

How to Cite:

Kubal, A., Rao, P., Rathod, V., Parikh, A., & Srivastava, M. (2022). Comparative evaluation of effect of diode laser as an adjunct to scaling and root planing and scaling and root planing alone on clinical parameters and gingival crevicular fluid interleukin-10 level in chronic periodontitis. *International Journal of Health Sciences*, 6(S6), 7316–7330.
<https://doi.org/10.53730/ijhs.v6nS6.11377>

Comparative evaluation of effect of diode laser as an adjunct to scaling and root planing and scaling and root planing alone on clinical parameters and gingival crevicular fluid interleukin-10 level in chronic periodontitis

Dr. Aishwarya Kubal

Post Graduate, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, India

*Corresponding author email: kubalaishwarya@gmail.com

ORCID: 0000-0002-3772-0365

Dr. Prajakta Rao

Professor, Department of Periodontology Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, India

Email: drprajaktarao22@gmail.com

ORCID: 0000-0002-4611-4711

Dr. Varsha Rathod

Professor and Head of the Department, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, India

Email: drvarshavora92@gmail.com

ORCID: 0000-0002-6945-2086

Dr. Antara Parikh

Post Graduate, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, India

Email: antara1231.ap@gmail.com

ORCID: 0000-0003-3409-9048

Dr. Manvi Srivastava

Post Graduate, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, India

Email: manvisrivastava25@gmail.com

ORCID: 0000-0002-2553-7547

Abstract--The aim of the study was to compare and evaluate the effect of diode laser as an adjunct to scaling and root planing and

scaling and root planing alone on clinical parameters and Gingival Crevicular Fluid (GCF) interleukin-10 level in chronic periodontitis. Ten chronic periodontitis patients were selected. As it was a split mouth study, two sites with deepest pocket one in each quadrant were randomly assigned for scaling and root planing (SRP) and SRP+ diode laser treatment. Plaque index, gingival index, probing depth, and clinical attachment level were measured at baseline and at 3 months after treatment. The GCF levels of interleukin-10 (IL-10) were analyzed by enzyme-linked immunosorbent assay. Test group showed significantly better outcome compared to the control group in all clinical parameters (PI, GI, PD and CAL). The total amount of IL-10 increased after treatment in both test and control groups. From baseline to three months, the SRP with Diode laser group demonstrated a substantial rise in IL-10 levels, demonstrating how lasers can improve nonsurgical periodontal treatment results. Thus, diode laser when used in adjunct to SRP, the levels of IL-10 which is an anti-inflammatory cytokine, significantly increased which indicated reduced inflammation of the tissues.

Keywords--SRP (scaling and root planing), IL-10 - Interlukin-10, GCF (gingival crevicular fluid), PD (probing depth), CAL (clinical attachment loss).

Introduction

Chronic periodontitis 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 Periodontal treatment aims to remove supragingival and subgingival deposits from root surfaces in order to prevent development and progression of periodontal disease.² Mechanical debridement therapy includes supra- and subgingival scaling and root planing (SRP), which has a high clinical effectiveness but sometimes may fail to completely remove pathogenic bacterial species. Nonsurgical periodontal therapy, one of the earliest indicated therapies for periodontal disease management, is defined as the removal of pathogenic biofilm with hand and ultrasonic devices. Although systematic and critical reviews have shown an improvement in clinical periodontal parameters, it has been demonstrated that conventional therapy via scaling and root planing (SRP) does not completely remove periodontal pathogens, especially in deep periodontal tissues, and can thus stimulate infective events.³

