



Effect of Low-Level Laser Therapy as an Adjunct to Nonsurgical Periodontal Therapy on Salivary Interleukin -1 Beta Levels in Smokers with Periodontitis: A Case Control Study

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Abstract

Background: The aim of this study was to evaluate the effect of Low-Level Laser Therapy (LLLT) as an adjunct to Scaling and Root Planning (SRP) on salivary interleukin -1 β levels in Generalized Stage II Grade B periodontitis patient.

Methods: All 45 systemically healthy patients who were included in the study initially received SRP. The LLLT group (n=25) received diode laser therapy as an adjunct to SRP. A diode laser with a wavelength of 940nm was used for LLLT. Energy density of 12 J/cm² was applied at the orifice of the gingival margin from distance of 1 cm using as a continuous wave with setting 1.5 W for period of 5s to 10s of exposure for anterior teeth and premolars and 20s of exposure for posterior teeth. Saliva samples were collected from all patients and clinical parameters were recorded on baseline and 21 days after treatment. Interleukin-1 β levels in the collected saliva were measured.

Results: The clinical variables like Plaque index, Gingival index, probing pocket depth and clinical attachment loss were reduced more in LLLT group than control group. IL-1 β levels decreased significantly more in the LLLT group after 21 days.

Conclusion: LLLT as an adjunctive therapy to SRP improves periodontal parameters.

Keywords: Interleukin 1 β ; Low Level Laser Therapy; Periodontitis; Scaling and root planning

Introduction

Periodontitis is a multifactorial disease that is associated with loss of the alveolar bone and periodontal ligament around the tooth [1]. Conventional periodontal therapy includes both non-surgical and surgical approaches that involve instrumentation of the inflamed dentogingival complex [2]. To control periodontitis non-surgical therapy by mechanical instrumentation is the primary recommended approach [3].

Periodontal disease is associated with widespread systemic inflammation, as demonstrated by the increase in circulating inflammatory cytokines and C reactive proteins [4,5]. This important topic is reinforced by evidences suggesting that non-surgical periodontal treatment should be able to restore the anti-atherogenic properties of the endothelium and to reduce serum cytokines levels [6,7].

Interleukin (IL) IL-1 β , IL-6, IL-8 and IL-10 are the main cytokines identified in chronic periodontal disease, with further influence on the activity of immune cells [8]. The IL-1 family of cytokines has a central role in triggering and perpetuating the immune and inflammatory responses [9]. Pro-inflammatory cytokines, such as interleukin 1 β , play a key role in the initiation and regulation of immune responses in periodontium [10]. It is well documented, that interleukin 1 β has been involved in the pathogenesis of inflammation-induced bone resorption [10].

In the last decade, the application of lasers as an adjunctive or alternative to scaling and root planning had a great run in the treatment of gingival inflammation [11,12]. Among laser applications, Low-Level Laser Therapy (LLLT) is recommended for its anti-inflammatory, wound healing promoter, pain-reducing effects [13].

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There are a limited number of studies that evaluated the effect of LLLT as an adjunct in chronic periodontitis. To the best of our knowledge no studies have been done so far to evaluate the effect of LLLT on salivary IL-1 β levels.

Thus, the present study evaluates the effect of LLLT, as an adjunct to SRP on periodontal clinical and biochemical parameter, i.e., Salivary Interleukin- 1 beta.

Participants and study design

Forty-five patients (Case group =25, Control group =20) who were classified as Generalized Stage II Grade B Periodontitis according to the 2017 American Academy of Periodontology workshop were included in the study. Based on the sample size estimation a minimum sample of 18 was required in each group. However, keeping in view about the drop outs, a total of 25 subjects were included in the case group and 20 subjects in the control group.

Patient were referred to Department of Periodontology, School of Dental Sciences, KIMS Karad, for periodontal treatment between January 2020 to September 2020. Written informed consent was obtained from all subjects. The study protocol was approved by Ethical Committee and Institutional Review Board of Krishna Institute of Medical Sciences Karad (Ref. No. KIMSDU/IEC/09/2018).

To be included the patients had to be 35 to 65 years of age with periodontal pocket depth of 4 mm to 7 mm. The exclusion criteria were as follows: Tobacco users, pregnant and lactating mothers, systemic diseases like diabetes mellitus, obesity, osteoporosis, rheumatoid arthritis etc. which could affect periodontal treatment outcomes, use of immunosuppressive agents and patient who had any antibiotics or anti-inflammatory drugs taken within the preceding 3 month and periodontal treatment within the past 6 month.

