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Clinical efficacy of diode laser and photodynamic therapy as an adjunct to scaling and root planing in the treatment of periodontitis: A randomized clinical trial.

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Aim & Background: Photodynamic therapy (PDT) is a new, non-invasive therapeutic procedure that employs photosensitizers, light of precise wavelength, and which leads to production of singlet oxygen to eradicate pathogenic microbes. The present study is a randomized split mouth prospective double blinded 6 month followup trial evaluating the efficacy of single application of PDT, Diode laser as an adjunct to conventional SRP in the treatment of chronic periodontitis patients receiving initial periodontal therapy.

Methods: This study was a single center double blind randomized clinical trial. The study included 15 participants who were diagnosed with periodontitis and were randomly divided in to 3 groups, i-e., Test group 1 Photodynamic therapy + scaling and root planing, Test group II Diode laser irradiation + scaling and root planing, control Group III scaling and root planing alone. 0.01% Toluidine blue O dye was

used as a photosensitizer and the area is irradiated with a wavelength of 660nm for 1 min. Clinical parameters such as Oral Hygiene Index Simplified, Sulcus Bleeding Index, probing pocket depth, clinical attachment level were recorded at baseline, 3 and 6 months. Analysis of data for Intra and Inter group differences was done using Multiple Measures ANOVA.

Results: Significant reduction (p<0.05)was observed among all the groups, but Test group I - Photodynamic therapy + scaling and root planing has showed highly statistically significant (p<0.001)results in probing pocket depth and clinical attachment level at 3 and 6 months when compared with other groups.

Conclusion: Photodynamic therapy using 0.01% Toluidine blue 'O' could be a better adjunctive therapy to scaling and root planing in the treatment of periodontitis.

Clinical significance: TBO used as a photosensitizer reacts with lipopolysaccharides in the cell membrane of gram negative bacteria, there by producing a bactericidal effect in the presence of light source.

Keywords: Photosensitizing Agents, Photodynamic therapy, Laser Therapy, microbiota, Chronic Periodontitis.

Introduction:

Periodontitis is an infectious, complex condition having a bidirectional relationship with a few systemic conditions. The fundamental cause of the disease is due to the disharmony between host responses and periodontopathogenic bacteria, which leads to periodontal tissue damage and tooth loss.Thus, the therapeutic goal of periodontal treatment was to eliminate periodontal inflammation by eradicating periodontal pathogens and restoring tissue to health. Scaling and root planing (SRP) has long been considered the gold standard for treating chronic periodontitis. However, SRP has shortcomings as it cannot entirely eradicate pathogenic microorganisms, particularly those located in deep periodontal pockets, furcation areas. As a result, microbes from the non-eliminated subgingival plaque recolonize.

The rapid progress of scientific and technical breakthroughs in the area of dentistry has resulted in a plethora of alternative treatment methods that could prevent recolonization.

An non-invasive approach by using lasers , mainly diode laser had gained much attention in treating oral conditions[1]. The advantage of the laser in treating inflammatory conditions like periodontitis has been also attributed to their host immuno-modulatory effects. The use of therapeutic lasers facilitate healing of treated sites[2], stimulate natural biological events and effects cells in decreased redox stage. Healthy cells cannot change their redox stage and therefore are not affected by lasers. Romanos has stated that Lasers in periodontal therapy have been demonstrated to be beneficial for control of bacteremia, better removal of the pocket epithelium in the pockets, bacteria reduction, efficient subgingival calculus removal and improvement of periodontal regeneration in animals and humans without damaging the surrounding bone and pulp tissues.

Photodynamic therapy (PDT) is another adjunctive modality which enhances the effect of low level laser therapy which works on the principle that light of a suitable wavelength activates photosensitizers which produce free oxygen radicals able to destroy bacteria and their products. Seyyedi SA et al.[3] stated that Photodynamic therapy with a low power laser enables the laser to indirectly decontaminate the periodontal pocket by activation of a photosensitizer agent, thus potentiating the bactericidal effect. Soft tissue thermolysis and bacterial decontamination can be done.

Toluidine blue O (TBO) is a substance that gives blue/violet color. It stains mast cells granules and proteoglycans/glycosaminoglycans of connective tissues. Methylene blue and toluidine blue O function well in inactivating both gram-positive and gram-negative periodontopathic bacteria. TBO reacts with lipopolysaccharides in the cell membrane of gram negative bacteria[4]. However, it is still questionable due to the limited amount of data, therefore this study is designed to evaluate the efficacy of

single application of PDT, Diode laser as an adjunct to conventional SRP in the treatment of periodontitis patients receiving initial periodontal therapy.

