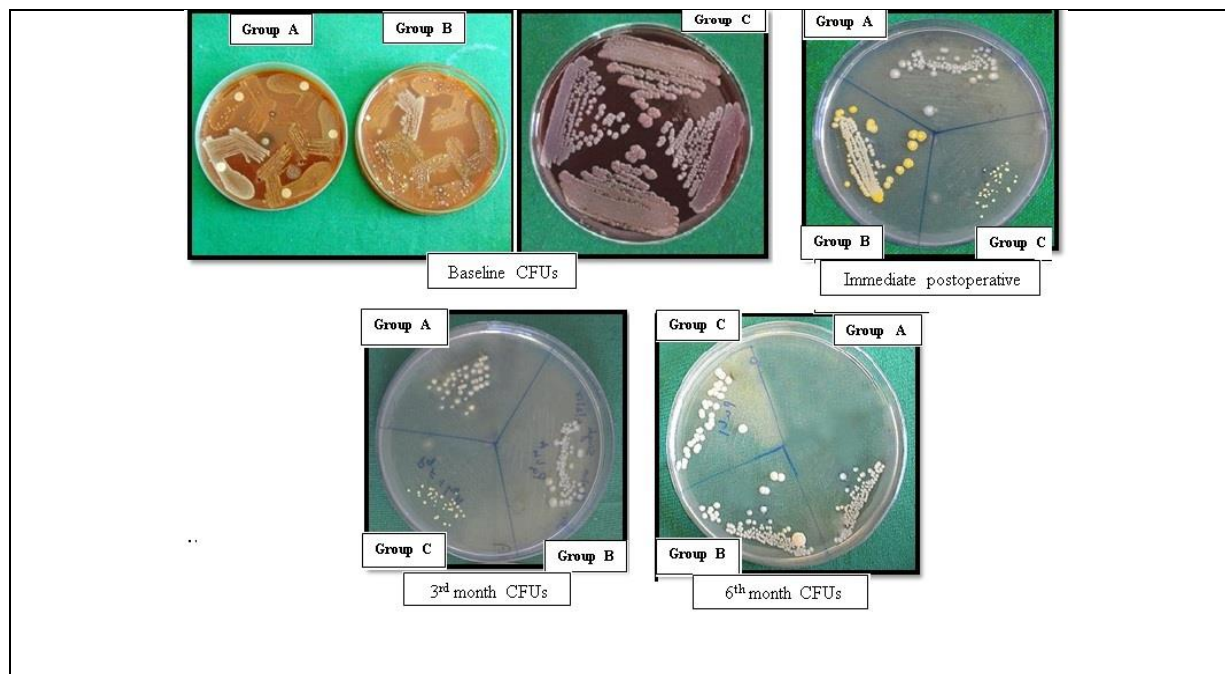


FIGURE 10

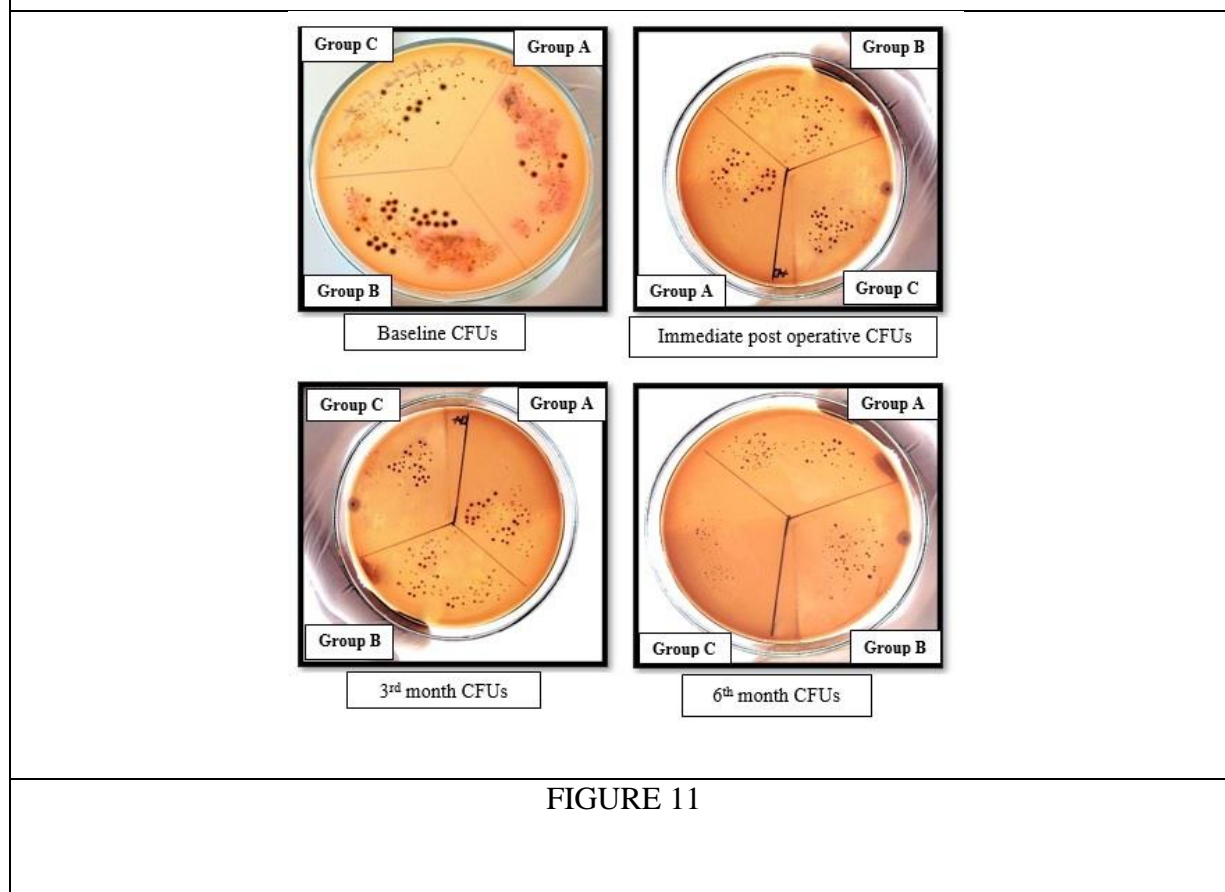


FIGURE 11

LEGENDS

Figure 1 – Armamentarium

Figure 2 – Acrylic stents and reduced transport medium

Figure 3– Doctor smile®, wiser soft tissue laser

Figure 4 – Group A baseline

Figure 5 – Group B baseline

Figure 6 – Group C baseline

Figure 7 – Group A post operatively at 3 and 6 months

Figure 8 – Group B post operatively at 3 and 6 months

Figure 9– Group C post operatively at 3 and 6 months

Figure 10 – Colony forming units of *Aggregatibacter actinomycetemcomitans* of Group A,B and C at baseline, 3 and 6 months postoperatively

Figure 11 – Colony forming units of *Porphyromonas gingivalis* and *Prevotella intermedia* of Group A, B and C at baseline, 3 and 6 months postoperatively

Table 1 – Comparison of clinical parameters of patients among three sampling stages (Baseline and Post-treatments) in groups (Intrasampling Stage Comparison)

Table 2 – Comparison of clinical parameters among groups at baseline, 3rd And 6th month (Intragroup Comparison)

Table 3 – Multiple comparison of Plaque index and Gingival index between two groups using post hoc test

Table 4 – Multiple comparison by post hoc test of PPD And CAL between two groups

Table 5 – Comparison of microbial load in patients among sampling stages (Baseline and Post Treatments: immediate post-operative , at 3rd and 6th month) in groups (intrasampling stage comparison)

Table 6 – Assessment and comparison of microbial load among groups at all sampling stages (Pre-Treatment, Immediate post operatively, and post treatment) [Intragroup Comparison]

Table 7 – Multiple Comparison of microbial load by post hoc test between two groups at baseline, immediate post operative, at 3rd and 6th month post-treatment

Table 8 – Comparison of Healing Index among sampling stages at pre (baseline) and post treatments: at 3rd and 6th month in groups (Intrasampling Stage Comparison)

Table 9 – Comparison of Healing Index among three groups at all sampling stages (pre and post treatments: at 3rd and 6th month) (Intragroup Comparison)