Original Article

Influence of Adjunctive Photodynamic Therapy on Interleukin-6, Interleukin-8, and Interleukin-10 Gingival Crevicular Fluid Levels in Chronic Periodontitis – A Randomized Controlled Trial

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Abstract

Background: Numerous studies have assessed the effect of photodynamic therapy (PDT) both as a primary mode of treatment and as an adjunct to scaling and root planing in the treatment of periodontitis. Some results were nondefinitive and, in part, inconsistent with respect to the clinical and biochemical effects. Hence, the aim of this study was to evaluate the effect of PDT as an adjunct to nonsurgical periodontal therapy (NSPT) on the gingival crevicular fluid (GCF) interleukin-6 (IL-6), IL-8, and IL-10 levels in the treatment of chronic periodontitis (CP). **Materials and Methods:** In 21 patients with CP, two contralateral sites (premolar and molar) were randomly divided into: control sites (treated with NSPT only) and test sites (treated with NSPT + PDT). Clinical parameters including bleeding on probing (BOP), probing pocket depth, clinical attachment level were evaluated at baseline, 1- and 3 months and biochemical parameters of GCF levels of IL-6, IL-8, and IL-10 were evaluated at baseline and 3-month post-therapy through enzyme-linked immunosorbant assay. **Results:** A greater improvement in BOP score at 1 month (41.10% ± 3.58%) and 3-months (38.00% ± 3.62%) posttherapy was found in the test site as compared to control site. Regarding cytokines, test sites exhibited significant reductions in IL-6 (4.29 ± 0.67 pg/ml) and IL-8 (308.16 ± 36.04 pg/ml) levels and increase in IL-10 (14.25 ± 0.83 pg/ml) level at 3 months ($P < 0.0001$). **Conclusion:** Additional application of PDT, adjunctive to NSPT, resulted in a significant reduction in BOP score as well as GCF pro-inflammatory cytokine levels along with an increase in anti-inflammatory cytokine levels, compared to NSPT alone.

Keywords: *Chronic periodontitis, gingival crevicular fluid, interleukin-10, interleukin-6, interleukin-8, photodynamic therapy*

Introduction

Periodontal disease is microbial infections within periodontal tissues that activate the complex inflammatory process. The ongoing interactions involving microorganisms, host defense assembly, environmental and genetic factors have been known to influence the rate of progression and destruction, which leads to the clinical expression of disease.[1] If left uncontrolled, the disease in their severe forms precipitates progressive destruction, ultimately leading to tooth loss and edentulism.[2] Microorganisms, through an indirect mechanism, trigger different components within the host defense assembly, leading to the destruction of the periodontal tissues.[3] Among them, cytokines the inflammatory and immune mediators have generated particular interest and suspicion regarding inflammation-related repair and destruction of periodontal tissues. Various studies have revealed that pro-inflammatory cytokines are known as initial responses to microbial aggression, which increases their respective concentration within the gingival crevicular fluid (GCF).[4]

Though scaling and root planing (SRP) is contemplated as the gold standard for the non-surgical treatment of periodontitis, but it is ineffectual in the total eradication of subgingival microorganisms and calculus from teeth with deep narrow intrabony defects, furcation involvement, anatomical aberrations like root curvatures and invaginatus.[5] To overcome this problem, local and systemic antibiotics have been additionally prescribed with nonsurgical periodontal therapy (NSPT). Nonetheless,

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the success of such therapies can and should be debated. However, still, such therapeutic protocols underlined the role of antimicrobial therapies and host restoration in the effective management of periodontal diseases.[2]

Photodynamic therapy (PDT) is a new, novel, noninvasive treatment approach for infection control and is synonymously known as photoradiation therapy or photochemotherapy.[6] PDT is a term coined by Von Tappeiner in 1904 to describe photosensitization initiated oxygen-dependent chemical reaction.[7] PDT involves the use of visible light through a diode laser in combination with photosensitizer.[6] The photosensitizer activated with the light of specific wavelength combines to the target cells, leads to the production of reactive agents and singlet oxygen. These substances are extremely toxic to certain cellular elements and bacteria, thereby causing their neutralization.[8]

