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EFFECT OF DIODE LASER IN THE TREATMENT OF PERIODONTAL POCKETS- A CLINICAL AND MICROBIOLOGICAL STUDY USING POLYMERASE CHAIN REACTION

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ABSTRACT

Aims and objectives: The aim of this study was to compare the clinical efficiency of a Diode laser as an adjunct to SRP with SRP alone in the treatment of chronic periodontitis patients and also to evaluate the changes in the clinical and microbiological parameters.

Materials and methods: Total number of 40 deepest sites in 10 chronic periodontitis patients was selected in this split mouth designed study. In each patient upper and lower right quadrants were assigned into one group and upper and lower left quadrants into the other. Treatment for each group was decided using a coin toss method, where **Group A (Control)** received only conventional SRP and **Group B (Test)** received conventional SRP and Laser assisted pocket debridement. The clinical parameters (Plaque index, Bleeding on probing, Probing pocket depth, Clinical attachment level) were recorded at baseline and 90th day and the microbiologic assessment for Aa, Pg and Pi were done on 7th, 21st and 90th day for both the groups.

Results: Out of the 10 patients, 2 patients who were irregular for the treatment protocol were excluded. When both groups were compared there was statistically significant reduction in Plaque index, Bleeding on probing, Probing pocket depth and gain in Clinical attachment level in Group B when compared to Group A. There was statistically significant reduction in *Porphyromonas gingivalis* from baseline to 90th day in Group B when compared to Group A. There was no statistically significant reduction in *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* in both the groups.

Conclusion: From the results observed in this study it can be concluded that use of Diode laser as an adjunct to SRP was found to be efficacious and safe.

INTRODUCTION

Chronic periodontitis is initiated by microbial plaque, which accumulates on the tooth surface at the gingival margin and induces an inflammatory reaction. The inflammatory response in patients with chronic periodontitis results in the destruction of the periodontal tissues. With a constant bacterial challenge the periodontal tissues are continuously exposed to the specific bacterial components that have the ability to alter many local cell functions.1

Basically the aim of the periodontal treatment is to restore the biological compatibility of the periodontally diseased root surfaces for subsequent attachment of periodontal tissues to the treated root surfaces.2

Complete removal of the bacterial deposits and their toxins from root surface and within the periodontal pockets is not necessarily achieved with conventional mechanical therapy and access to areas such as furcations and grooves is limited owing to the complicated root anatomy.2

There has been growing interest in recent years to search for new machine driven therapeutic devices which are capable of improving and simplifying mechanical root surface management and displaying antibacterial properties in order to decrease the number of bacteria associated with periodontal pockets. In this respect laser radiation at different wavelengths has been investigated as a novel system and an adjunct to conventional therapy.2

Although dental plaque harbours a great number of bacterial species, only a limited group of organisms has truly pathogenic potential. Dzink3 and Takeuchi4 et al in their studies have shown that Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are responsible for destructive periodontal diseases. Yano-Higuchi5 et al stated that the proportion of Porphyromonas gingivalis is significantly correlated to the aggrevation of the clinical parameters. Lopez6 et al in their study have shown the elevated levels of Prevotella intermedia in progressing sites of chronic periodontitis. Tseng7et al stated that besides eliminating Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, subgingivally, it has been affirmed that laser can enhance SRP.

This study was designed to compare the clinical efficiency of a Diode laser as an adjunct to SRP with SRP alone in the treatment of chronic periodontitis patients and also to evaluate the changes in the clinical and microbiological parameters.

MATERIALS AND METHODS

Ten Chronic periodontitis patients both males and females, aged 30 to 55 years, having a probing pocket depth of ≥5mm in 4 to 6 sites of all the quadrants were selected for the study. Patients were selected from the patient pool of the Department of Periodontics, Meenakshi Ammal Dental College, Chennai. Ethical clearance for the study was obtained from the ethical committee of the MAHER University. Written informed consent was obtained from the patients. Patients with age group 30 to 55 years, Teeth with ≥5mm probing pocket depth, Patients who can maintain good oral hygiene were included and Patients with systemic disease, Use of tobacco, Use of antibiotics or any form of periodontal treatment in the previous 6 months were excluded from the study.

Total number of 40 deepest sites in 10 chronic periodontitis patients was selected in this split mouth designed study. In each patient upper and lower right quadrants were assigned into one group and upper and lower left quadrants into the other. Treatment for each group was decided using a coin toss method. Where Group A received only conventional SRP and Group B received conventional SRP and Laser assisted pocket debridement. Table 1 illustrates the treatment schedule that determined the different examination and treatment steps.

