Asian Pac. J. Health Sci., **2018; 5(3):203-211** **e-ISSN: 2349-0659, p-ISSN: 2350-0964**

Document heading **doi: 10.21276/apjhs.2018.5.3.28** **Original Article**

Evaluation of interleukin-6 in non surgical periodontal therapy with & without laser: a clinico-biochemical study

Pankaj Kumar Sinha*, Varun Dahiya, Pradeep Shukla, Gaurav Malhotra, Prerna Kataria, Chander Shekhar Joshi

Divya Jyoti College of dental science and Research, Modinagar GZB, UP, India

Received: 01-08-2018 / Revised: 02-09-2018 / Accepted: 20-09-2018

ABSTRACT

The aim of this clinical trial was to examine the clinical and biochemical efficacy of diode laser as an adjunct to scaling and root planing (SRP). 30 subjects were selected on basis of inclusion criteria and were categorized into two groups. After selection of subjects, 15 patients were included under control group and 15 patients were included under test group randomly. Plaque index, gingival index, bleeding on probing, probing depth, and clinical attachment level were measured at baseline, 1 month and 3 months after treatment. Interleukin-6 (IL-6), component of gingival crevicular fluid were analyzed by enzyme-linked immunosorbent assay. Better outcome was observed in test group compared to control group in full-mouth clinical parameters. The total amount of IL-6 value was decreased ($p<0.05$) after treatment in both the test as well as the control groups ($p<0.05$). The Diode laser provided significant improvements in clinical parameters which showed its positive effect on non surgical periodontal therapy

Keywords: Chronic periodontitis, Gingival crevicular fluid, Interleukin-6, Diode laser

Introduction

Chronic periodontitis (CP) is an infectious disease caused by periodontal pathogens resulting in inflammation, attachment loss, bone resorption, and characterized by pocket formation and/or gingival recession[1].The essential objective of periodontal treatment is to decrease or eliminate the responsible periopathogens, by means of removing bacterial deposits from the tooth surface. Conventional mechanical debridement (i.e SRP) is considered to be the gold standard for inflammatory periodontal disease treatment[2].Mechanical therapy alone may fail to eliminate pathogenic bacterial niches in the soft tissue and in areas that are inaccessible to periodontal instruments, such as deep pockets, furcation areas, and root depressions[3]. In deep pockets, periodontopathic bacteria can persist after SRP. This situation can lead to the recolonization of treated sites.

**Correspondence*

Pankaj Kumar Sinha

Divya Jyoti College of dental science and Research, Modinagar GZB, UP, India.

Moreover, the use of SRP in the treatment of CP may result in a moderate and temporary shift in the composition of the microbial flora particularly in deep pockets where periodontopathic bacteria can persist after SRP. This situation can lead to the recolonization of treated sites[4] Limitations in conventional non-surgical periodontal therapy have led to the exploration of other treatment options to improve clinical outcomes. Lasers of various wavelengths have been proposed as an alternative treatments for non-surgical periodontal therapy[5].The bactericidal and detoxifying effects of the diode laser during non-surgical periodontal treatment have been documented. Lasers can be effective on oral microbial species and "disinfect" the periodontal environment. Lasers may also modulate the oral inflammatory response. Diode laser (780 nm) has been shown to inhibit IL-6 in lipopolysaccharide stimulated macrophages[1]

The biochemical analysis of GCF offers a non invasive approach for assessing the host response in individuals with periodontitis[6] Spontaneous production of IL-6 has been found in mononuclear cells isolated from inflamed gingival tissues of patients with Periodontitis. IL-6 m-RNA expressing cells are observed in both lymphocytes and non- lymphoid cells in inflamed gingival tissues, but not in healthy gingival tissue. IL-6

is produced in inflamed gingival tissues and is involved in the initiation or prognosis of periodontitis[7]

IL-6 is a pleiotropic cytokine with a broad range of humoral and cellular immune effects, relating to inflammation, host defense and tissue injury promoting the osteoclast differentiation from progenitor cells.[8] Thus the aim of the study was to assess the efficacy of diode laser as an adjunct to the non-surgical periodontal therapy (scaling and root planing) for the treatment of chronic periodontitis and to compare the efffectiveness of SRP+laser and with SRP alone and check the biochemical analysis of IL6 present in both the groups in patients of chronic periodontitis.