Literature search and analysis of several controlled clinical trials, the adjunctive use of PDT exhibited a greater reduction in probing pocket depth (PPD), bleeding on probing (BOP), and gain in clinical attachment levels (CALs) when compared with monotherapy of SRP in patients with periodontitis.[5,7-9] However, these observations were not reflected in a systematic review, where the authors concluded that PDT offered no additional benefits in clinical parameters when used as an independent therapy or as an adjunct to SRP over SRP alone.[8,10] Such contradictory findings and the uncertain understanding of PDT in the management of periodontal diseases remain particularly due to a lack of randomized controlled trials (RCT).[11] In view of the above, the present study was aimed to evaluate the influence of PDT as an adjunct to NSPT on the GCF IL-6, IL-8, and IL-10 levels in chronic periodontitis (CP).

Materials and Methods

The present RCT comprised 21 patients affected by CP, with a mean age of 44.95 ± 8.51 years, selected from the Department of Periodontics and Implantology of the VSPM Dental College and Research Centre, Nagpur, India. The study was approved by the Institutional Ethics Committee and was performed from September 2018 to October 2019 in accordance with the Helsinki Declaration of 1975, as revised in 2013 and was registered at Clinical Trial Registry–India (CTRI/2018/09/015737). Before the initiation of the study, written informed consent was signed by the patients.

The included patients presented with CP as defined by Armitage.[12] Systemically healthy patients, aged >35 years, with at least 20 natural teeth in the oral cavity. Patients affected and diagnosed with moderate to severe CP, as assessed with PPD >5 mm and CAL >5 mm with alveolar bone loss affecting >30% of the teeth as detected on radiograph at the time of initial diagnosis and the patients exhibiting similar periodontal pocket depths in contralateral premolars (PM) and molars (M) were included in the study. Patients with known systemic diseases or allergy to toluidine blue O, pregnancy, who were on antibiotics, were excluded from the study. The selection process is depicted in the study flow chart [Figure 1].

Clinical examination

The clinical parameters included were PPD, CAL, plaque index (PI),[13] gingival index (GI),[14] BOP,[15] and radiographic evidence of bone loss. The measurements of PPD and CAL were recorded on six sites around each tooth and were rounded off to the nearest millimeter. The intraoral examination was conducted by a single examiner (VK) using a manual periodontal probe (PCP-UNC 15; Hu Friedy, Chicago, IL, USA). Readings were repeated by the same examiner (VK) to perform intra-observer reproducibility analysis.[1]

Two contralateral sites (PM and M) per patient were randomly divided into:

- Control sites (NSPT): Treated with NSPT alone
- Test sites (NSPT + PDT): Treated with NSPT along with PDT.

Randomization was done using computer generated random number table.

Site selection and gingival crevicular fluid collection

GCF samples were collected from contralateral PM and M in each patient after drying the area. The area was isolated with sterile gauze after the removal of supragingival plaque. Microcapillary pipette (Labo Glass Scientific Supply Co. Haryana, India) was placed at the entry point of periodontal pockets for the collection of GCF. Sites that did not secrete GCF, which were inadvertently mixed with blood and/or saliva were excluded from the study.[16]

An approximate quantity of 10 µL of GCF was collected from each patient. Collected GCF samples were promptly