The aetiology of periodontal diseases is complex.⁴ More than 600 bacteria⁵, nonbacterial species⁶ and virulence factors such as lipopolysaccharide, fimbria, and capsule make up the microbial community surrounding the periodontium. Microbial components together elicit host responses, which are influenced by genetic and environmental variables.⁷ In inflamed periodontal tissues, a complex network of pro and anti-inflammatory cytokines is at work. Interleukin-10 (IL-10) is a multifunctional cytokine that is essential among others. An increase or decrease in IL-10 levels produced by genetic polymorphisms in the host is crucial for individual management of the balance between inflammatory, humoral, and

microbiological stresses. Treatment of periodontal diseases, as well as the stability of outcomes, must take these factors into account, while novel techniques and modalities should be examined. To this end, diode lasers could provide a good option.⁸

The use of lasers in dentistry has grown in popularity in recent years. Dental lasers are categorized according to the kind of gain medium used, wavelength, delivery system, emission modes, tissue absorption, and clinical applications. The diode laser is a semiconductor laser that converts electrical energy into light energy by using a combination of gallium (Ga), arsenide (Ar), and additional components such as aluminium (Al) and indium (In). The wavelength range is around 800–980 nm. As the diode laser does not interact with dental hard tissues, it is ideal for soft tissue procedures such as cutting and coagulating gingiva and oral mucosa, soft tissue curettage, and sulcular debridement.⁹ Moritz et al. have demonstrated significant bacterial decrease and reduction of inflammation when using a diode laser of 805 nm wavelength combined with SRP.¹⁰ Lasers can be effective on oral microbial species and “disinfect” the periodontal environment. Lasers may also modulate the oral inflammatory response.

There is limited evidence that diode laser can lower periodontal inflammation and matrix metalloproteinase-8 (MMP-8) levels while having no effect on gingival crevicular fluid (GCF) IL-1 levels. The Nd:YAG laser can also considerably lower IL-1 and MMP-8 levels.¹¹ Moreover, a recent systematic review by Slot et al.¹² reported that a diode laser when used as an adjunct to SRP offers only moderate benefits during the nonsurgical approach to periodontal disease. Hence, its clinical relevance remains a matter of debate IL10 is an immune-regulatory cytokine which can inhibit a series of pro-inflammatory signals. IL10 were found to be significantly lower in disease site as compared to control site.⁸ As there are no studies conducted in relation to levels of interleukin10 in GCF of chronic periodontitis patients treated with laser as an adjunct to SRP, this study was performed to compare and evaluate the clinical parameters and levels of Interlukin10 in GCF of patients with chronic periodontitis before and after SRP with diode laser and SRP alone.

Materials and Method

This study was a randomized and controlled 3-month clinical trial. A total of 10 patients with chronic periodontitis, who were referred for periodontal treatment at the Department of Periodontology at Bharati Vidyapeeth dental college and hospital in Navi Mumbai were selected based on inclusion and exclusion criteria and each patient was randomly allotted (coin toss method) 2 sites (Site A [control group] – scaling and root planing; Site B [test group] – Diode Laser debridement as an adjunct to scaling and root planing). Written informed consent was obtained from all patients. The study protocol was approved by the institutional scientific review committee and institutional ethical committee (IEC244122019). Exclusion criteria were patients with physical disability who were unable to maintain good oral hygiene, patients who used tobacco in any form, pregnant and lactating women, patients with known systemic disease or metabolic disorders and patients who had received any anti-inflammatory drugs and antibiotics in the

previous 6 months. The inclusion criteria were patients with age group 18 years and above of either sex having minimum 20 teeth and patients suffering from Chronic periodontitis with pocket probing depth (PPD) $>4\text{mm}$ and $<7\text{mm}$

Clinical procedure

All the patients were enrolled according to the inclusion and exclusion criteria. After enrolment of the patients, case history was recorded, clinical parameters including Plaque Index (Silness & Loe, 1964), Gingival Index (Loe & Silness, 1963), Probing depth and Clinical attachment level (PI, GI, PD & CAL) were recorded at baseline. As this was a split mouth study, one site with the deepest pocket was selected in each of the two assigned quadrants. The two sites in each quadrant were randomly assigned for scaling and root planing (SRP) and SRP+ laser treatment and stent was fabricated for selected probing depths using putty at baseline. Patient were recalled the next day for GCF collection.