Material and method

Participants and study design

Patients with in age range of 18-55 years of both the sexes were selected from the Out Patient Department of Periodontology, after the approval of the ethical committee of the DJ College of Dental Sciences and Research, Modinagar, Uttar Pradesh. Each patient was given a detailed verbal and written description of the study. They were required to sign an informed consent form prior to commencement of the study. Patients who were diagnosed with chronic periodontitis in the Oral Diagnosis and Radiology Department were randomly assigned in 30 subjects and divided into 2 groups. After subject selection 15 patients were randomly assigned to first group that is the contol group, and remaining 15 patients were assigned as second group. The patients will be categorized into 2 groups- GROUP 1: patients with chronic generalized periodontitis receiving therapy using diode laser and SRP. GROUP 2: patients: receiving therapy using only SRP. Patients were included both male and female from the range of 18-55 years and presence of chronic periodontitis (at least 7 teeth with periodontal pocket deeper than 4 mm.). Exclusion criteria were periodontal treatment received for the last 1 year; systemic diseases that could influence the outcome of the therapy, pregnancy, smoking, History of medication in previous 5 months.

Clinical parameters

This would be followed by evaluation on the basis of periodontal parameters and biochemical study. Clinical parameters include plaque index, gingival index, gingival bleeding index, probing depth and Clinical attachment level. Follow up would be done at baseline, 1 month and 3 months. Biochemical sampling will be performed for the evaluation of IL-6 levels using ELISA kit at baseline, 1 month and 3 month.

Clinical procedure

In control group, at baseline Patient came to the department of periodontology and implantology and had undergone supragingival scaling, and GCF sample was taken. This was followed by a comprehensive phase I therapy which included patient education and motivation, plaque control, scaling and root planing. The patients were given oral hygiene instructions and then advised of meticulous home care including mechanical plaque control and patient were recalled after 1 month and 3 month. In test group One week after baseline, subgingival scaling and root planing under local anesthesia was performed in a single appointment for each patient in all groups using an ultrasonic scaler and hand instruments. SRP and diode laser therapy was performed in the same visit.

Clinical measurements

On the first visit, detailed case history including clinical parameters [gingival index, plaque index, gingival bleeding index, probing pocket depth, clinical attachment level (with the help of UNC-15 probe to the nearest millimeter), GCF samples were taken and Interleukin-6 levels were recorded. Scaling and root planning were performed in both the groups. No antibiotics or anti-plaque and anti-inflammatory agents were prescribed after treatment. This was followed by a comprehensive phase I therapy which included patient education and motivation, plaque control, scaling and root planning.One month and three month later these measurements (GI, PI, GBI, PD, CAL) and GCF sampling were repeated and Interleukin-6 were assessed by means of commercial Enzyme linked immunosorbent assay.

Laser procedure

In test group, 1 week before treatment, supragingival scaling was performed for each patient in using hand instruments (Gracey curettes, Hu-Friedy, Chicago, IL, USA) and ultrasonic devices. Full-mouth subgingival scaling and root planing under local anesthesia was performed in a single appointment for each patient in all groups using an ultrasonic scaler and hand instruments. SRP and diode laser therapy was performed in the same visit. All treatments were performed under local anesthesia. Laser treatment was performed by using a 980 nm indium– gallium–aluminum–phosphate diode laser. The periodontal pocket was set at 1.5 W with a pulse interval of 20 ms and pulse length of 20 ms delivering 20 s/ cm2 and 15 J/cm2 of energy. Irradiation was accomplished with a 300 μm fiber optic delivery system. The fibre was inserted into the periodontal pocket base in parallel alignment with the root surface, the device was activated, and the fiber was slowly moved from apical to coronal in a sweeping motion during the laser light emission. This was done mesially to distally at the buccal aspect for 10 s and distally to mesially at the

lingual aspect for 10 s reaching a total of 20 s for each tooth. The periodontal pocket was irrigated with saline solution after each session of irradiation. In order to control for the same conditions, pockets were also rinsed with saline after SRP in the control group. Both patients and the operator wore protective glasses during laser application were performed.

Collection of GCF

Each GCF sample was collected for 15 seconds by calibrated volumetric microcapillary pipettes which were inserted in the gingival sulcus immediately after the area has been isolated with cotton rolls, air dried, and supragingival plaque has been removed with a sterile Gracey curette. The pipettes with GCF were coded for patient details and were placed in polypropylene tube and immediately transferred to plastic vial and stored at -20^0 C prior to transportation to the lab. Polypropylene tube were immediately transferred to plastic vial then stored at -70°C till the time of assay. Microcapillary pipettes contaminated with blood and saliva were excluded from the sampled group. GCF sample were collected again after one month and 3 month respectively.

GCF analysis

Biochemical analysis of GCF samples was done to estimate the level of Interleukin-6.