TABLE I. TREATMENT SCHEDULE		
	Group A (Control)	Group B (Laser)
0 DAY	Evaluation of periodontal parameters & SRP	Evaluation of periodontal parameters & SRP
7th day	Sample collection for microbiologic examination	Sample collection for microbiologic examination & LASER assisted pocket debridement
21st day	Sample collection for microbiologic examination	Sample collection for microbiologic examination
90th day	Sample collection for microbiologic examination & Evaluation of periodontal parameters	Sample collection for microbiologic examination & Evaluation of periodontal parameters

Plaque index (Silness and Loe), Bleeding index (Ainamo and Bay), Probing Pocket depth and Clinical attachment level were evaluated on baseline and 90th day. Sub-gingival plaque samples were collected from both the groups. The total count of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia were assessed at different time points using PCR.

Sub-gingival plaque sample collection: The tooth with deepest pocket of the quadrant was isolated with a cotton roll and air dried and an ISO Standardized absorbent paper points of 25 size were placed within the pocket using a sterile tweezer for a period of 30 sec and then immediately transferred into a sterile ependroff tube containing thioglycollate broth and transported immediately to the laboratory.

At baseline, all periodontal parameters were assessed and full mouth scaling and root planing was done. (Fig 1a and 1b shows the pre operative measurements of Pocket depths in both groups)

On 7th day, sub gingival plaque samples were collected from both the groups before laser assisted pocket sterilization was done in group B. Diode laser (SIRO Laser, class IV Diode Laser, with an active semiconductor medium (Gallium, Aluminium, Arsenide) with a wavelength of 970+/-15nm) is used for the pocket debridement with an optical fiber of 320µm diameter, with an output power of 1 watts in a continuous mode with all the other parameters in a preset mode. The calibration of the fiber was the depth of the treatment site minus 1mm. This measurement allows for the laser energy to penetrate the tissue and reduce the bacterial load without the fiber actually touching the epithelial attachment at the bottom of the pocket. The fiber is placed on the tissue at the top of the sulcus, directing the laser energy away from the tooth structure, and moved towards the bottom.(Fig 2.Laser therapy in Group B) The fiber is moved both horizontally and vertically, and contact is maintained with the soft tissue down to the calibrated depth of the fiber. The pocket is sterilized with the laser for around 30 sec per tooth. The fiber must be inspected frequently and any accumulated tissue and debris must be wiped off to avoid inefficiency. Bacterial reduction is complete when signs of fresh bleeding occur. Post operative instructions following laser soft tissue procedures may include: analgesics as needed, such as Ibuprofen, avoidance of foods that could cause

irritation to healing tissue, for three to five days.

On 21st day sub-gingival plaque samples were collected from both the groups

On 90th day all the periodontal parameters were assessed and sub-gingival plaque samples were collected from both groups for microbiological assessment. (Fig 3a and 3b shows the pre operative measurements of Pocket depths in both groups)

Microbiological assessment

Assessment of Aa, Pg and Pi were carried out by PCR (Peq star universal gradient Thermocycler). After collecting the subgingival plaque sample the paper points were transferred into 2ml ependroff tube containing 1ml of thioglycolate broth and kept for overnight incubation in an anaerobic dessicator. Then the DNA samples were isolated

Isolation of genomic DNA

1ml of plaque sample containing TG broth was taken in a 15ml Falcon tube. 3ml of reagent A (0.01M Tris – HCl, 20mM Sucrose (Merck),5mM MgCl2 1% Triton x 100 (Bio Basic)) was added and vortexed in a cyclomixer for 4min at room temperature. Then centrifuged at 4000rpm for 5min in a research centrifuge. Without disturbing the cell pellet the supernatant was discarded. The remaining moisture was removed by inverting the tube and blotting onto the tissue paper. 1ml of reagent B (1M Tris-HCl 400ml pH 7.6, 0.5M EDTA 120ml pH 8, NaCl 8.76g pH 8 (Bio Basic)) was added and vortexed briefly resuspend the cell pellet. 250µl of 3M NaCl was added and mixed by inverting the tube several times. The tubes were placed in a water bath for 15 to 20 min at 650C. The tubes were cooled to room temperature. 2ml of ice-cold chloroform was added and mixed on an orbital shaker for 30 to 60 min. Centrifuged at 3200rpm for 2 min in a research centrifuge. Upper phase was transferred to the eppendrof tube using micropipette, 0.5ml of ice cold ethanol was added and the tubes were inverted gently to allow the DNA to precipitate and then centrifuged at 10000rpm for 10min in cooling centrifuge. Ethanol was discarded carefully without disturbing the pellet and then dried. 50µl of TE buffer was added to dissolve the DNA. This DNA is stored at -200C for further use.