GCF Collection and analysis

The two deepest pocket with probing depth of $\geq 4\text{ mm}$ and $\leq 7\text{mm}$ were chosen for GCF sampling from two assigned sites. GCF was collected using micropipette [MICROFILL (10-100ul)]. Cotton rolls were used to isolate and dry the area surrounding the deepest pocket. GCF was then collected from both the selected sites by lightly contacting the gingival margin with the micropipette at the entrance of the gingival sulcus. The volume of GCF collected in the micropipette was then ejected into an Eppendorf tube. The Eppendorf tube was then put in an airtight ice box until it was transferred to the laboratory on the same day for the Interlukin10 ELISA kit (ImmunoTag by GBioscience) test process. The biochemical analysis was carried out at DY Patil University's School of Biotechnology and Informatics in Navi Mumbai using commercially available kit in accordance with the manufacturer's instructions.

After GCF collection, on the same day periodontal therapy was done in both the quadrants, as per the allotment for site A and site B i.e SRP and SRP+ Diode laser respectively. All treatments were performed under local anesthesia. Diode laser treatment was performed by using a 940 nm indium- gallium- aluminum- phosphate diode laser (EpicX, Biolase, USA). Diode laser application in the pocket was done in contact mode with a thin flexible 300 μm fiber-optic delivery system and was set at 1.5 W with a pulse interval of 20 ms and pulse length of 20 ms delivering 20 s/ cm^2 and 15 J/ cm^2 of energy. The fiber 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. The same was repeated on 3rd and 7th day. Both patients and the operator wore protective glasses during laser application. After 3 months from the 1st day of treatment all the clinical parameters (PI, GI, PD and CAL) were assessed and GCF samples were collected again from the same sites for revaluation.

Statistical analysis

All the data was entered into MS Excel sheet (Microsoft Corp.) which was prepared and validated for the data form. Data was entered and checked for errors and discrepancies. Mean and standard deviation were calculated using IBM SPSS software (Version 20.0 Chicago IL, USA). Data for PD and interleukin10 levels in GCF was expressed as mean, median with standard deviation (SD) and standard error of mean (SEM), 95% confidence intervals (C.I.). The scores were compared between before and after periodontal therapy using paired t-test and student t-test. All analysis was done using two-sided tests at alpha 0.05 (95% confidence level). The data was interpreted at a confidence interval of 95% and the levels of significance were as follows: $p \geq 0.05$ – Not significant, $p < 0.05$ – Significant, $p \leq 0.001$ – Highly significant.

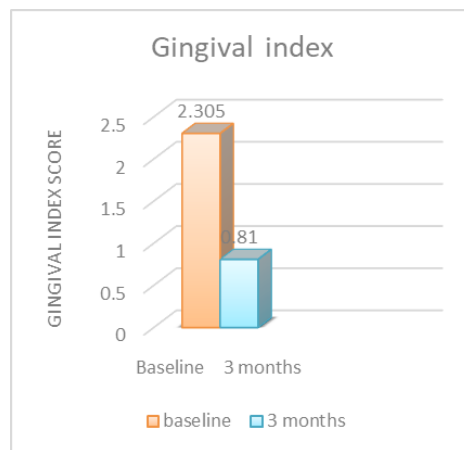
Results

All patients completed the entire study. In all cases, the healing process was uneventful. No patients reported any adverse effects such as discomfort, burning sensations, dentin hypersensitivity, or pain from laser irradiation.