Initially, all participants received basic periodontal treatment including scaling and root planning and oral hygiene instructions. Baseline measurement of the probing pocket depth, clinical attachment loss, gingival index (Loe and Silness in 1963) and Plaque index (Silness J. and Loe H 1964) were recorded before SRP. Saliva sample, for analyses of IL-1 β were taken before SRP. Case group received LLLT after SRP. All the treatment were performed by single trained clinician.

LASER treatment

Laser therapy was performed after scaling and root planning in LLLT group using a 940 nm diode laser (Photon plus; Zolar technology co inc., Canada). The laser was fired at the orifice of the gingival margin from distance of 1 cm using in a continuous wave with 1.5 W for period of 5s to 10s of exposure for anterior teeth and premolars and 20s of exposure for posterior teeth. Six sites were fired around each tooth. The energy density was 12 J/cm².

All LASER safety Guidelines and precautions were strictly followed.

Patients were recalled at end of 21 days for reassessment of periodontal parameters.

Sample collection

Unstimulated whole expectorated saliva was collected from each subject. The subjects were made to sit comfortably in a calm and isolated room. He/she were asked to rinse the mouth thoroughly using distilled water or deionized water to remove any food debris, tilt their head forward and then expectorate whole saliva into a

sterile Eppendorf tube [14]. Collection was done at standard time, preferably between 8 am to 11 am and before doing periodontal examination. The subjects were preferably in the fasting state or two hours after breakfast [15]. Collected samples were placed on an ice pack immediately then it was transported to the laboratory and were centrifuged at 5000 rpm for 15 min. The supernatant structure was kept frozen at <-20°C as aliquots until assayed. Freezing and thawing cycles were avoided.

Laboratory analyses

The assay of IL-1 β was performed using human (IL-1 β) ELISA kit (Abbkine ELISA, Elikine Human IL-1 β ELISA Kit, Inc. China) by quantitative sandwich immunoassay technique. The overall inter assay co-efficient of variation has been reported to be <10%.

Statistical analysis

All the collected data were tabulated and entered into MS-Excel. The collected data were analyzed using a statistical software package for social sciences SPSS, IBM, INDIA 20.0. The Mean values for PI, SI, PPD, and CAL were calculated to compare the difference in the healing response using the site of the tooth as a unit of analysis. Differences between groups and between different time points within each group were tested by the Wilcoxon signed-rank test. Statistical significance was set at the 95% confidence level for Wilcoxon signed-rank test and statistical significance was set at p<0.05.

Results

The current study was conducted in 45 patients, case (25) and control (20) and no patients were lost during follow up.

Age and gender wise distribution

Table 1 shows that among 20 controls, 13 (65%) patients were males and 7 (35%) were females with an average age of 43.5 \pm 6.38 years. Whereas, out of 25 cases, 19 (76%) were males and 6 (24%) were females with an average age 47.44 \pm 9.68 years. There was no significant difference among the groups.

Comparison of Plaque Index (PI) and Gingival Index (GI) at baseline and after 21 days in case and control group

In case and control group there was statistically significant difference in PI and GI values from the baseline to 21st day (p<0.05). Thus, observed reduction in PI and GI significantly more in the case group (Table 2).

Comparison of PPD and CAL at baseline and after 21 days in case and control group

In case and control group there was statistically significant difference in PPD and CAL values from the baseline to 21st day (p<0.05). Thus, observed reduction in PPD and CAL significantly more in the case group (Table 3).

Comparison of IL-1 β (pg/ml) Levels at baseline and after 21 days in case and control group

In case and control group there was statistically significant decline in IL-1 β levels from the baseline to 21st day (p<0.05). And this

Table 1: Age and gender wise distribution.

Study Group	Gender		Age (in years) *	
	Males (%)	Females (%)	Mean	SD
Control Group (n=20)	13 (65%)	7 (35%)	43.5	6.38
Case Group (n=25)	19 (76%)	6 (24%)	47.44	9.68

*Not Significant (p>0.05) in control and case groups

Table 2: Comparison of Plaque index and gingival index at baseline and after 21 days in case and control group with Wilcoxon signed-rank test.

	Control Group				Case Group			
	PI		GI		PI		GI	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	1.265	0.517	1.17	0.5	1.504	0.575	1.53	0.539
After 21 days	0.76	0.4297	0.73	0.313	0.94	0.322	0.912	0.293
Difference \$	0.505	0.0877	0.435	0.187	0.564	0.252	0.624	0.245
Wilcoxon W- value	153		136		276		276	
p-value	<0.0001*		<0.0001*		<0.0001*		<0.0001*	

\$ Wilcoxon test P<0.05; *Significant when p<0.05; † Unpaired t- test P<0.05

Table 3: Comparison of PPD and CAL at baseline and after 21 days in case and control group with Wilcoxon signed-rank test.