IL-6 concentration was measured according to manufacturer's instructions using commercially available sensitive ELISA kit (Boster Biological Technology co.LTD)

Principle of the assay

Boster's human IL-6ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IL-6 specific-specific polyclonal antibodies has been precoated onto 96-well plates. The human specific detection monoclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human IL-6 amount of sample captured in plate.

Results

All subjects completed the entire study. Healing was uneventful in all cases. No adverse effects, such as discomfort, burning sensation, dentin hypersensitivity, or pain related to the laser irradiation were reported by any of the subjects. The baseline demographic data of the male and female volunteers are given per group in Table 1.

Clinical assessments

The results of the whole-mouth clinical measurements (mean±SD) between baseline and time points in test and control groups are displayed in Figs. 1 and 2. In both groups, all clinical parameters showed statistically significant reductions at all time points compared to baseline ($p<0.05$). The mean PD at baseline was 5.20±0.36 in the laser group and 5.50±0.54 in the control group. After treatment, these values became 3.42±0.32and 3.72.±0.41 at 1 month, 2.97±0.26 and 3.13±0.24 at 3 months, respectively. The mean CAL at baseline was 5.20±0.45 in the laser group and5.43±0.52 in the control group. After treatment, these values decreased to3.83±0.33 and 3.95±0.28 at 1 month, 2.03±0.30 and 2.33.±0.47 months, respectively. The reduction in PD and CAL was significantly higher for the test group ($p<0.05$). The mean PI at baseline was 1.96±0.11 in the laser group and 2.04±0.11 in the control group. After treatment, these values were 1.20±0.08 and 1.44±0.08 at 1 month, 1.05±0.11 and 1.26±0.08 at 3 months, respectively. The mean GI at baseline was1.92±0.12 in the laser group and 1.91±0.11 in the control group. After treatment, these values were reduced to1.19±0.08 and 1.31±0.07 at 1 month, 0.99±0.08 and1.15±0.09 at 3 months, respectively. The mean BOP percentage at baseline was 82.24±4.87 in the laser group and 84.40±5.12 in the control group. After treatment, these percentages changed into 17.55±1.77 and 29.77±1.95 at 1 month, 11.53±3.50 and 17.60±2.06 at 3 months, respectively.

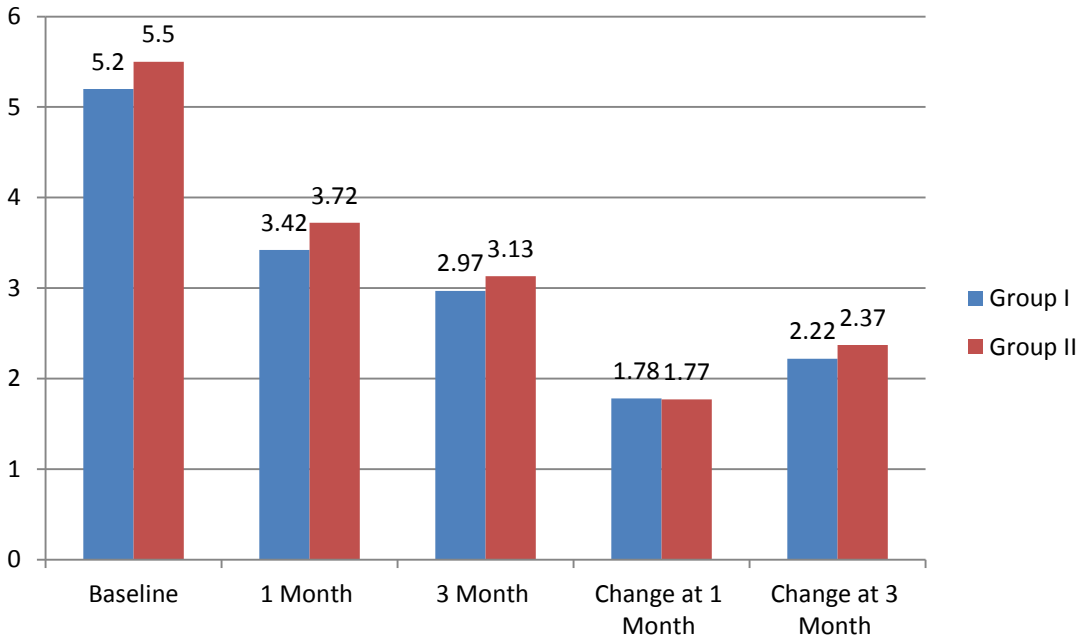

Fig 1:shows that Scaling and Root-Planing with laser are more efficient in reducing Probing depth.