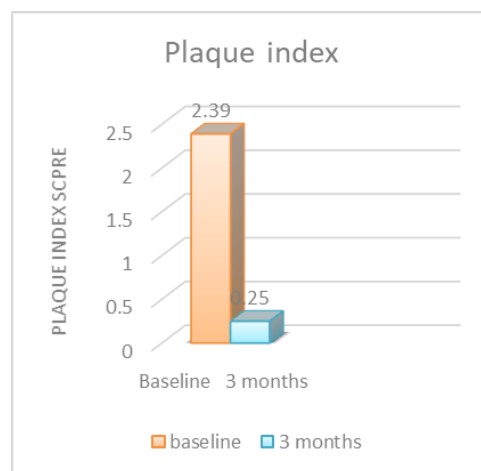
Clinical assessment

At baseline, the mean Gingival index score was 2.3050 ± 0.41730 (SD). After 3 months follow up, the values were found to have reduced to 0.8100 ± 0.19692 . When the baseline and 3 months follow up data was analyzed, these changes were statistically significant ($p < 0.001$) at 95% confidence interval (Graph 1). Significant reduction in gingival score was seen during the 3 months follow up with reduced gingival inflammation and bleeding. At baseline, the mean Plaque index scores were found to be 2.3900 ± 0.40125 (SD). After 3 months follow up, the values were decreased to 0.25000 ± 0.13540 . When the baseline and 3 months follow up data was analyzed, changes were statistically significant ($p < 0.001$) at 95% confidence interval. (Graph

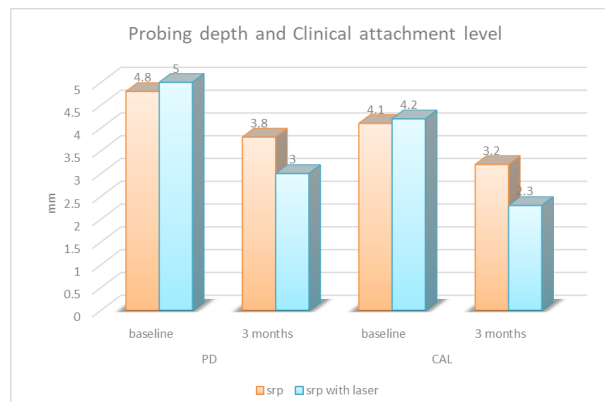
At baseline, the mean probing depth in SRP group were found to be 4.8000 ± 0.78881 (SD). After 3 months, the values were reduced to 3.8000 ± 0.78881 (SD). At baseline, the mean probing depth in SRP with diode laser group were found to be 5.0000 ± 0.81650 (SD) which reduced to 3.0000 ± 0.66667 (SD) at 3 months follow up (Graph 3). At baseline, the mean clinical attachment level in SRP group was 4.1000 ± 0.73786 (SD). After 3 months follow up, the values were reduced to 3.2000 ± 0.63246 (SD). At baseline, the mean clinical attachment loss in SRP with diode laser group were found to be 4.2000 ± 0.78881 (SD) which reduced to 2.3000 ± 0.82327 (SD) at 3 months follow up (Graph 3). Paired t test was used for intra group analysis and independent t test was used for intergroup analysis in both the groups. When compared to baseline values and after 3 months follow up, intragroup analysis in SRP with diode laser group revealed that the changes in probing depth and clinical attachment level gain were statistically significant ($p < 0.05$) at 95% confidence interval. Inter group analysis at 3 months follow up showed that the changes in probing depth were statistically significant ($p < 0.05$) at 95% confidence interval.



Graph 1. Mean gingival index scores at



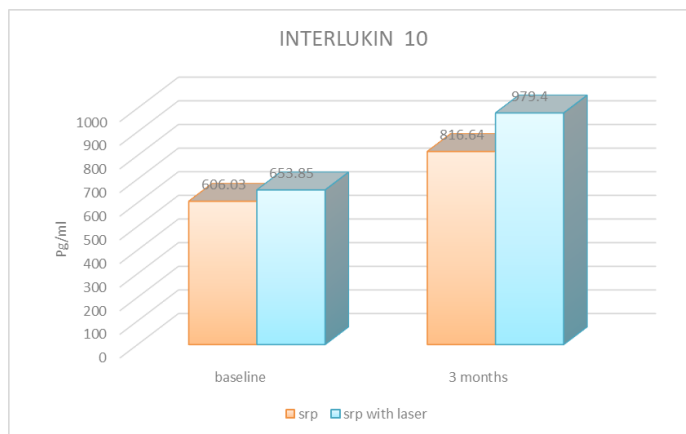
Graph 2. Mean plaque index scores at baseline and 3 months baseline and 3 months