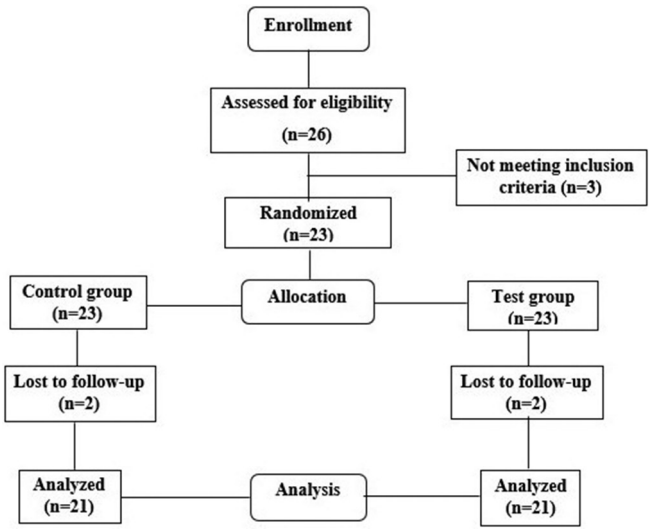

Figure 1: Study flow chart

relocated to airtight plastic vials (Eppendorf tubes) and stored at −20°C until assayed.[17] Samples were investigated using commercially available enzyme-linked immunosorbant assay (ELISA) (Krishgen Biosystems Human IL-6 (KB1068), IL-8 (KB1070), IL-10 (KB1072) ELISA, Mumbai, Maharashtra, India) (Enzyme-linked immune-sorbent assay) for assessment of GCF IL-6, IL-8, and IL-10 levels.

Clinical procedure

After clinical examination and allocation of sites, GCF-samples were collected. Full-mouth SRP was performed using hand and ultrasonic instruments. At the PDT allocated sites, TBO photosensitizer 0.01% was placed within the periodontal pockets beginning from the most apical portion in an incremental manner toward the coronal direction. A duration of 60-seconds was fixed for photosensitization, after which the sites were cleansed with distilled water irrigation. At this point of time, the sites were ready for illumination through continuous-wave diode laser (Diode laser [Biolitec, Germany]) operating at 810-nm over a 60-s preprogrammed treatment cycle with the total energy as 6 J, at low power (<2 W), where it was moved in coronal direction using sweeping motion for 1-minute followed by additional vertical movements in apical and coronal directions for 30-s.[18]

The patients were re-examined at 1- and 3-month posttherapy. Second application of PDT was performed at 1-month. Using acrylic stents, the clinical parameters were recorded at both recall visits with the UNC-15 periodontal probe. GCF collection was done at 3-month.

Statistical analysis

The sample size was determined using immunological cytokine level changes as the primary outcome. It was estimated that a sample size of 20 sites in each group would permit a Type-II error level of $\beta = 0.20$ (80% power) and a Type-I error level of $\alpha = 0.05$ (5% probability). It was decided to have 23 sites in each group to allow for potential dropouts.

The clinical parameters like PI and GI were presented in terms of mean and standard deviations and compared across times using repeated measure analysis of variance (ANOVA). Other parameters such as BOP, PPD, and CAL were compared between Test and Control sites using paired *t*-test. Furthermore, the comparisons were performed for each parameter across time at each site using ANOVA. The cytokines namely IL-6, IL-8, and IL-10 were also compared between two sites using paired *t*-test. The comparison of each parameter at each site was also performed between two-time points using a paired *t*-test. Further, Pearson's correlation coefficient was also obtained to estimate the relationship of parameters between different times. The analysis was performed independently for PM and M specimens. The data analyses were performed using a Statistical Package (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA) and the statistical significance was tested at 5% level.

Results

Twenty-one patients completed the study with 3 months of follow-up and were included in the data analyses. There were no complications associated with treatment during the follow-up period. The PI and GI showed statistically significant reduction across time. Furthermore, a statistically significant difference in BOP observed between test and control sites at 1 and 3 months, favoring the test sites ($P < 0.001$) [Table 1].

Statistically significant improvements ($P < 0.001$) were found for PPD and CAL when baseline values were compared with those obtained at the 1- and 3-month follow-up evaluation for both sites at PM and M. At 1- and 3-month follow-up, there were no statistically significant differences in clinical measures at either site. However, PPD for PM showed a statistically significant difference between both the sites at 1- and 3-month. The mean changes in clinical parameters (PPD at M, CAL at PM and M) were slightly better in the NSPT + PDT versus NSPT group, but these differences failed to reach any level of significance [Tables 2 and 3].

At 3 months post-therapy, lower concentrations of pro-inflammatory cytokines (IL-6 and IL-8) and higher concentrations of anti-inflammatory cytokine (IL-10) were observed in the Test sites ($P < 0.0001$) [Table 4].