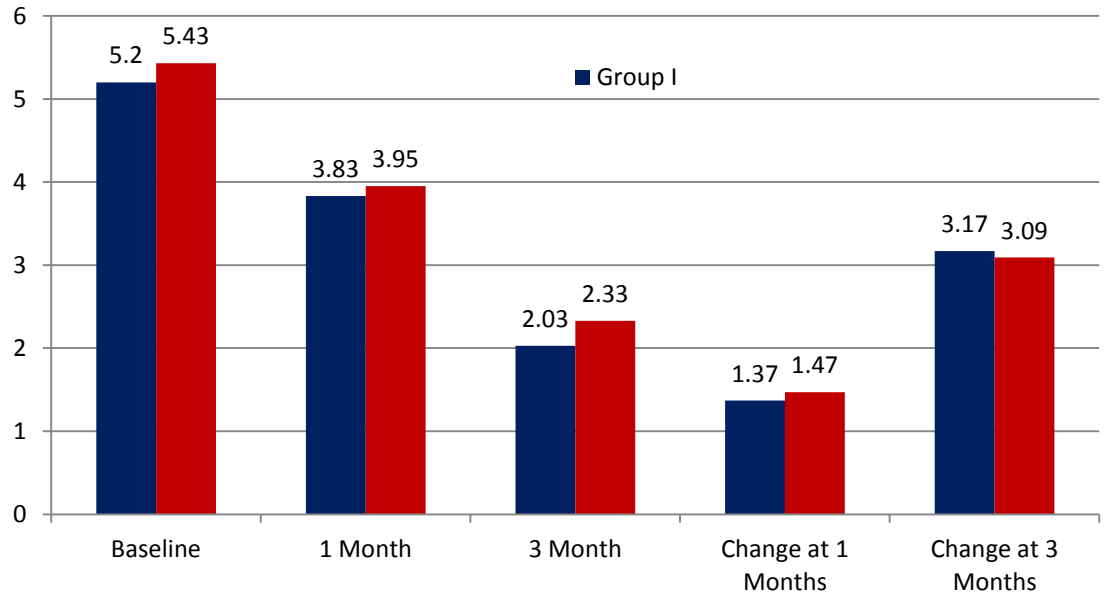

Fig 2:shows that Scaling and Root-Planing with laser are more efficient in gaining Clinical attachment level.

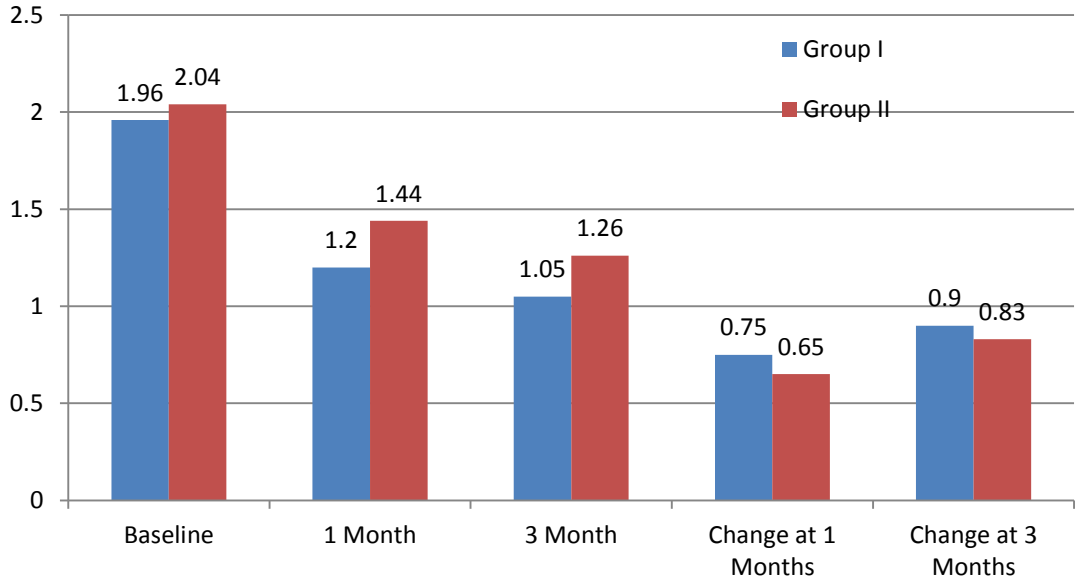

Fig 3:shows that Scaling and Root-Planing with Laser are more efficient in reducing Plaque index.