Methods:

Ethical statement: The study was approved by the institutional study board SSDC & RI/IRB/IEC/2020-21/409/8/2 (Sree Sai Dental College and Research Institute, Srikakulam) and the study trial was registered in ClinicalTrials.gov (CTRI/2021/10/037074) before study commencement. The trial was executed according to the Helsinki Declaration of 1975, modified in 2008. The nature and process of the study was explained and a written consent form was obtained from the participants.

1.Study design

The present study was a split mouth, single center, double blinded clinical trial with a 6 months followup from Dec 2021 to July 2022 and sample size estimation was done using G* power software version 3.1.9.2, α error probability 0.05 with a sample size of 15 patients who were diagnosed with periodontitis of stage I / II grade A/B/C recruited from the outpatient Department of Periodontology.

2. Inclusion criteria

Age range of 25 -40 years. Systemically healthy individuals. Probing depth of 4 -6 mm in 3 different quadrants of the mouth.

3. Exclusion criteria

Smokers & alcoholics, pregnant and lactating women, medically compromised patients, history of antibiotics and periodontal therapy in the past 6 months were excluded from the study.

4. Clinical parameters

An experienced periodontist calibrated the clinical parameters. The clinical parameters which were evaluated are OHI-S, Sulcus bleeding index(SBI), probing pocket depth(PPD), and clinical attachment level(CAL) were recorded at baseline, 3 & 6 months using University of North Carolina No. 15 periodontal probe.

To standardize the measurement of site-specific PPD, a custom-made acrylic stent was used. PPD was calculated as the distance from marginal gingiva to base of the periodontal pocket. CAL was measured as the distance between the cemento-enamel junction and the base of the periodontal pocket. PPD, CAL was recorded at 6 sites per tooth. Test group I PDT+SRP, test group II Diode laser therapy + SRP, control group SRP alone.

5. Treatment procedure

Full-mouth SRP was performed with both hand (Gracey Curettes; Hu-Friedy) and an ultrasonic instruments(SATELEC). Following SRP, patients were randomized via sequentially numbered envelop method in to 3 groups. Photosensitizer used was 0.01% Toluidine blue 'O' (Fig 2b) was employed to the base of the pocket for 1 min and laser irradiation (Fig 2c) (zolar diode laser ZOLAR Technology & Mfg Co. Inc) with a wavelength of 660nm, 0.6 watt power output was used to irradiate for 1 min. Test group II diode laser irradiation (Fig 3b) was performed with a wavelength of 660nm, with a power output of 0.6 for 1 min. In control group only scaling and root planing was performed.

The patients were randomized via sequentially numbered envelop method in to 3 groups. The participants and the statistician were blinded in the study.

6. Statistical analysis:

Entire data was transferred to Microsoft excel spread sheet and subjected to statistical analysis done using SPSS 25.0 (IBM Inc. Chicago, IL, USA). Intra and Inter group differences were analyzed using Multiple Measures ANOVA. Statistical significance was defined as $p < 0.05$.

Results

The demographic data of test and control groups were summarized in table 1 consisted of 15 subjects of which 5 subjects (33.3%) were females and 10 males (66.7%) with an age group 25- 40 years and the mean age is 31.27. Mean ± standard deviation values of OHI-S baseline, 3 and 6 months was summarized in table 2. There

was no statistically significant difference at baseline between test and control groups. Inter and Intra group comparison there was high statistically significant difference regarding sulcular bleeding index (table 3, 4), PPD (table 5, 6) and CAL (table 7, 8) after 3 and 6 months post-treatment. Test group I - PDT + SRP has showed statistically significant results(p>0.000) in PPD [3.40 (Fig 2d), 3.67 (Fig 2e)] and CAL (6.50, 6.73) when compared with test group II PPD [3.67 (Fig 3c), 3.93 (Fig 3d)] CAL (6.73, 6.96) and control group PPD [3.73 (Fig 4b), 4.13(Fig 4d)] CAL (6.93, 7.20) at 3 and 6 months respectively.