Graph 3. Mean probing depth (PD) and Clinical attachment level (CAL) at baseline and 3 months

Changes in IL-10 levels in GCF

At baseline, the mean interleukin-10 levels in SRP group were found to be 606.03 ± 96.78644 (SD). After 3 months follow up, the values were found to have increased to 816.64 ± 83.61571 (SD). At baseline, the mean interleukin-10 levels in SRP with diode laser group were found to be 653.85 ± 159.31450 (SD). After 3 months, the values were found to have increased to 979.40 ± 101.92800 (SD). (Table 1 and Graph 4). In both the groups, the paired t test was used for intragroup analysis while the independent t test was utilized for intergroup analysis. When compared to baseline values and after 3 months, intragroup analysis in both the groups revealed that these changes were statistically significant ($p < 0.001$) at 95% confidence interval. Inter group analysis at 3 months follow up showed that the change in levels of IL-10 were statistically significant ($p < 0.001$) at 95% confidence interval.



Graph 4. Mean Interlukin-10 levels at baseline and 3 months

Discussion

Periodontal disease severity is determined not only by the presence and composition of the biofilm, but also by the host's reaction to the biofilm bacteria. Although scaling and root planing (SRP) is considered the “gold standard” for non-surgical periodontal therapy, it is not adequate for every patient.¹³ Hence, many additional treatments have been done in adjunct with SRP such as Local drug delivery (LDD), soft tissue lasers and full mouth disinfection. It has been noted that diode lasers have the potential to provide additional benefit, but their effectiveness in dental treatments, such as non-surgical periodontal therapy is still debatable. The present split mouth study had been carried out to evaluate and compare the effect of diode laser used as an adjunct to SRP and SRP alone in chronic periodontitis patients. Here the clinical parameters such as GI, PI, PD and CAL with IL-10 levels were evaluated at baseline and after 3 months. Results from this study showed that the postoperative healing was uneventful, with no complications such as abscesses, infections, or dentin hypersensitivity.

After periodontal therapy, we discovered that both treatment modalities resulted in considerable improvements in all clinical parameters. In this study, Plaque

index given by Silness & Loe, (1964)¹⁴ was used as it is the most widely accepted and recognized among the plaque indices that has demonstrated good validity and reliability. It can be used on all surfaces of all or selected teeth. Gingival index given by Loe H. and Silness J. (1963)¹⁵ was used for the purpose of assessing the severity of gingivitis and its location in four possible areas by examining only the qualitative changes of the gingival soft tissue. Because of its demonstrated validity, reliability, and ease of use, the gingival index is one of the most widely accepted.

Our study revealed significant reduction in Plaque index and Gingival index after both treatment modalities which was similar to the results obtained by Qadri et al¹¹ in their study. In this study, inter group analysis demonstrated mean decrease of 2 mm in the probing depths at the laser sites from baseline to 3 months as compared to the SRP site where mean decrease of 1mm was seen. The decrease in the probing depths in laser with SRP group were statistically significant. In the laser with SRP group there was a mean attachment gain of 2 mm from baseline to 3 months as compared to SRP group alone where the mean attachment gain was less than 1 mm. These results were also statistically significant In a similar study done by Kreisler et al.¹⁶ they examined the clinical efficacy of laser periodontal pocket irradiation as an adjunct to conventional scaling and root planing in twenty-two systemically healthy patients with a need of periodontal treatment. Their study revealed that after use of lasers there was a larger reduction in PD which was most likely due to the de-epithelization of periodontal pockets, resulting in improved connective tissue attachment.