Polymerase chain reaction

The Primer designs (Eurofins) used were

Aggregatibacter actinomycetemcomitans

Forward primer: ACGCAGACGATTGACTGAATTTAA (24)

Reverse primer: GATCTTCACAGCTATAGGCAGCTA (24)

Porphyromonas gingivali

Forward primer: CCTACGTGTACGGACAGAGCTAT (23)

Reverse primer: AGGATCGCTCAGCGTAGCATT (21)

Prevotella intermedia

Forward primer: CAGCACCCACAACGATATGA (20)

Reverse primer: TTCCATCTTCTCTGCCTGTC (20)

Preparation of PCR Master Mix

10X Reaction buffer - 2µl
dNTP - 0.2µl (Qiagen)
Taq polymerase - 0.2µl (Qiagen)
PCR Grade water - 15.9µl (Bio-Sciences)
Forward primer - 0.4µl
Reverse primer - 0.4µl
Template DNA - 1µl

17µl of the master mix was mixed with 2µl of each of forward and reverse primers and 1µl of template DNA and the reaction mixture was loaded in the PCR machine and programmed.

PCR Programme setup:

Initial denaturation: 940c for 7 seconds
Secondary denaturation: 940c for 30 seconds
Primer annealing: 550c for 45 seconds
Extension: 720c for 45 seconds
All the 4 steps were repeated for 32 cycles

10µl of PCR amplified samples was mixed with 1µl of dye and loaded into the gel. Electrophoresis was run at 50 to 100v till the tracking dye reached 2/3rd of the distance of gel length and gel was then visualized in UV gel documentation.

Detection and analysis of the reagent product

The amplified strands were observed and compared with DNA ladder (with the known molecular weight) to find out the molecular weight in base pairs of the unknown DNA. The molecular weight of Aa – 232 bps, Pg – 130 bps, Pi – 108 bps. (Fig 4 Comparison of Aa, Pg and Pi with the standard marker gel)

Statistical analysis: results were expressed as mean ± SD and proportions as percentages. Intragroup comparisons were made by paired t-test and unpaired t-test for intergroup comparisons. Categorical data were analyzed by chi test.

For all the tests, a P-value of 0.05 or less was considered for statistical significance.

RESULTS

Clinical parameters

At the beginning of the study 10 patients were selected. 2 patients who were irregular for treatment protocol were excluded. Hence, in this study a total number of 32 deepest sites in 8 chronic periodontitis patients were selected. Intergroup comparisons of change in clinical parameters were shown in table 2 and 3.

Table 2. Mean change in bleeding sites in both groups

TIME POINT	GINGIVAL BLEEDING INDEX	GROUP A		GROUP B		P Value Intergroup
		No.	%	No.	%	
DAY 0	+ve	16	100%	16	100%	
	-ve	-	-	-	-	
DAY 90	+	11	68.8%	5	31.2%	0.034 (S)
	-	5	31.2%	11	68.8%	

Table 3. Intergroup comparison of change in clinical parameters

	Group	N	Mean	Std. Dev	P-Value
Plaque Index - Base line	Group A	16	2.50	0.632	0.761?(NS)
	Group B	16	2.56	0.512	
Plaque Index - 90th day	Group A	16	1.94	0.574	0.012(S)
	Group B	16	1.37	0.619	
Probing Pocket Depth - Base line	Group A	16	6.31	1.195	0.140(NS)
	Group B	16	5.69	1.138	
Probing Pocket Depth - 90th day	Group A	16	4.69	0.946	<0.001(S)
	Group B	16	2.69	1.195	
Clinical Attachment Level - Base line	Group A	16	7.19	1.940	0.420(NS)
	Group B	16	6.69	1.493	
Clinical Attachment Level - 90th day	Group A	16	5.56	1.209	<0.001(S)
	Group B	16	3.56	1.632	

At baseline no statistically significant differences in any of the investigated parameters were found between the two groups. Within the groups there was statistically significant reduction in Plaque scores, Bleeding sites and Probing pocket depth from baseline to 90th day in both the groups. When both groups were compared there was statistically significant reduction in Plaque scores, Bleeding sites and Probing pocket depth in Group B when compared to Group A. There was statistically significant gain in Clinical attachment level from baseline to 90th day in both the groups. When both the groups were compared there was statistically significant gain in Group B when compared to Group A.