Discussion

Impact of SRP alone, SRP + PDT, SRP + Diode therapy on clinical parameters were examined and the results were compared. Split mouth design helps to facilitate comparison of all groups via eliminating patient specific factors. Toluidine blue O dye was used as a photosensitizer for this investigation since it has been shown to be one of the safest photosensitizers for treating periodontal conditions. Annaji S et al.[5] reported that there is significant improvement in both microbiological and clinical parameters in sites treated with PDT+SRP using TBO dye.

All the three groups resulted in significant improvements in all the clinical parameters. Oral hygiene improved significantly after 3 months and 6 months which could be due to the oral hygiene maintenance due to hawthorn effect stated by Segarra-Vidal M et al.[6]

TonettiM.S et al.[7]BOP is regarded as an objective indicator to assess gingival inflammation. A BOP-positive site can be considered to be associated to have further attachment loss. Lang et al.[8] stated that BOP could be used as a prognostic indicator for assessing periodontal inflammation. The results of the present study are in accordance with the studies done by Annaji S et al.[5] , Christodoulides et al.[9] and Chondros et al.[10], Monzavi A et al.[11], Alwaeli HA et al.[12]which showed reduction in case of BOP in PDT group.

Improvement in CAL occurred because of significant reduction in probing pocket depth[6]. The reduction in PPD after SRP depended on the initial PPD.

Under the experimental conditions test group I SRP+PDT showed statistically significant improvement in all the clinical parameters when compared with the other groups. The results of the present study showed significant reduction of PPD was in accordance with Gandhi KK et al.[13] , Pal et al.[14]and Meimandi M et al.[15] stated that the positive effect of PDT on treatment outcome is that it has a focal effect, and the use of high concentration photosensitizer plays a critical role in reducing bacterial load by triggering type 1 and type II reactions; Rajesh S et al.[16] stated that these ROS are capable of inactivating bacteria by irreversibly altering their protein structure, deactivation of membrane transport system and changing their nucleic acid without causing any adverse effects to the host; it also reduces bacteremia and eliminates the need for systemic antibiotics.

Komerik et al.[17] reported that even at the lowest light energy dose employed enough photons were supplied to activate all of the photosensitizer molecules present in the gingival crevice and released sufficient ROS to allow killing of most (approximately 90%) of the bacteria. Moritz et al.[18] stated that diode laser might have similar properties as that of Nd:YAG laser which is in the range of infra red with a similar wavelength. Annaji S et al.[5], Lulic et al.[19] reported that multiple episodes of PDT showed greater significant reduction of clinical parameters with a long term effect. In the present study single episode of exposure was performed as an adjunct to SRP which showed statistically significant reductions in all the clinical parameters.

Although there are many supportive studies that highlight the effectiveness of PDT as an adjunct, there are some contrary studies stating that there is no significant change when compared with SRP and the reason could be because of difference in variance of study design, photosensitizer, exposure duration and power output in PDT and diode laser therapy. Bundidpun P et al.[20], Pourabbas R et al.[21], Carvalho VF et al[22], Chondros et al.[10] and christodoulides et al.[9] , Azarpazhooh Amir et al.[23]failed to show positive results in PPD & CAL.

The results of the present study using diode laser irradiation showed statistically significant improvements of PPD and CAL when compared with control group. These results coincided with the results of De Micheli et al.[24] and Dukic et al.[25], Mohammadreza Talebi et al.[15]. The limitations of the present study might be (1)small sample size, (2) short time period, (3) Lack of GCF and microbial analysis due higher cost. Further randomized controlled clinical trials with large sample size and long term followups should be carried out for concluding the affirmative role of PDT, Diode laser therapy as adjunct to SRP.

Conclusion: The results of the present study showed that the additional application of single episode of photodynamic therapy to scaling and root planing have shown additional effects on clinical parameters in patients diagnosed with chronic periodontitis and could be a better adjunctive modality.

Clinical significance:

Damage to healthy cells is very much limited in photodynamic therapy as photosensitizer tend to build up more in abnormal cells in the presence of light source. Rapid elimination of bacteria because of its bactericidal effect, with selective photocytotoxicity.