These findings suggest that the laser treatment results in de-epithelization, decreased epithelial migration, and enhanced connective tissue development. The findings of our study were similar to their results, indicating that laser penetration shows a significant inflammatory difference when compared to mechanical therapy alone, in addition to improve clinical healing. The clinical findings in our study were in agreement with the studies done by Walter Dukic et al¹⁷ where they concluded that the laser group showed significant PD reduction in moderate pockets from baseline to 18-week and Qadri et al¹¹ who also found significant reduction in PD in the laser group over conventional therapy. Contrary to our findings, De Micheli et al¹⁸ in their study had found out that as compared to conventional therapy, diode laser had no greater therapeutic benefit. Also according to Euzebio Alves et al¹⁹ who used a diode laser (808 ± 5 nm) as an adjuvant to SRP and SRP alone showed no significant difference in the clinical parameters in both the groups at 6 weeks and at the end of 6 months. Along with clinical parameters we also did the biochemical analysis of IL-10 in GCF. Generally GCF collection is done using filter paper which results in non-specific attachment of the analyte, resulting in a misleading decrease in measurable biomarkers' level.

Microcapillary tubes, well-known for their ease of collection, measurement, and standardization of samples, can also be used for collection. One of the major drawbacks for using this for collecting GCF was the likelihood of damage to the marginal gingiva.²⁰ In our study we have used micropipettes which allows precise collection of GCF from a given site in a fixed volume. Though this procedure delivers pure native GCF, the collection of adequate samples may take more than

30 minutes, posing a risk of tissue harm. Sometimes it is difficult to extract the entire sample from the tube.²¹ Considering, the loss of GCF due to sticking of the sample to the tube walls, a sample of 10µl of GCF was collected from each site.

In this study GCF analysis was carried out using ELISA (enzyme-linked immunosorbent assay) kit as it is widely used for detecting and quantifying proteins and antigens from various samples including IL-10. Mehmet Saglam and his colleagues had studied the effect of diode laser as a nonsurgical periodontal therapy with scaling and root planing. Plaque index, gingival index, bleeding on probing, probing depth, and clinical attachment level were measured at baseline and at 1, 3, and 6 months after treatment. Group treated with diode laser with SRP showed significantly better clinical outcome when compared to the SRP alone. Their studies also showed increase in levels of anti-inflammatory cytokine, interleukin-8 (IL-8) after the treatment in both the groups.⁷

IL-10 is a pluripotent cytokine with effects on numerous cell populations, in particular circulating and resident immune cells as well as epithelial cells. This cytokine originates from immune cells such as monocytes, macrophages, and T and B-cells and acts by reducing the synthesis of the pro-inflammatory cytokines. It down-regulates the macrophage activation and impacts the immune response by limiting the duration and extent of inflammation. With its potent immunoregulatory capacities, its main biological function is the limitation and termination of inflammatory responses.²²In this study, IL-10 levels increased significantly in both groups after treatment when compared to baseline. As compared to SRP alone, diode laser group showed highly significant results. Studies conducted by Andrade *et al.*²³ and Grover *et al.*²⁴ reported an increased level of GCF IL-10 at 3 months after non-surgical periodontal therapy which is in agreement with the results of our study. The rise in anti-inflammatory cytokine concentrations appears plausible, since IL-10 may play an essential regulatory function in reducing the duration and extent of the acute inflammatory response.