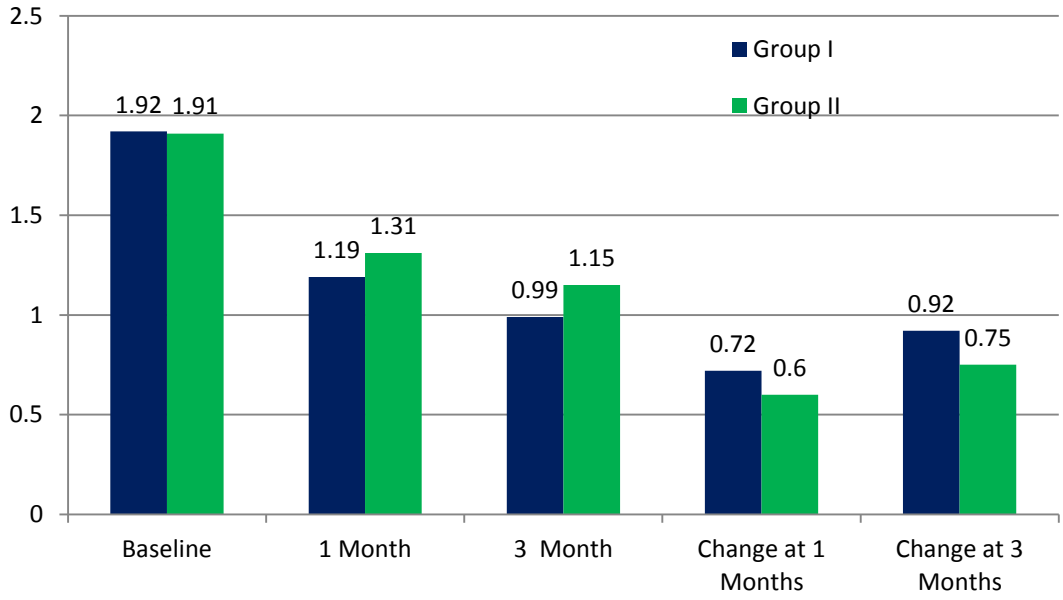

Fig 4:shows that Scaling and Root-Planning with laser are more efficient in reducing gingival index.

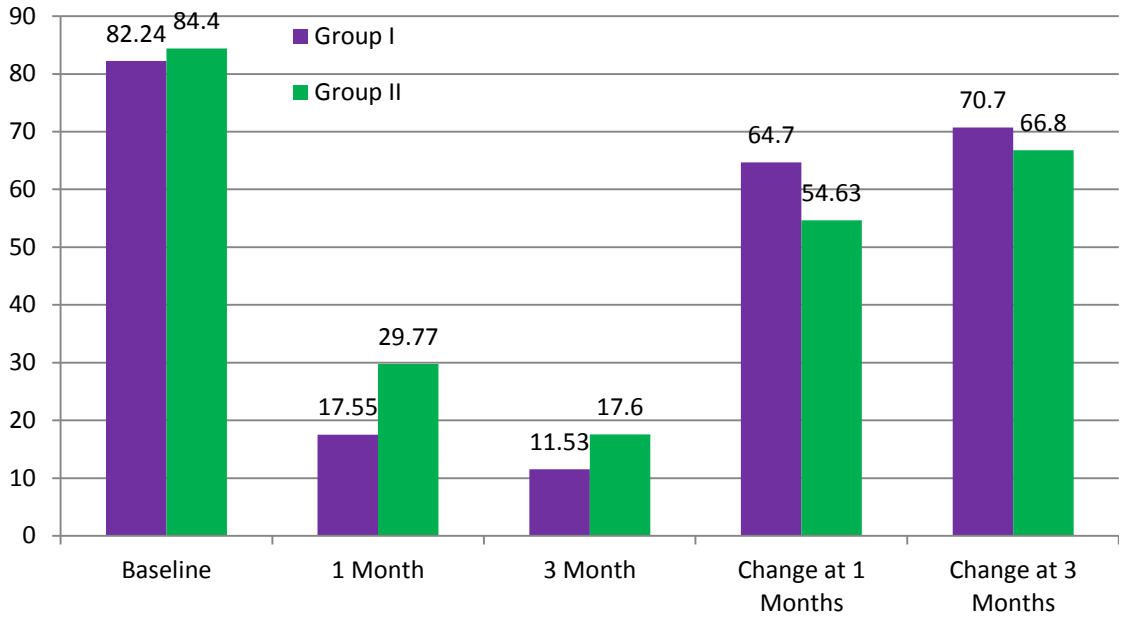

Fig 5:shows that Scaling and Root-Planning with Laser are more efficient in reducing gingival bleeding index.