Abbreviations

SRP - Scaling and Root Planing

PDT - Photodynamic therapy

TBO - Toluidine blue O

OHI-S - Oral Hygiene Index- Simplified

SBI - Sulcus bleeding index

PPD - probing pocket depth

CAL - clinical attachment level

ANOVA - Analysis of variance

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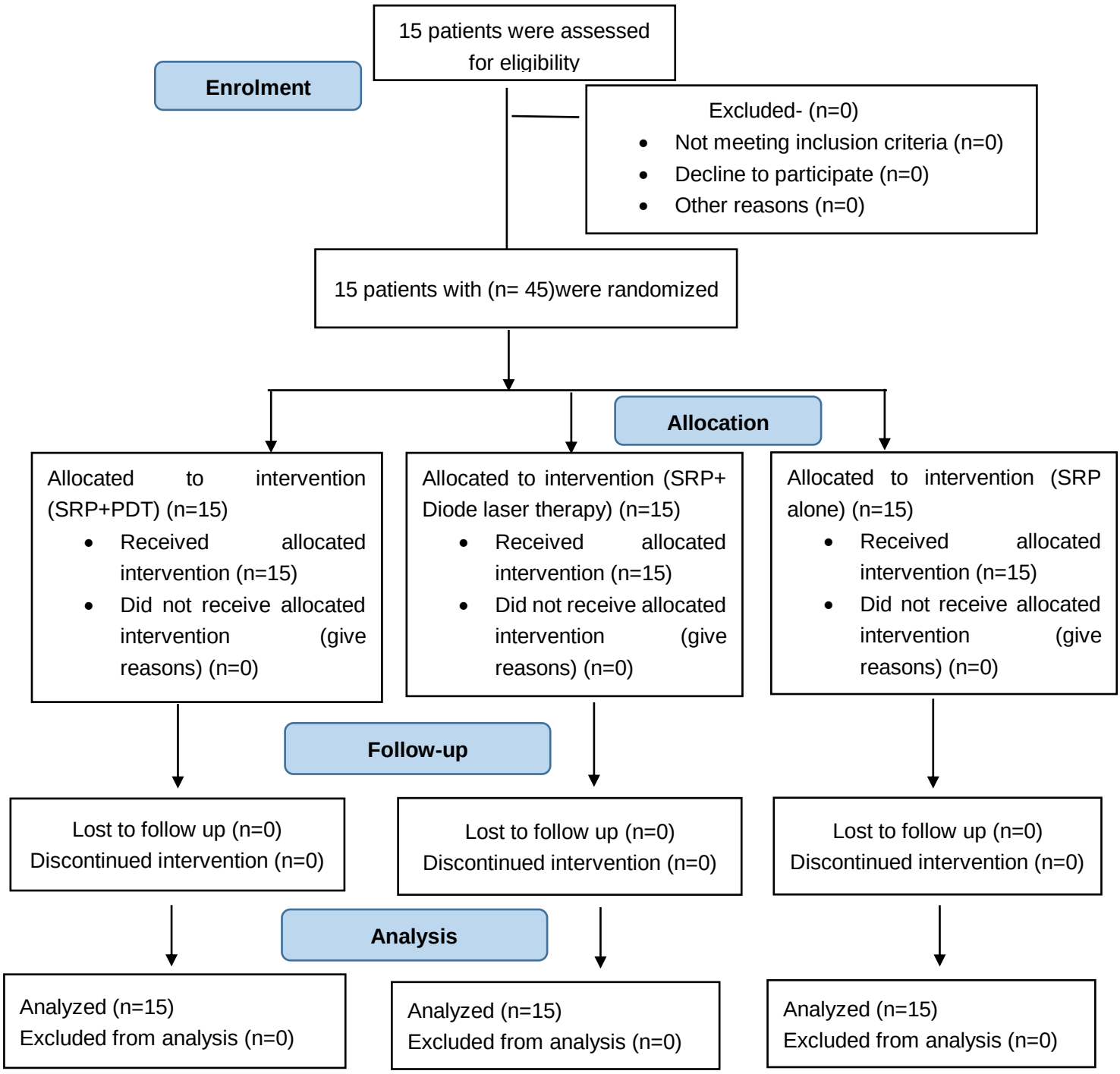
Figure 1 consort flow chart

CONSORT 2010 Flow Diagram



CONSORT flow chart depicting screening, enrolment, allocation, follow-up, and analysis