Microbiological parameters

Changes in amounts of Aggregatibacter actinomycetemcomitans

The number of samples positive for Aa are shown in Table 4 and Graph 1 Both intergroup and intragroup analyses demonstrated a reduction which was not statistically significant.

Table 4. Percentage of Aggregatibacter actinomycetemcomitans (Aa) present in Group A and Group B at different Time points (n=16)

	Baseline n (%)	21st Day n (%)	90th Day n (%)	P VALUE (Intragroup)		
				0-21	21-90	0-90
GROUP A	8 (50%)	7 (44%)	8 (50%)	0.723 (NS)	0.723 (NS)	1.000 (NS)
GROUP B	7 (44%)	4 (25%)	5 (31%)	0.264 (NS)	0.694 (NS)	0.465 (NS)
P VALUE (Intergroup)	0.723 (NS)	0.264 (NS)	0.280 (NS)			

Changes in amounts of Porphyromonas gingivalis

The number of samples positive for Pg is shown in Table 6 and Graph 2. An intergroup analysis demonstrated a statistically significant reduction in Group B. Intragroup analysis of Group A demonstrated reduction which was statistically not significant whereas intragroup analysis of Group B demonstrated a statistically significant reduction from baseline to 21st and from baseline to 90th day.

Table 5. Percentage of Porphyromonas gingivalis (Pg) present in group A and group B at Different time points (N=16)

	Baseline N (%)	21st Day N (%)	90th Day N (%)	P Value (Intragroup)		
				0-21	21-90	0-90
GROUP A	12 (75%)	9 (56%)	10 (63%)	0.264 (NS)	0.719 (NS)	0.446 (NS)
GROUP B	13 (81%)	6 (38%)	6 (38%)	0.012 (S)	1.000 (NS)	0.012 (S)
P VALUE (Intergroup)	0.669 (NS)	0.288 (NS)	0.157 (NS)			

Changes in amounts of Prevotella intermedia

The numbers of samples positive for Pi are shown in table 6 and Graph 3. Both Intergroup and Intragroup analyses demonstrated a reduction which was not statistically significant

Table 6. Percentage of Prevotella intermedia (Pi) present in group A and group B at Different time points (N=16)

	Baseline N (%)	21st Day N (%)	90th Day N (%)	P Value (Intragroup)		
				0-21	21-90	0-90
GROUP A	9 (56%)	8 (50%)	8 (50%)	0.723 (NS)	1.000 (NS)	0.723 (NS)
GROUP B	8 (50%)	3 (19%)	4 (25%)	0.063 (NS)	0.669 (NS)	0.144 (NS)
P VALUE (Intergroup)	0.723 (NS)	0.063 (NS)	0.144 (NS)			

DISCUSSION

Conventional nonsurgical periodontal therapy consists of mechanical supra and sub gingival tooth debridement and instruction in self-administered oral health care measures. These measures are directed towards reducing the bacterial load and altering the microbial composition towards a flora more associated with health. In turn, these microbiologic changes result in lower levels of inflammation and relative stability in periodontal attachment levels.8

However conventional mechanical debridement using curettes is still technically demanding and time consuming and power scalers cause uncomfortable stress to the patients from noise and vibration. Although systemic and local antibiotics are occasionally administered into periodontal pockets for the purpose of disinfection, with frequent use of antibiotics there is a potential risk of producing resistant micro-organisms.2

Therefore development of novel systems for scaling and root planing, as well as further improvement of currently used mechanical instruments is required.2

As Lasers can achieve excellent tissue ablation with strong bactericidal and detoxification effects, they are one of the most promising new technical modalities for nonsurgical periodontal therapy. Another advantage of Laser is that they can reach the sites that conventional mechanical instrumentation cannot.2