Changes in cytokine levels in GCF

IL-6 were assessed in GCF as marker of cytokine-mediated inflammatory response. At baseline, no significant differences were found between test and control groups. The mean IL-6 level at baseline was 21.08±4.37 pg/30 s in the laser group and 16.79±3.86 pg/30 s in the control group. After treatment, these values were 8.87±1.77and7.63±1.95 pg/30 s at 1 month, 3.99±1.16 and 4.84±1.96 pg/30 s at 3 months, respectively. After treatment, levels of IL-6 significantly reduced at 1 month ($p<0.05$), stayed low at 3 months ($p<0.05$). There were no statistically significant differences between groups.

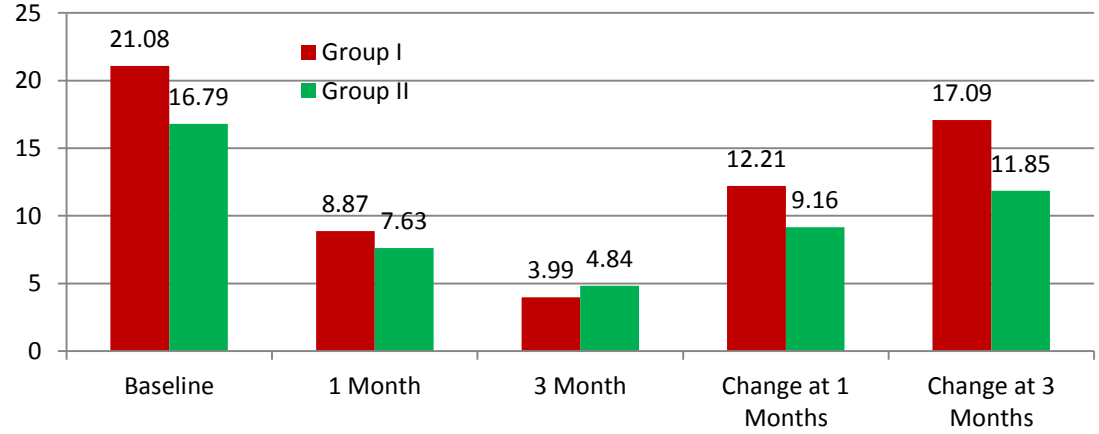

Fig 6:shows that Scaling and Root-Planing with laser are more efficient in reducing Interleukin-6.

Discussion

The essential objective of periodontal treatment is to decrease or eliminate the responsible periopathogens by means of removing bacterial deposit from the tooth surface[9]The cytokine interleukin-6 (IL-6) is a major mediator of the host response to tissue injury and infection. IL-6 plays a major role in B-cell differentiation in the immune system. It is also accepted that this cytokine has multiple biological activities, such as the enhancement of cell proliferation and the acceleration of bone resorption[10] It is produced by activated macrophages, lymphocytes, and adipose tissue**,** and pivotal cytokine involved in the regulation of host response to infection and tissue injury. It is synthesized by different cells, for instance, monocytes, fibroblasts, osteoblasts and vascular endothelial cells in response to inflammatory challenges[11]

The diode laser is a semiconductor laser that generally includes a combination of gallium (Ga), arsenide (Ar), and other elements such as aluminum (Al) and indium (In) to convert electrical energy into light energy. The wavelength range is about 800–980 nm. The diode laser does not interact with dental hard tissues making it convenient for soft tissue operations; cutting and coagulating gingiva and oral mucosa, soft tissue curettage, or sulcular debridement. **Moritz et al.** have demonstrated significant bacterial decrease and reduction of inflammation when using a diode laser of 805 nm wavelength combined with SRP[1]Diode laser is used in the current study because of its lower cost, portability, perceived ease of use. It can be applied to treat soft tissues due to its bactericidal and detoxification effects, but in treatment of chronic periodontitis, it may be useful as an adjunct to SRP because it is unable to remove calculus and bacterial deposit[2]

The goal of periodontal therapy is to eliminate the supra and subgingival deposits from the root surfaces in order to prevent disease initiation and progression. Mechanical treatment for removal deposits involves supra and subgingival scaling and root planing (SRP) with substantial clinical efficacy. Mediators of inflammation promote extracellular matrix destruction in periodontium and stimulate bone resorption. Successful treatment of the periodontal diseases and the stability of the outcomes have to take these issues into account while new approaches and modalities should be considered. To this end, lasers could provide a good option. Lasers can be effective on oral microbial species and "disinfect" the periodontal environment. Lasers may also modulate the oral inflammatory response[1]