To the best of our knowledge there has not been any study conducted to check the efficacy of diode laser with SRP on IL-10 levels in GCF. This is a novel study done to evaluate the effect of diode laser with SRP on clinical parameters and IL-10 levels in chronic periodontitis patients. Results of our study demonstrates that the clinical parameters as well as IL10 levels showed significant improvements in diode laser group as compared to SRP group suggesting that use of diode laser in periodontal therapy as an adjunct can be more effective than SRP alone. However, one of the limitations of our study was that the number of participating subjects was limited. A longer period follow up and larger sample size may authenticate our result and serve as a basis for future research. As sometimes only clinical parameters may not suffice to detect the nature and extent of periodontal disease, chair-side tests to detect IL-10 levels may offer higher diagnostic and /or prognostic value. Thus, from the findings of our present study it can be concluded that Diode laser as an adjunct to SRP demonstrates a superior outcome as compared to SRP alone in treating chronic periodontitis patients. Diode laser when used in adjunct to SRP, the levels of IL-10 which is an anti-inflammatory cytokine, significantly increased which indicated reduced inflammation of the tissues.



Site A

Figure 1. Pre-operative probing depth with putty stent



Site B

Figure 2. Pre-operative probing depth with putty stent



Site A

Figure 3. GCF collection with micropipette



Site B

Figure 4. GCF collection with micropipette



Site A

Figure 5. SRP with ultrasonic scaler



Site B

Figure 6. SRP with ultrasonic scaler



Site B

Figure 7. Diode laser application in periodontal pocket



Site A

Figure 8. Post-operative probing depth using putty stent



Site B

Figure 9. Post-operative probing depth using putty stent

Acknowledgement

I would like to express my sincere appreciation and gratitude to my professor, Head of Department, Dr. Varsha Rathod for her guidance and support. Her loving and affectionate assistance with inspiring suggestions and critical review have been precious for the completion of this study. This study would not have been possible without the support, motivation and innovative ideas of my guide, Dr. Prajakta Rao. I am thankful for her guidance and her help in formulation of aims and research goals. I am honoured and blessed to be her student.

Funding Information

Study was financed by Dr. Aishwarya Kubal

Author Contributions

Conceptualization - Dr. Aishwarya Kubal, Dr. Prajakta Rao

Methodology - Dr. Aishwarya Kubal, Dr. Prajakta Rao

Writing – original draft preparation - Dr. Aishwarya Kubal

Writing – review and editing - Dr. Prajakta Rao, Dr. Varsha Rathod

Conflicts of Interest statement

No conflicts of interest.

References

1. Andrade P, Euzebio Alves V, Carvalho V, De Franco Rodrigues M, Pannuti CM, Holzhausen M, et al. Photodynamic therapy decrease immune-inflammatory mediators levels during periodontal maintenance. *Lasers Med Sci.* 2017;32:1–9.
2. Aoki A, Sasaki KM, Watanabe H, Ishikawa I. Lasers in nonsurgical periodontal therapy. *Periodontol 2000* 2004, 36:59–97
3. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontology/Am Acad Periodontology* 1999, 4(1):1–6
4. Batara, R., Ardani, I. G. A. I., Wardani, I. A. K., Windiani, I. G. A. T., Adnyana, I. G. A. N. S., & Setiawati, Y. (2022). Psychogenic dysphagia in children, and the success of family-based treatment: Case report. *International Journal of Health & Medical Sciences*, 5(1), 163-168. <https://doi.org/10.21744/ijhms.v5n1.1894>
5. Bibi, T.; Khurshid, Z.; Rehman, A.; Imran, E.; Srivastava, K.C.; Shrivastava, D. Gingival Crevicular Fluid (GCF): A Diagnostic Tool for the Detection of Periodontal Health and Diseases. *Molecules* 2021, 26, 1208.
6. Cobb CM. Non-surgical pocket therapy: mechanical. *Ann Periodontology/Am Acad Periodontology* 1996, 1(1):443–490
7. De Micheli G, de Andrade AK, Alves VT, Seto M, Pannuti CM, Cai S Efficacy of high intensity diode laser as an adjunct to non-surgical periodontal treatment: a randomized controlled trial. *Lasers Med Sci* 2011, 26(1):43–48
8. Dental Scaling and Root Planing for Periodontal Health: A Review of the Clinical Effectiveness, Cost-effectiveness, and Guidelines [Internet]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2016 Oct 17.