Contrary to mechanical treatment with conventional instruments, the excellent ablation of tissues with laser treatment is expected to promote healing of periodontal tissues, ablating the inflamed lesions and epithelial lining of the soft tissue wall within the periodontal pockets. Part of laser energy scatters and penetrates during irradiation into periodontal pockets. The attenuated laser at a low energy level might then stimulate the cells of surrounding tissue, resulting in reduction of the inflammatory conditions, in cell proliferation, and in increased flow of lymph, improving the periodontal tissue attachment and possibly reducing postoperative pain.2

Since the diode basically does not interact with dental hard tissues, the laser is an excellent soft tissue surgical laser, indicated for cutting and coagulating gingiva and oral mucosa, and for soft tissue curettage or sulcular debridement. The

FDA approved oral soft tissue surgery in 1995 and sulcular debridement in 1998 by means of a diode laser (GaAlAs 810nm).2 The diode laser exhibits thermal effects using the 'hot-tip' effect caused by heat accumulation at the end of the fiber, and produces a relatively thick coagulation layer on the treated surface.2

The results of the present study indicated that the use of Diode laser as an adjunct to SRP led to statistically significant improvements in all the investigated clinical parameters at 90th day following treatment.

In this study evaluating the clinical assessments of Diode as an adjunct to SRP a total number of 16 periodontal pockets in 8 chronic periodontitis patients were treated. Each subject was evaluated on baseline and 90th day.

In the laser group there was a statistically significant reduction in Plaque scores and Gingival sites because of reduction in the degree of gingival inflammation or the laser irradiation per se. Study done by Qadri et al (2005)9 reported a decrease in the amounts of MMP-8 on the laser irradiated sites which is responsible for gingival inflammation. So the reduction in Bleeding index at laser sites might be because of this reason.

In the laser group there was statistically significant reduction in Pocket depth as well as gain in attachment level. This is because the laser irradiation reduces prostaglandin PGE2 (Sakurai et al, 2000)10 and stimulates cellular ATP. (Karu et al, 2003).11

Romanos et al (1994)12 stated that laser irradiation of periodontal pocket eliminated the pocket epithelium without damaging the collateral blood supply that helps for periodontal healing as well as for attachment. which might also be, because of the increased stimulation and proliferation of periodontal ligament fibroblast by laser irradiation, which was in accordance with the study done by Eun-Jeong Choi et al (2010).13

Polymerase chain reaction (PCR) was the method of quantifying the bacteria in this study. Other studies refer to the use of DNA probes or bacterial cultures. Detection levels are not necessarily the same when using PCR, cultures or DNA probes. Thus, a sample that is negative using one of the other techniques can at times be positive when analyzed by PCR. It has been shown that PCR is much more sensitive than bacterial culture and affords a better detection of the microorganisms. Therefore PCR has been presented as the ''gold standard'' of periodontal pathogen identification techniques. It is presently quite a suitable method and very promising for bacterial diagnosis.8

In laser group there was statistically significant reduction of Pg, which is because of the bactericidal and detoxification effects of Diode laser. There was reduction of Aa and Pi by from 7th to 21st day but there was a recurrence of pathogensby 90th day which might be because of the laser irradiation of periodontal pockets was done only for once where as in other studies done by Mortiz et al (1998)14 the laser irradiation was done repeatedly within the treatment protocol.

It is not always possible to select the optimal laser and treatment parameters for laser therapy because of lack of adequate studies. The parameters used in this study seem to have been within the "therapeutic window" of dosage but not necessarily optimal.

Limitations of the present study include small sample size, single episode of laser irradiation and short time period.

However, long term studies are required with larger sample size and longer time

period with repeated laser irradiation of periodontal pockets after initial periodontal therapy and in the maintenance stage and along with microbiological analysis to evaluate the effectiveness of Diode laser in the treatment of periodontal pockets

CONCLUSION

In the present study, there were no adverse events observed with the use of Diode laser. Therefore it can be concluded that Diode Laser is safe and effective in the treatment of periodontal pockets.