	Control Group				Case Group			
	PPD		CAL		PPD		CAL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	6.18	0.81	3.22	0.26	6.43	0.86	3.12	0.5
After 21 days	5.42	0.78	2.56	0.26	4.17	0.65	2.27	0.34
Difference \$	0.75	0.2	0.66	0.19	2.26	0.54	0.85	0.34
Wilcoxon W- value	36585		496		63190		4656	
p-value	<0.0001*		<0.0001*		<0.0001*		<0.0001*	

\$ Wilcoxon test P<0.05; *Significant when p<0.05; † Unpaired t- test P<0.05

Table 4: Comparison of IL-1β Levels (pg/ml) at baseline and after 21 days in case and control group with Wilcoxon signed-rank test.

Salivary IL-1β Levels	Baseline		After 21 days		Difference		Wilcoxon W-value	p-value
	Mean	SD	Mean	SD	Mean	SD		
Control Group (n=20)	0.83	0.32	0.77	0.31	0.06	0.55	36	<0.0001*
Case Group (n=25)	0.91	0.28	0.66	0.43	0.23	0.62	30	<0.0001*

P-value for Difference; † Unpaired t-value =3.35, 0.0017*; \$ Wilcoxon test P<0.05; *Significant when p<0.05; † Unpaired t- test P <0.05

reduction was significantly higher in the case group (Table 4).

Discussion

Periodontitis is a peripheral chronic inflammatory disease, caused by an imbalance between destruction and repair of tooth supporting tissues, triggered by periodontal bacteria of the dental plaque [16,17]. Periodontal disease has been associated with increased systemic inflammatory markers such as cytokines, which play an important role in the pathogenesis and progression of the disease, by determining the strength, nature and duration of the immune response [18,19]. Cytokines in serum, plasma, GCF, and saliva have been identified as inflammatory indicators of periodontal disease and can be used as biomarkers of chronic periodontitis [20].

The interrelationship between chronic periodontitis and IL-1b levels in GCF and gingival tissues has been investigated in a considerable number of studies [21-26], but only some studies have explored IL-1b in saliva [27-31].

Recently, the use of lasers in the medical field is more pronounced. Today, low-level lasers are also used in medicine to improve wound healing [32]. The dose applied during laser application is one of the important treatment parameters to benefit from LLLT. However, a precisely determined dose has not been proved for each indication. In many literatures it has been reported that bio-stimulation with doses between 0.001 and 10 J/cm² as a therapeutic window [33]. In our study we used a Diode laser with a wavelength of 940 nm, output power of 1.5 W, and 12 J/cm² energy density on the first day after the SRP.

The outcome of periodontal therapy is conventionally based on an assessment of clinical parameters. Laser biostimulation brings about a marginal improvement of such clinical parameters. To understand the dynamics at the molecular level, IL-1β was estimated along with the clinical parameters.

The present investigation aimed at demonstrating the beneficial effects of a combined therapy, LLLT plus nonsurgical treatment, in the improvement of periodontal indexes and in the reduction of salivary inflammatory biomarkers, particularly interleukin 1β in generalized stage II grade B periodontitis patients.

The results of our study show that there is a statistically significant improvement in clinical parameters after non-surgical periodontal treatment in each group. The beneficial effects of scaling and root planning combined with personal plaque control in the treatment of periodontitis have been well documented [34-36]. These include reduction of clinical inflammation, microbial shifts to a less pathogenic subgingival flora, reduction of PPD and gain of clinical attachment.

In our study, the PI score showed a statistically significant reduction from baseline to after 21 days in both groups. Also, the PI score was reduced more in case group than control group. Michelli in 2011 [37], performed a study, where PI score demonstrated a significant decrease. This result is in accordance with the present study. The decrease in PI score was greater on the case group, which agrees with the study done by Qadri et al [38]. It is questionable whether this is because of a reduction in degree of gingival inflammation or the laser irradiation per second. However, our results are contrary to the

study by Lui et al. [39] which showed no significant difference in PI scores between test and control.

Our study showed a significant difference in GI scores of case and control group at baseline and after 21 days suggesting beneficial effects of LLLT on reducing inflammation. Previous studies on the adjunct use of LLLT in treating chronic periodontitis are few and have discrete results. In accordance with our results, Qadri et al. [38] reported statistically significant decrease in GI levels for the LLLT group compared with SRP alone.