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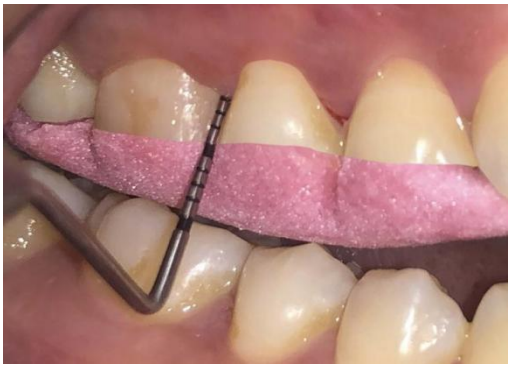
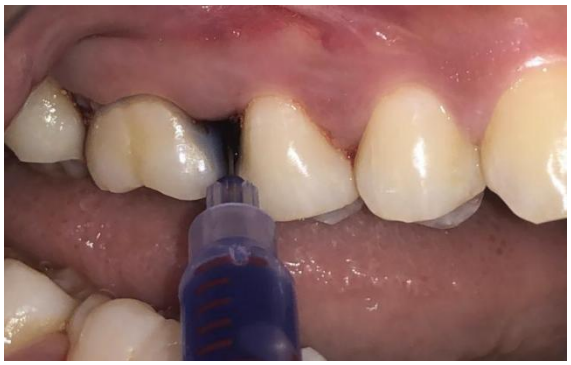
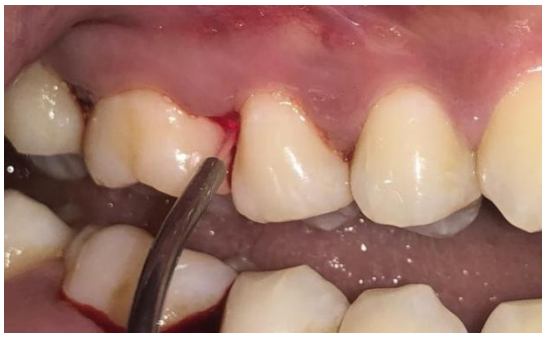
Figures

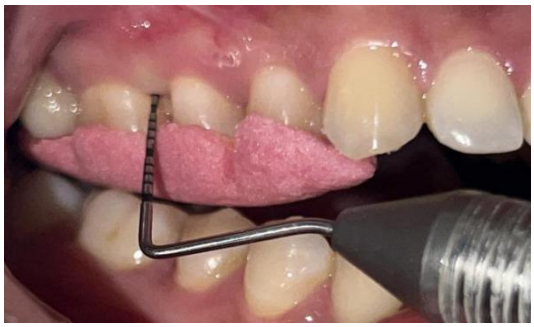
Test group I Photodynamic therapy + SRP



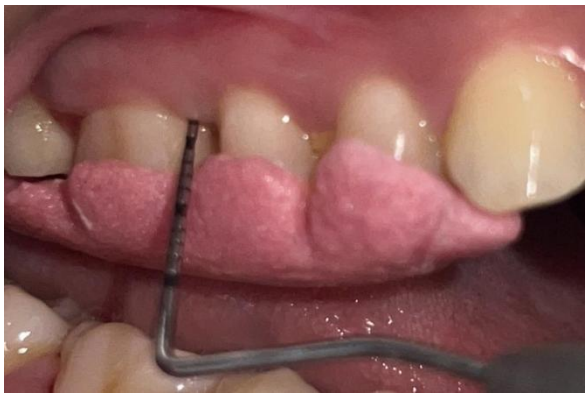


Test group 1 (2a) Pre-operative PPD irt 16 (2b) 0.01% Toluidine blue is adminstered