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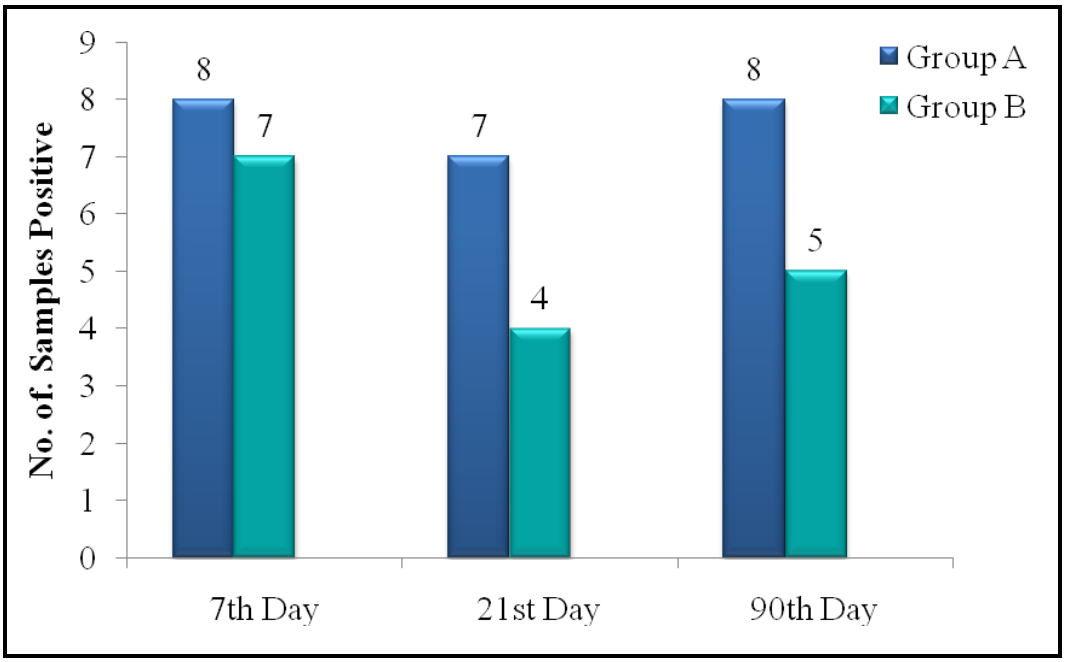
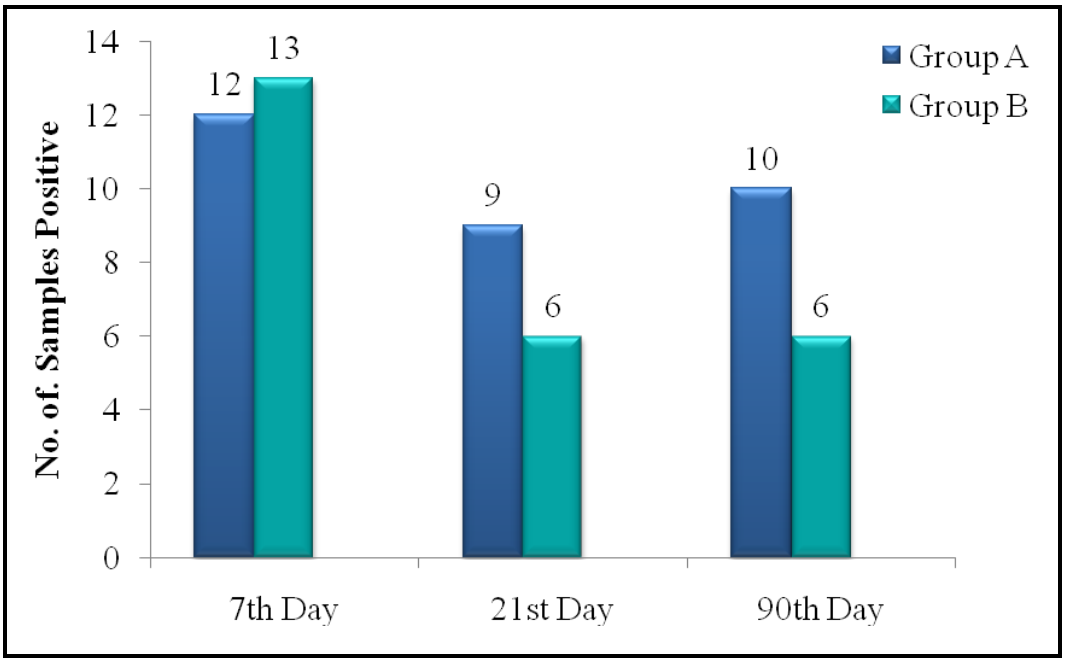
Legends