Discussion

The impetus of PDT lies in its potent ability to kill bacteria in planktonic solution and biofilms.[2] The present split-mouth RCT aimed to assess the efficiency of PDT as an adjunct to NSPT and its effects on GCF levels of IL-6, IL-8, and IL-10 in CP.

A significant reduction in full mouth PI and GI can be attributed to the fact that there was a reduction in supragingival plaque after SRP and oral hygiene instructions received during preliminary visits. This is in

Table 1: Comparison of bleeding on probing between two sites and across time at each site

BOP	Mean±SD		*P*
	Test site (*n*=21)	Control site (*n*=21)	
Baseline	64.10±7.67	64.43±7.58	0.888 (NS)#
1 month	41.10±3.58	45.29±3.90	0.001 (S)#
3 month	38.00±3.62	46.81±4.33	<0.0001 (S)#
P	<0.0001 (S)**	<0.0001 (S)**	

#Obtained using paired *t*-test; **Obtained using repeated measures of ANOVA; $P<0.05$ implicating statistically significant difference; BOP: Bleeding on probing; NS: Not significant; S: Significant; SD: Standard deviation

Table 2: Comparison of probing pocket depth between two sites and across time at each site

PPD (mm)	Mean±SD		*P*
	Test site (*n*=21)	Control site (*n*=21)	
Premolar			
Baseline	5.23±0.65	5.14±0.70	0.444 (NS)#
1 month	4.10±0.64	4.37±0.69	0.021 (S)#
3 month	3.42±0.63	3.66±0.66	0.022 (S)#
P	<0.0001 (S)**	<0.0001 (S)**	
Molar			
Baseline	5.92±0.71	5.78±0.54	0.134 (NS)#
1 month	4.82±0.71	4.80±0.67	0.836 (NS)#
3 month	4.12±0.71	4.15±0.68	0.722 (NS)#
P	<0.0001 (S)**	<0.0001 (S)**	

#Obtained using paired *t*-test; **Obtained using repeated measures of ANOVA; *P*<0.05 implicating statistically significant difference; PPD: Probing pocket depth; NS: Not significant; S: Significant; SD: Standard deviation

Table 3: Comparison of clinical attachment level between two sites and across time for each site

CAL (mm)	Mean±SD		*P*
	Test site (*n*=21)	Control site (*n*=21)	
Premolar			
Baseline	6.67±0.91	6.46±0.93	0.319 (NS)#
1 month	5.55±0.92	5.67±0.93	0.570 (NS)#
3 month	4.72±0.87	5.06±0.93	0.088 (NS)#
P	<0.0001 (S)**	<0.0001 (S)**	
Molar			
Baseline	7.58±1.09	7.52±1.47	0.790 (NS)#
1 month	6.48±1.09	6.80±1.51	0.171 (NS)#
3 month	5.78±1.09	6.00±1.50	0.345 (NS)#
P	<0.0001 (S)**	<0.0001 (S)**	

#Obtained using paired *t*-test; **Obtained using repeated measures of ANOVA; *P*<0.05 implicating statistically significant difference; CAL: Clinical attachment level; NS: Not significant; S: Significant; SD: Standard deviation

accordance with the finding of a previous study by Raj *et al*. and Theodoro *et al*.[19,20]

The possible explanation for the higher reduction of BOP scores at Test sites was the additional benefits provided by low-level laser therapy, favoring the repair of tissues and diminishing periodontal inflammation as a result of the potential bio-modulatory effects, such as stimulation and proliferation of cells and the collagen synthesis. Similar results were found in the study of Campos *et al*.[21] However, in a study done by Theodoro *et al*.[20] no significant difference in the BOP score after a single session of PDT and NSPT was observed. While in the present study, a statistically significant BOP reduction at the test site could be due to the application of two sessions of PDT along with NSPT.