(2c) 660nm Diode laser irradiation (2d) 3 months PPD followup



(2e) 9 months followup

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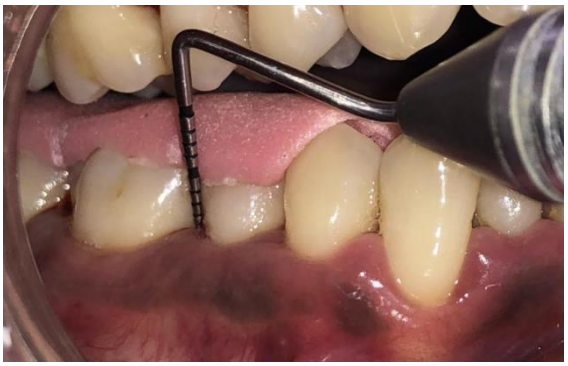
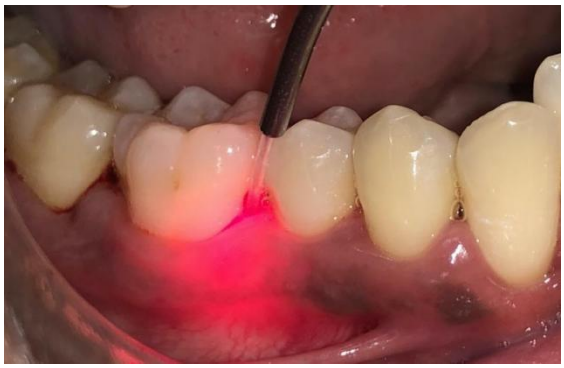
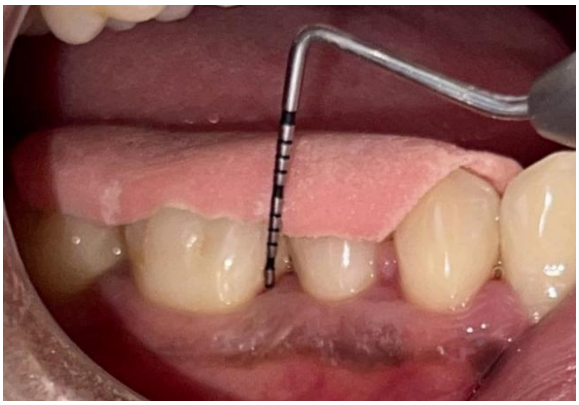
Test group II Diode laser therapy + SRP

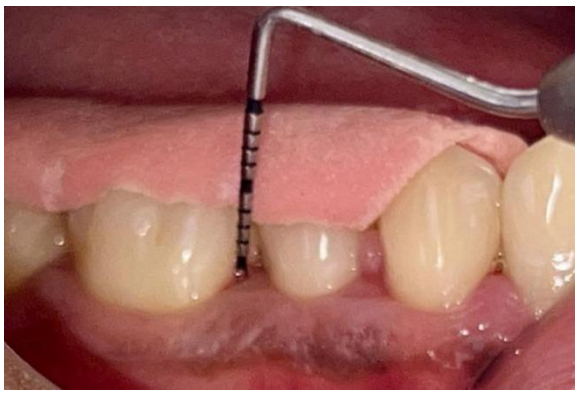




Test group II(3a) Preoperative view irt 46

(3b) diode laser irradiation





(3c) 3 months followup

(3d) 9 months followup

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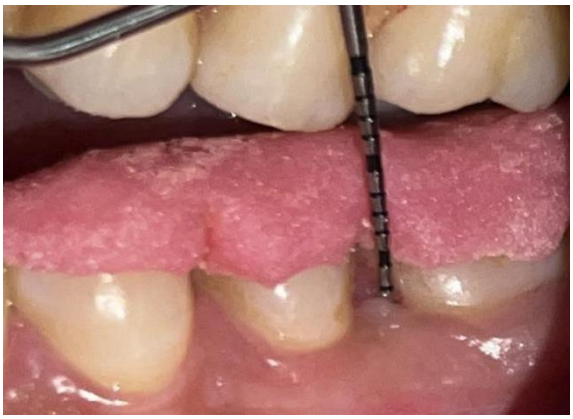
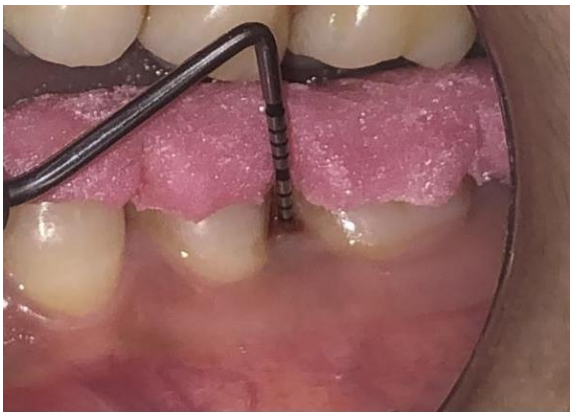
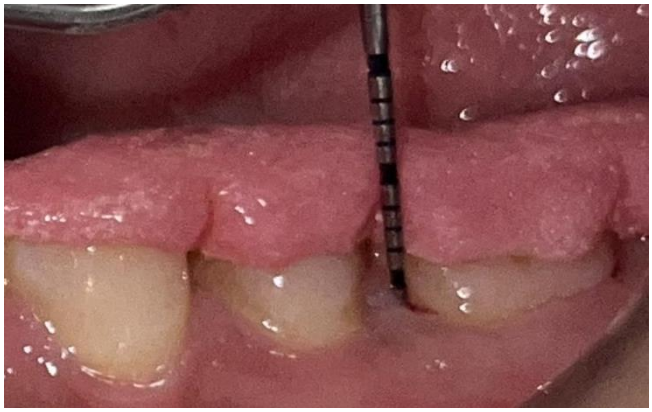
Control group SRP alone