Similarly, on inter-group comparison, the mean GI difference from baseline to 21 days, the case group showed more reduction in GI score. This result was in accordance with the study done by Kreisler et al. [40] where GI scores ($p < 0.001$) decreased from baseline to 3 months in LLLT and control groups. This showed that adjunctive use of LLLT is beneficial than SRP alone in the reduction of GI and PI scores.

Anti-inflammatory effects and edema reduction can partially be explained by improved circulation or stimulation immediately after laser therapy. The increase in blood flow caused by low level laser application is not the result of a heat effect, but of an increase and normalization of homeostasis in tissue metabolism [41-44]. Frenzen and Koort et al. [45-47], suggest that anti-inflammatory effects laser treatment increases microcirculation, causing vasoconstriction of blood vessels, which directly affects the inflammatory process. It has been previously shown that lipopolysaccharides from periodontal pathogenic bacteria can penetrate into gingival tissue and stimulate production of Prostaglandin PGE₂ [48]. Clinical observations suggest that low level laser may favorably influence growth factors, prostaglandin E₂ production, acute phase protein and cellular function [49,50].

The comparison of mean PPD in laser and control sites in our current study, showed statistically significant difference from baseline to 21 days. Case group demonstrated a higher reduction in PPD values when compared to the control group. One probable reason could be Laser irradiation reduces PGE₂ [51] and stimulates cellular ATP [42]. Combining laser therapy with conventional procedures is in fact achieved a more effective decontamination of the pocket, with also a recolonization slower than sites treated only mechanically; some authors attribute this phenomenon to clot formation in the pocket, that would act as a seal to it.

This result was in accordance with the study done by Kreisler et al. [40] where PD decreased from baseline to 3 months in both groups. Michelli in 2011 [37] observed a similar change at 6 weeks.

On the comparison of mean PPD in laser and control sites, there was statistically significant difference observed from baseline to 1st month and 1st month to 3rd month in the study by Sudhakar et al. [52]. He also found that irradiation with low-level laser was better than SRP alone. Its effect was greatest on the GI and PPD. Our results are contrary to the study by Lui et al. [39] which showed no significant difference in reduction of PPD after LLLT. The reduction in PPD might be attributed to the improved maintenance of oral hygiene. This could be possible because of the better comfort and less pain on the laser side.

There was a statistically significant decrease in CAL in our current study from baseline to 21 days in both the groups. LLLT resulted in a statistically significant decrease in CAL levels after 21 days. This result was in accordance with the studies done by Michelli in 2011 [37], with the difference in CAL being, -1.7 mm for the test and -2.9 mm for the

control group, six weeks postoperatively ($p < 0.001$). Similar findings were reported by Balasubramaniam et al. in 2014 [53], who observed the same, with decrease in CAL two months postoperatively. Another parallel-designed study by Aykol et al. [54] reported that LLLT results in a statistically significant decrease in PPD and CAL levels in the 1st, 3rd, and 6th months.

In this study, the mean reduction of IL-1 β was highly significant in case group as compared to control group. There was statistical significance from the baseline to 21 days. Our results are in accordance with the studies by Lui et al. [39] who showed significant differences between the case group and control group.

However, our results are contrary to study by Qadri et al. [38] who concluded that a difference between SRP and SRP with laser with respect to cytokines IL-1 β level showed no significant differences. Safavi et al. [55] evaluated the effect of low level He-Ne laser on gene expression of Interferon γ (IFN- γ), IL-1 β and Growth Factors (PDGF, TGF- β , bFGF) to provide an overview of the effect of low level He-Ne laser on their interactive role in the inflammation process. The findings suggest that low-level He-Ne laser irradiation decreases the amount of inflammation and accelerates the wound healing process by changing the expression of genes responsible for the production of inflammatory cytokines.

Within its limits, the results of the present study demonstrate that the adjunct use of LLLT promotes clinical parameters. In addition, LLLT has the ability to promote IL-1 β levels modulation. With respect to clinical outcomes, the present study demonstrated improvements in terms of PI, GI, PPD and CAL in patients treated with the LLLT + SRP compared to patients treated with SRP alone. The IL-1 β levels are decreased more in case group than control group, suggesting LLLT might have an advantage in controlling periodontal inflammation during early healing period.

Limitations of the present study include small sample size, lack of established protocol of adjunctive LLLT with SRP. Long-term studies with more sample size and longer follow-up are required to determine the efficacy of LLLT in the treatment of chronic periodontitis and its effect on inflammatory cytokines.

Conclusion

The LLLT group showed more reduction in all parameters than the control group. The results showed that the patient with additional laser therapy showed greater reduction in IL-1 β levels than the SRP alone.

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