For PM, in test site mean PPD showed a significant reduction at 1 and 3-months follow-up than control site. The positive clinical outcome obtained in the present study

Table 4: Comparison of interleukin-6, interleukin-8, and interleukin-10 levels between two sites and across times

	Mean±SD		*P*
	Test site (*n*=21)	Control site (*n*=21)	
IL-6 (pg/ml)			
Baseline	8.65±0.64	8.48±0.70	0.087 (NS)#
3 month	4.29±0.67	5.83±0.65	<0.0001 (S)#
P	<0.0001 (S)#	<0.0001 (S)#	
IL-8 (pg/ml)			
Baseline	456.53±9.42	454.25±8.51	0.161 (NS)#
3 month	308.16±36.04	379.51±7.32	<0.0001 (S)#
P	<0.0001 (S)#	<0.0001 (S)#	
IL-10 (pg/ml)			
Baseline	11.77±0.75	11.56±0.73	0.141 (NS)#
3 month	14.25±0.83	12.48±0.82	<0.0001 (S)#
P	<0.0001 (S)#	<0.0001 (S)#	

#Obtained using paired *t*-test; *P*<0.05 implicating statistically significant difference; IL-6: Interleukin; NS: Not significant; S: Significant; SD: Standard deviation. IL-6-Interleukin-6; IL-8-Interleukin-8; IL-10-Interleukin-10

are similar to previously reported findings.[5] Nevertheless, several patient-related and tooth site-related factors may influence the healing response to periodontal therapy. Periodontal pockets associated with furcation involvements or in multi-rooted teeth responded less favorably to SRP than pockets at non-molar teeth.[22,23]

Statistically, significant improvements were observed for PPD and CAL at 1 and 3-month follow-up for both sites. However, no significant differences were observed in the measurements of these clinical parameters (PPD at M, CAL at PM and M) at both sites during follow-up. Similar results were obtained in a previous study by Chondros *et al*.[24] A systematic review[10] analyzing the data from 5 RCTs concluded that PDT as an independent treatment or as an adjunct to SRP was not superior to control the treatment of SRP. In a very recent study by Ahuja *et al*.,[25] where the authors concluded that NSPT was effective in improving clinical parameters and glycemic status. However, contrasting results were shown in a study by Campos *et al*.[21] The variation in the reported treatment outcome to PDT, in the literature, can be attributed to several factors such as drug ion concentration, period of retention of the drug within the tissue, mode of drug application, pH of the environment (tissue/tooth interface), presence of exudates and GCF and time for biological response. A likely concern for the clinical application of PDT is the potential photo-cytotoxicity to host cells. However, it has been revealed that the doses of light needed for killing bacteria in PDT are much lower than those that are toxic for fibroblasts and keratinocytes.[26]

In the present investigation, we also compared the effects of NSPT with or without PDT on biomarkers of inflammation. The analysis on inflammatory markers indicated that lower concentrations of pro-inflammatory

cytokines (IL-6 and IL-8) and higher concentrations of anti-inflammatory cytokine (IL-10) were observed in the Test sites compared to control sites at 3-months. Inconsistent and scarce data are available concerning the role of PDT in the immune-inflammatory mediator profile during periodontal therapy. The effect of PDT as an adjunct to mechanical therapy in furcations was investigated by Luchesi *et al*.[27] where authors reported that pro-inflammatory mediators exhibited reduced levels in PDT group at 3-months. Conflicting results were reported by Kolbe *et al*.[28]

Increasing amounts of data from various studies specified that the intensity of inflammation as well as destruction within periodontal tissues can be assessed objectively by the analysis of GCF components because the several inflammatory and immune mediators have been recognized in GCF.[29] GCF appears as an attractive oral diagnostic fluid due to its ease of collection and site-specificity.[30] Hence, the present study was formulated to utilize GCF as an avenue for evaluating IL-6, IL-8, and IL-10 levels in periodontitis.

The bactericidal effect of PDT is based on the preferential binding of the photosensitizer to the bacterial cell surface. The photosensitizer molecule absorbs light and generates highly reactive O_2 species (singlet O_2), which can damage a wide variety of proteins, carbohydrates, and lipids. As singlet O_2 has a very short half-life and therefore, its destructive radius is small, resulting in photoreactive effects only in very close proximity makes PDT highly bactericidal with little damage to the surrounding tissue. PDT treatment has the ability to inhibit destructive host responses, which may contribute to its clinical usefulness as an adjunctive therapy.[2]

The literature suggests the beneficial effect of PDT as an adjunct to NSPT in controlling periodontal inflammation. However, this study plays a pivotal role in demonstrating the effect of PDT as an adjunct to NSPT and NSPT alone in the reduction of periodontal inflammation in patients with CP and also in evaluating the GCF levels of IL-6, IL-8 and IL-10 at both sites, and their levels after PDT and NSPT.