1a. Group A - Preoperative view of Probing Pocket depth (Baseline)
1b. Group B - Preoperative view of Probing Pocket depth (Baseline)
2. Group b- Laser assisted pocket debridement using Diode laser
3a. Group A - Postoperative view of Probing Pocket depth (Baseline)
3b. Group B - Postoperative view of Probing Pocket depth (Baseline)
4. Comparison of Aa, Pg and Pi gels with the standard marker gel

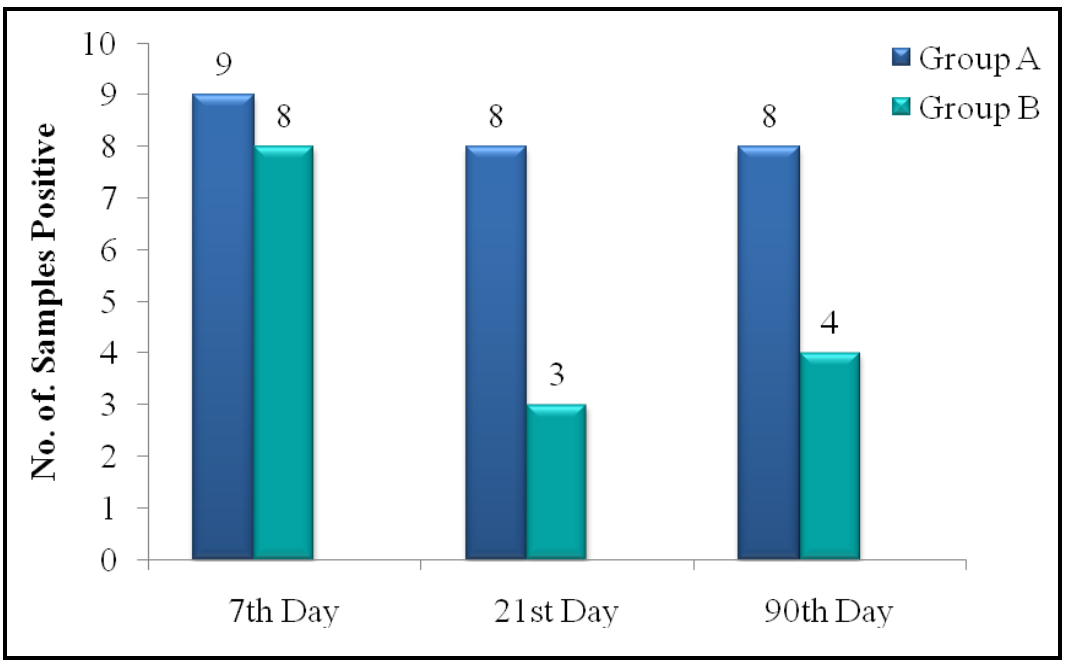
GRAPH 1: NUMBER OF SAMPLES POSITIVE FOR AGGREGATIBACTER ACTINOMYCETEMCOMITANS IN GROUP A AND GROUP B AT DIFFERENT TIME POINTS



GRAPH 2: NUMBER OF SAMPLES POSITIVE FOR PORPHYROMONAS GINGIVALIS IN GROUP A AND GROUP B AT DIFFERENT TIME POINTS



GRAPH 3: NUMBER OF SAMPLES POSITIVE FOR PREVOTELLA INTERMEDIA IN GROUP A AND GROUP B AT DIFFERENT TIME POINTS



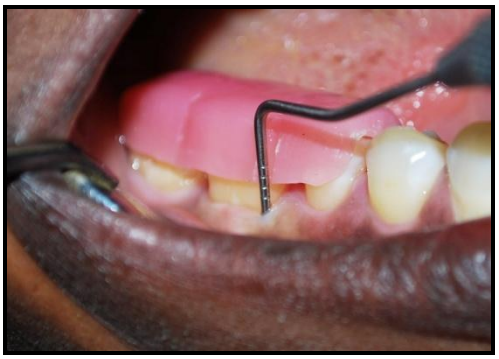


Fig 1a: Group A - Preoperative view of Probing Pocket depth (Baseline)

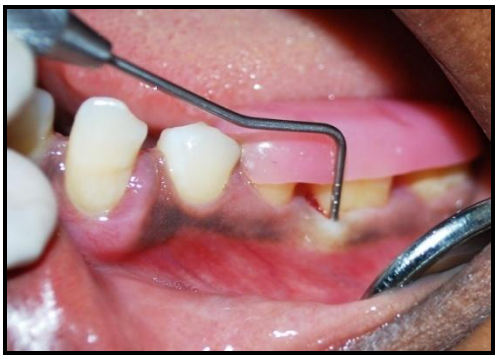


Fig 1b: Group B - Preoperative view of Probing Pocket depth (Baseline)