9. Dukić W, Bago I, Aurer A, Roguljić M. Clinical effectiveness of diode laser therapy as an adjunct to non-surgical periodontal treatment: a randomized clinical study. *Journal of periodontology*. 2013 Aug; 84(8):1111-7.
10. Euzebio Alves VT, de Andrade AK, Toaliar JM, Conde MC, Zezell DM, Cai S, Pannuti CM, De Micheli G. Clinical and microbiological evaluation of high intensity diode laser adjunct to non-surgical periodontal treatment: a 6-month clinical trial. *Clin Oral Investig*. 2013 Jan;17(1):87-95. doi: 10.1007/s00784-012-0703-7. Epub 2012 Mar 9. PMID: 22402617.
11. Griffiths GS. Formation, collection and significance of gingival crevicular fluid. *Periodontol* 2000. 2003; 31:32-42.
12. Grover HS, Kapoor S, Singh A. Effect of topical simvastatin (1.2 mg) on gingival crevicular fluid interleukin-6, interleukin-8 and interleukin-10 levels in chronic periodontitis-a clinicobiochemical study. *J Oral Biol Craniofac Res*. 2016;6:85-92
13. Loe, H. and Silness, J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. *Acta Odontologica Scandinavica*, 1963, 21, 533-551
14. Maheshwari S, Grover V, Gupta J, Kumar N, Jain A. Effect of non-surgical periodontal therapy on the serum and crevicular fluid interleukin-10 levels in chronic periodontitis - A systematic review and meta-analysis. *Int J Health Sci (Qassim)*. 2021 Nov-Dec;15(6):34-46. PMID: 34912186; PMCID: PMC8589830.
15. Mehmet Saglam & Alpdogan Kantarci & Niyazi Dundar & Sema S. Hakki Clinical and biochemical effects of diode laser as an adjunct to nonsurgical treatment of chronic periodontitis: a randomized, controlled clinical trial *Lasers Med Sci* (2014) 29:37-46
16. Mombelli A. Antimicrobial advances in treating periodontal diseases. *Frontiers Oral Biol* 2012, 15:133-148
17. Moritz A, Schoop U, Goharkhay K, Schauer P, Doertbudak O, Wernisch J, Sperr W. Treatment of periodontal pockets with a diode laser. *Lasers Surg Med* 1998, 22(5):302-311
18. Paster BJ, Dewhirst FE. Molecular microbial diagnosis. *Periodontol* 2000 2009, 51:38-44
19. Qadri T, Poddani P, Javed F, Tuner J, Gustafsson A. A shortterm evaluation of Nd:YAG laser as an adjunct to scaling and root planing in the treatment of periodontal inflammation. *J Periodontol* 2010, 81(8):1161-1166
20. Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB, Camargo LE, et al. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis. *J ClinPeriodontol* 2004, 31: 443-448.
21. Silness J, Løe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta odontologica scandinavica*. 1964 Jan 1;22(1):121-35.
22. Silness J, Løe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta odontologica scandinavica*. 1964 Jan 1;22(1):121-35. 33) Loe, H. and Silness, J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. *Acta Odontologica Scandinavica*, 1963, 21, 533-551
23. Slot DE, Jorritsma KH, Cobb CM, Van der Weijden FA. The effect of the thermal diode laser (wavelength 808-980 nm) in non-surgical periodontal therapy: a systematic review and meta-analysis. *J Clin Periodontol* 2014; 41:681-692.

24. Slots J, Slots H. Bacterial and viral pathogens in saliva: disease relationship and infectious risk. *Periodontol 2000* 2011, 55(1):48–69
25. Tunkel J, Heinecke A, Flemmig TF. A systematic review of efficacy of machinedriven and manual subgingival debridement in the treatment of chronic periodontitis. *J Clin Periodontol* 2002, 3:72–81.
26. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, 29(3), 550–561. <https://doi.org/10.1002/cae.22202>