There are certain limitations associated with the present study, where two applications of PDT was performed, which could have affected the clinical outcome. Further studies are required to ultimately explain to what extent multiple applications of PDT might improve the outcome of therapy. There is a lack of an established protocol for PDT with NSPT. Periodontal therapy aims at reducing the levels of periodontopathogens, but during the healing period, intraoral dissemination of periodontopathogens occurs, which may have affected treatment outcomes. A larger sample size with long-term observation period is desirable for substantiation of the findings.

Conclusion

Within the limitations of the study, it can be concluded that the applications of two sessions of PDT, adjunctive to NSPT, resulted in reduction in GCF levels of pro-inflammatory cytokines and increase in levels of anti-inflammatory cytokines in CP patients. Furthermore, adjunctive treatment modality promoted a significant reduction in BOP, signifying a potential positive effect on periodontal healing. However, both treatment modalities showed comparable results regarding PPD reduction and CAL gain. Considering the advantages like more patient compliance, safety, and the lack of side effects, the PDT treatment with NSPT is recommended as an efficient adjunctive modality for the treatment of CP.

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Conflicts of interest

There are no conflicts of interest.

References

1. Bawankar PV, Kolte AP, Kolte RA. Evaluation of stress, serum and salivary cortisol, and interleukin-1β levels in smokers and non-smokers with chronic periodontitis. J Periodontol 2018;89:1061-8.
2. Braham P, Herron C, Street C, Darveau R. Antimicrobial photodynamic therapy may promote periodontal healing through multiple mechanisms. J Periodontol 2009;80:1790-8.
3. Yucel OO. Inflammatory cytokines and the pathogenesis of periodontal disease. Immunome Res 2015;11:2.
4. Tawfig N. Proinflammatory cytokines and periodontal disease. J Dent Probl Solut 2016;3:012-7.
5. Alwaeli HA, Al-Khateeb SN, Al-Sadi A. Long-term clinical effect of adjunctive antimicrobial photodynamic therapy in periodontal treatment: A randomized clinical trial. Lasers Med Sci 2015;30:801-7.
6. Christodoulides N, Nikolidakis D, Chondros P, Becker J, Schwarz F, Rössler R, *et al*. Photodynamic therapy as an adjunct to non-surgical periodontal treatment: A randomized, controlled clinical trial. J Periodontol 2008;79:1638-44.
7. Thomas George V, John S, Sreejith CK, Varghese MM. Photodynamic therapy-A new ray of hope in periodontics. Int J Contemp Med Res 2017;4:425-9.
8. Saxena S, Bhatia G, Garg B, Rajwar YC. Role of photodynamic therapy in periodontitis. Asian Pac J Health Sci 2014;1:200-6.
9. Akram Z, Abduljabbar T, Sauro S, Daood U. Effect of photodynamic therapy and laser alone as adjunct to scaling and root planing on gingival crevicular fluid inflammatory proteins in periodontal disease: A systematic review. Photodiagnosis Photodyn Ther 2016;16:142-53.
10. Azarpazhooh A, Shah PS, Tenenbaum HC, Goldberg MB. The effect of photodynamic therapy for periodontitis: A systematic review and Meta-Analysis. J Periodontol 2010;81:4-14.
11. Pourabbas R, Kashefimehr A, Rahmanpour N, Babaloo Z, Kishen A, Tenenbaum HC, *et al*. Effects of photodynamic therapy on clinical and gingival crevicular fluid inflammatory biomarkers in chronic periodontitis: A Split-mouth randomized clinical trial. J Periodontol 2014;85:1222-9.

12. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1-6.
13. Silness J, Loe H. Periodontal Disease in Pregnancy. II. Correlation between Oral hygiene and periodontal condtion. Acta Odontol Scand 1964;22:121-35.
14. Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. Acta Odontol Scand 1963;21:533-51.
15. De Oliveira RR, Schwartz-Filho HO, Novaes AB, Garlet GP, De Souza RF, Taba M, *et al*. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: Cytokine profile in gingival crevicular fluid, preliminary results. J Periodontol 2009;80:98-105.
16. Karthikeyan BV, Pradeep AR. Gingival crevicular fluid and serum leptin: Their relationship to periodontal health and disease. J Clin Periodontol 2007;34:467-72.
17. Ghodpage PS, Kolte RA, Kolte AP, Gupta M. Influence of phase I periodontal therapy on levels of matrix metalloproteinase 1 and tissue inhibitor of metalloproteinase 1. Saudi Dent J 2014;26:171-5.
18. Mistry A, Pereira R, Kini V, Padhye A. Effect of Combined Therapy Using Diode Laser and Photodynamic Therapy on Levels of IL-17 in Gingival Crevicular Fluid in Patients With Chronic Periodontitis. J Lasers Med Sci 2016;7:250-5.
19. Raj KR, Musalaiah S, Nagasri M, Kumar PA, Reddy PI, Greeshma M. Evaluation of efficacy of photodynamic therapy as an adjunct to nonsurgical periodontal therapy in treatment of chronic periodontitis patients: A Clinico-microbiological study. Indian J Dent Res 2016;27:483-7.
20. Theodoro LH, Silva SP, Pires JR, Soares GH, Pontes AE, Zuza EP, *et al*. Clinical and microbiological effects of photodynamic therapy associated with nonsurgical periodontal treatment. A 6-month follow-up. Lasers Med Sci 2012;27:687-93.
21. Campos GN, Pimentel SP, Ribeiro FV, Casarin RC, Cirano FR, Saraceni CH, *et al*. The adjunctive effect of photodynamic therapy for residual pockets in single-rooted teeth: A randomized controlled clinical trial. Lasers Med Sci 2013;28:317-24.
22. Pihlstrom BL, Oliphant TH, McHugh RB. Molar and nonmolar teeth compared over 6 1/2 years following two methods of periodontal therapy. J Periodontol 1984;55:499-504.
23. Graetz C, Plaumann A, Bielfeldt J, Tillner A, Sälzer S, Dörfer CE. Efficacy versus health risks: An *in vitro* evaluation of power-driven scalers. J Indian Soc Periodontol 2015;19:18-24.
24. Chondros P, Nikolidakis D, Christodoulides N, Rössler R, Gutknecht N, Sculean A. Photodynamic therapy as adjunct to Non-surgical periodontal treatment in patients on periodontal maintenance: A randomized controlled clinical trial. Lasers Med Sci 2009;24:681-8.
25. Ahuja CR, Kolte AP, Kolte RA, Gupta M, Chari S. Effect of Non-surgical periodontal treatment on gingival crevicular fluid and serum leptin levels in periodontally healthy chronic periodontitis and chronic periodontitis patients with type 2 diabetes mellitus. J Investig Clin Dent 2019;10:e12420.
26. Mielczarek-Badora E, Szulc M. Photodynamic therapy and its role in periodontitis treatment. Postepy Hig Med Dosw (Online) 2013;67:1058-65.
27. Luchesi VH, Pimentel SP, Kolbe MF, Ribeiro FV, Casarin RC, Nociti FH Jr., *et al*. Photodynamic therapy in the treatment of class II furcation: A randomized controlled clinical trial. J Clin Periodontol 2013;40:781-8.
28. Kolbe MF, Ribeiro FV, Luchesi VH, Casarin RC, Sallum EA, Nociti FH Jr., *et al*. Photodynamic therapy during supportive periodontal care: Clinical, microbiologic, immuno-inflammatory, and patient-centered performance in a split-mouth randomized clinical trial. J Periodontol 2014;85:e277-86.
29. Konopka L, Pietrzak A, Brzezinska-Blaszczyk E. Effect of scaling and root planing on interleukin-1β, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients. J Periodontal Res 2012;47:681-8.
30. Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontol 2000 2016;70:53-64.