Control group (4a) Preoperative view (4b) 3 months followup



(4c) 6 months followup

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Tables

Table 1 Demographic data

Gender	Frequency	Percentage
Female	5	33.3
Male	10	66.7
Total	15	100

Table 2 Mean ± SD of OHI-S

Parameter	Duration	Mean	Std.deviation	P.value
OHI-S score	Baseline	3.06	0.83	0.000
	3 months	1.88	0.40	
	6 months	1.84	0.50	

Statistical significance is set at $p<0.05$.

Table 3 Mean ± SD and intragroup mean reductions of SBI scores at baseline, 3 and 6 months.

Group	**SBI**	**Mean**	**SD**	***p* value**
Control	Baseline	1.81	0.114	0.000
	3 months	1.54	0.089	
	6 months	1.57	0.139	
Test group 1	Baseline	1.78	0.208	0.000
	3 months	1.32	0.148	
	6 months	1.36	0.213	
Test group 2	Baseline	1.75	0.188	0.000
	3 months	1.45	0.140	
	6 months	1.49	0.071	

Statistical significance is set at $p<0.05$.

Table 4 Intergroup comparison of mean reductions of SBI at baseline, 3 and 6 months.

Time	**Test group 1 vs control**		**Test group 2 vs control**		**Test group 1 vs test group 2**	
Months	**Mean diff**	***p* value**	**Mean diff**	***p* value**	**Mean diff**	***p* value**
Baseline	-0.03	0.000	-0.06	0.000	0.03	0.000
3 months	-0.23	0.000	-0.09	0.000	-0.14	0.000
6 months	-0.21	0.000	-0.08	0.000	-0.13	0.000

Statistical significance is set at $p<0.05$.

Table 5 Mean ± SD and intragroup mean reductions of PPD scores at baseline, 3 and 6 months.

Group	**SBI**	**Mean**	**SD**	***p* value**
Control	Baseline	5.13	0.352	0.000
	3 months	3.73	0.516	
	6 months	4.13	0.516	
Test group 1	Baseline	5.27	0.458	0.000
	3 months	3.40	0.737	
	6 months	3.67	0.900	
Test group 2	Baseline	5.13	0.352	0.000
	3 months	3.67	0.488	
	6 months	3.93	0.799	

Statistical significance is set at $p<0.05$.

Table 6 Intergroup comparison of mean reductions of PPD at baseline, 3 and 6 months.

Time	**Test group 1 vs control**		**Test group 2 vs control**		**Test group 1 vs test group 2**	
Months	**Mean diff**	***p* value**	**Mean diff**	***p* value**	**Mean diff**	***p* value**
Baseline	0.14	0.000	0.0	0.000	0.14	0.000
3 months	-0.33	0.000	-0.06	0.000	-0.27	0.000
6 months	-0.46	0.000	-0.2	0.000	-0.26	0.000

Statistical significance is set at $p<0.05$.

Table 7 Mean ± SD and intragroup mean reductions of CAL scores at baseline, 3 and 6 months.

Group	**SBI**	**Mean**	**SD**	***p* value**
Control	Baseline	8.10	0.280	0.000
	3 months	6.93	0.495	
	6 months	7.20	0.527	
Test group 1	Baseline	8.27	0.458	0.000
	3 months	6.50	0.707	
	6 months	6.73	0.677	
Test group 2	Baseline	8.12	0.352	0.000
	3 months	6.73	0.495	
	6 months	6.96	0.667	

Statistical significance is set at $p<0.05$.

Table 8 Intergroup comparison of mean reductions of CAL at baseline, 3 and 6 months.

Time	**Test group 1 vs control**		**Test group 2 vs control**		**Test group 1 vs test group 2**	
Months	**Mean diff**	***p* value**	**Mean diff**	***p* value**	**Mean diff**	***p* value**
Baseline	0.17	0.000	0.03	0.000	0.14	0.000
3 months	-0.93	0.000	-0.2	0.000	-0.23	0.000
6 months	-0.47	0.000	-0.24	0.000	-0.23	0.000

Statistical significance is set at $p<0.05$.