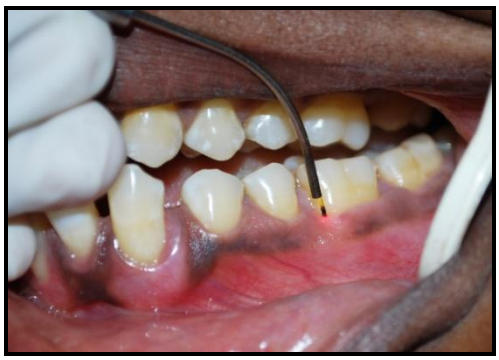


Fig 2: Group B: Laser assisted pocket debridement (SIROLASE)

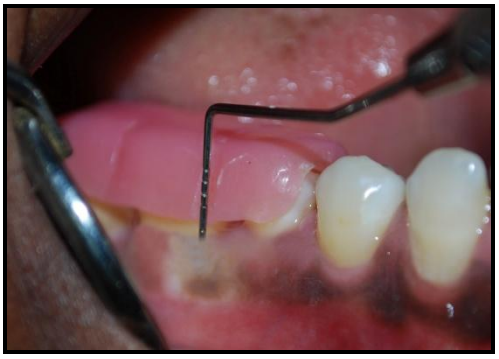


Fig 3a: Group A - Postoperative view of Probing Pocket depth (90th day)

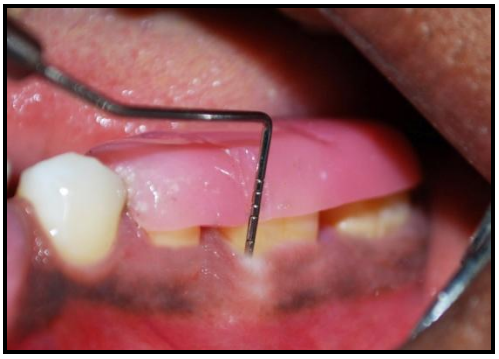


Fig 3b: Group B - Postoperative view of Probing Pocket depth (90th day

Comparison of Aa, Pg and Pi gels with the standard marker gel

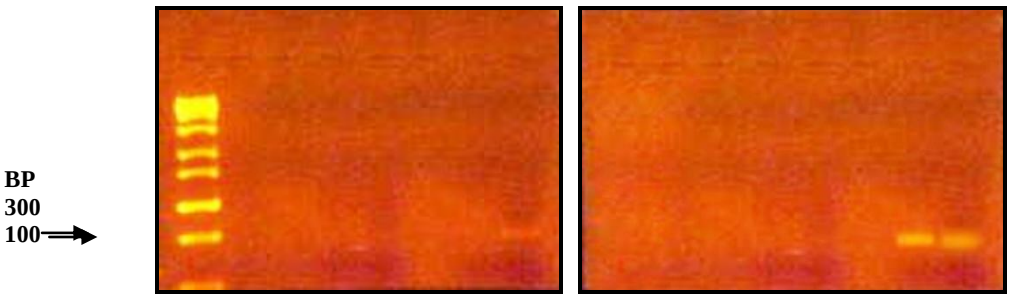
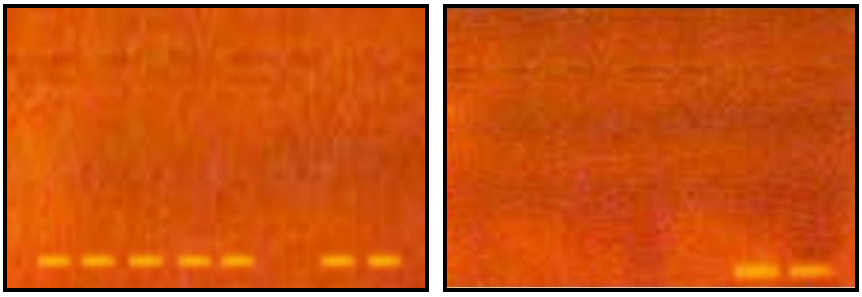


Fig 4: ***Standard marker*** ***Aggregatibacter actinomycetemcomitans***



Porphyromonas gingivalis ***Prevotella intermedia***