We found that both treatment modalities resulted in significant improvements in all clinical parameters after periodontal treatment. clinical reductions were greater in the test group compared to the control group. **Kreisler et al.** suggested that higher reduction in PD was probably related to the de-epithelization of the periodontal pockets leading to an enhanced connective tissue attachment. These studies confirmed that laser application provided de-epithelization and resulted in a reduced epithelial migration as well as an increased connective tissue formation. Our results supported this notion and suggested that the penetration of the laser may not evoke a substantial inflammatory difference compared to the mechanical treatment alone while resulting in an enhanced clinical healing. Our clinical results are in agreement with those obtained by **Kreisler et al.** who demonstrated differences between the groups for PD and CAL[12] In addition, our data also support findings by **Qadri et al[13]**who observed differences for laser group in PD, PI, and GI compared to conventional treatment. Likewise, **Moritz et al**. and **Lui et al.** who both demonstrated superior results for laser group in the terms of PD and BOP[14,15]According to **Dukic W** et al found that the results were significant PD gain in moderate pockets during the baseline to 18-week and 6 to 18 week periods, whereas no difference was found between Laser group and Control group in the remaining clinical parameters and indicated that when compared to SRP alone, multiple adjunctive applications of a 980-nm diode laser with SRP showed PD improvements only in moderate periodontal pockets[16]According to **Kamma et al**. demonstrated that the effect of 980-nm diode-laser-assisted treatment with SRP was superior to SRP or laser therapy alone for clinical (PD, CAL) and microbial parameters in patients with AgP[17]According to **Aykol G** showed that low level laser therapy have more improvement in sulcus bleeding index, clinical attachment level and probing depth compared to control group and showed better result[18]Contrary to our findings, **Yilmaz et al.** and **Micheli D et al.** suggested that diode laser did not result in any additional clinical benefit when compared with conventional treatment. These controversial reports might be the result of different wavelengths, application power densities (685 nm at 30 mW), and application time[19,20]In a recent study, **Gokhale et al**. Reported that diode laser application (980 nm, 2.5 W) as an adjunct to periodontal flap surgery did not improve clinical parameters but its bactericidal effect

was clearly evident by greater reduction of colony forming units of obligate anaerobes[21]

Caruso U showed that the additional treatment with diode laser may lead to a slight improvement of clinical parameters (PPD, CAL, GI, PI) after 4, 8,12 weeks, whereas the BOP indices were reduced more in the test group than in the control group. The use of diode laser as adjunctive therapy to scaling and root planing provided no additional clinical and microbiological benefit over conventional mechanical treatment[22]

Nguyen NT showed that by using the diode laser, intragroup analyses showed that sites treated with SRP + L or SRP alone had a statistically significant reduction of PD, gain in CAL, and reduction of BOP at 3 months after treatment but in intergroup analyses showed no statistically significant difference in the mean change of the clinical parameters between the SRP + L and SRP alone groups. SRP + L did not enhance clinical outcomes compared to SRP alone in the treatment of inflamed sites with 5 mm PD it is confirmed[5]Contrary to our findings, **Lui** suggested that low power laser did not result in any additional clinical benefit[15] The differences between these studies may be due to laser wavelength (635–830 versus 940 nm in our study), energy density (4.5– 8.75 versus 15 J/cm2 in our study) and study design (quadrants instead of the whole mouth).The intergroup comparison of percentage reduction in the IL6 levels at 1 month and 3 months (from Baseline) between the Group I and Group II. There was significantly higher reduction in the IL6 levels at 1 month and 3 month time interval for the Group I as compared to Group II. Further the intra group comparison of the IL6 levels between the three time intervals was statistically significant for Both Group I and Group II (p=0.001) In this study, GCF IL-6 levels decreased, significantly in both groups after treatment compared to baseline. **Saglam M** supported in favour of all these clinical parameter which are present in this study[1]The current study is designed to test if the diode laser will enhance the treatment outcomes of the SRP in periodontitis and this will be accompanied by the changes in the levels of mediators of inflammation and tissue turnover. More vigorous work needs to be done to confirm the usefulness of the diode laser with SRP which would greatly facilitate the treatment of periodontal diseases.

Conclusion

Use of the diode laser as an adjunct to scaling and root planning produces significant improvement in the whole-mouth clinical parameters compared to conventional treatment. When biochemical parameters were compared between groups, laser with SRP group has been shown to be more effective than the mechanical treatment in reducing the GCF Interleukin-6 levels. Therefore, within the limits of this study it can be concluded that diode laser as an adjunct to the non-surgical periodontal therapy is effective for the treatment of chronic periodontitis. The limitation to this study could be small sample size and observation period. Hence, more rigorous work needs to be done to confirm the usefulness of the diode laser with SRP which would greatly facilitate the treatment of periodontal diseases.

References

1. Saglam M, Kantarci A, Dundar N, Seema S, Hakki. Clinical and biochemical effects of diode laser as an adjunct to nonsurgical treatment of periodontitis: A randomized, controlled clinical trial. Lasers Med Sci 2014; 29: 37–46.
2. Birang R et al. Effect of Nonsurgical Periodontal Treatment Combined With Diode Laser or Photodynamic Therapy on Chronic Periodontitis: A Randomized Controlled Split-Mouth Clinical Trial Journal of Lasers in Medical Sciences 2015; 6(3):2
3. Paster BJ, Dewhirst FE. Molecular microbial diagnosis. Periodontol 2000 .2009; 51:38–44
4. Sbordone L, Ramaglia L, Gulleta E, Iacono Y. Recolonization of the subgingival microflora after scaling and root planning in human periodontitis. J Periodontol 1990; 61:579–584
5. Nguyen NT et al. Adjunctive Non-Surgical Therapy of Inflamed Periodontal Pockets During Maintenance Therapy Using Diode Laser: A Randomized Clinical Trial J Periodontol 2015; 86:1133-1140.
6. Giannopoulou C et al. Effect of Photodynamic Therapy, Diode Laser, and Deep Scaling on Cytokine and Acute-Phase Protein Levels in Gingival Crevicular Fluid of Residual Periodontal Pockets J Periodontol 2012; 83:1018-1027.
7. Guillot JL, Pollock SM, and Johnson RB Gingival Interleukin-6 Concentration Following Phase I Therapy / Periodontol 1995; 66:667- 672.
8. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med 1998; 128(2):127–137
9. Zhang L, Henson BS, Camargo PM & Wong DT. The clinical value of salivary biomarkers for

periodontal disease. 2009; Periodontology 2000; 51: 25-37.

10. Takahashi K. Assessment of Interleukin-6 in the Pathogenesis of Periodontal Disease J Periodontol 1994; 65:147-153.

11. Alwan AH et al. Determination of Interleukin-1β (IL-1 β) and Interleukin-6(IL6) in Gingival Crevicular Fluid in Patients with Chronic Periodontitis IOSR Journal of Dental and Medical Sciences. 2015; 11(14): 81-90.

12. Kreisler M et al. Effect of Diode Laser Irradiation on the Attachment Rate of Periodontal Ligament Cells: An In Vitro Study. J Periodontol 2001; 72:1312-1317.

13. Qadri T, Miranda L, Tuner J, Gustafsson A The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation. J Clin 2005; 32(7): 714-719.

14. Moritz A et al. Treatment of Periodontal Pockets with A Diode Laser. Laser in Surgery and Medicine 1998; 22:302–311.

15. Lui J, Corbet EF, Jin L Combined photodynamic and low-level laser therapies as an adjunct to nonsurgical treatment of chronic periodontitis J PERIODONTAL RES 2011; 82(3): 48-88.

16. Dukic W , Bago I, Aurer A, Rogulji M. Clinical Effectiveness of Diode Laser Therapy as an Adjunct to Non-Surgical Periodontal Treatment: A Randomized Clinical Study J Periodontol 2013; 84:1111-1117.

17. Kamma JJ et al. The effect of diode laser treatment on aggressive periodontitis: evaluation of microbial and clinical parameters. Photomed laser surgery. 2009;3(4):23

18. Akyol G et al.The effect of low-level laser therapy as an adjunct to non-surgical periodontal treatment J Periodontol 2011; 82(3): 148-188.

19. Micheli D, de Andrade AK, Alves VT, Seto M, Pannuti CM,Cai S Efficacy of high intensity diode laser as an adjunct tonon-surgical periodontal treatment: a randomized controlled trial. Lasers Med Sci 2011; 26(1):43–48.

20. Yilmaz S, Kuru B, Kuru L, Noyan U, Argun D, Kadir T Effect of gallium arsenide diode laser on human periodontal disease: a microbiological and clinical study. Lasers Surg Med2002; 30 (1):60–66(10):598–603.\

21. Gokhale SR, Padhye AM, Byakod G, Jain SA, Padbidri V,Shivaswamy S. A comparative evaluation of the efficacy of diode laser as an adjunct to mechanical debridement versus conventional mechanical debridement in periodontal flap surgery: a clinical and microbiological study. Photomedicine Laser Surg . 2012:98

22. Caruso Uet al. Use of diode laser 980 nm as adjunctive therapy in the treatment of chronic periodontitis. A randomized controlled clinical trial New Microbiological 2008; 3:513-518.

Conflict of Interest: None
Source of Support: Nil