Aim: The aim of this study was to evaluate the effect of nonsurgical periodontal therapy on serum levels of interleukin-1β (IL-1β) and interleukin-8 (IL-8) in smokers and nonsmokers with chronic periodontitis before and after scaling and root planing (SRP). Materials and Methods: This was a comparative interventional study including a total of 52 (26 smokers and 26 nonsmokers with chronic periodontitis) subjects. Clinical parameters (gingival index [GI], plaque index [PI], recession, probing pocket depth [PPD], and clinical attachment level [CAL]) were recorded at baseline and 4 weeks after initial periodontal therapy. Initial periodontal therapy included oral hygiene instructions and full mouth SRP. Venous blood sample of 5 mL was collected from each subject at baseline and 4 weeks after initial periodontal therapy to evaluate serum IL-1β and IL-8. These biochemical parameters were assayed using enzyme-linked immunosorbent assay (ELISA) method. Results: The periodontal parameters such as PI, GI, recession, PPD, and CAL were reduced after nonsurgical periodontal therapy. Smokers with chronic periodontitis showed statistically significant lower GI and higher PI, gingival recession, PPD, and CAL as compared to nonsmokers with chronic periodontitis. Statistically significant reduction in periodontal parameters was seen in both groups after periodontal therapy. IL-1β and IL-8 were increased in both groups at baseline; after SRP both groups showed statistically significant reduction in IL-1β and smokers with chronic periodontitis showed statistically significant increase in IL-8 after SRP. Conclusion: Smokers with chronic periodontitis showed more periodontal destruction and systemic inflammatory markers compared to nonsmokers with chronic periodontitis. After periodontal therapy both groups showed statistically significant improvement in clinical parameters and biochemical parameters excluding IL-8.

Keywords: Chronic periodontitis, interleukin, scaling and root planing, smoking

INTRODUCTION

Periodontitis is a chronic infectious disease that affects the supporting tissues of the teeth. Patients with periodontitis develop gradual loss of tooth attachment, which leads to periodontal pocket, gingival recession, loose teeth, and eventually tooth exfoliation.[1] The pathogenesis of periodontal disease is associated with dental plaque biofilm and host response.[2] The microbial challenge stimulates host responses, which result in gingivitis or initiation of periodontitis. Protective aspects of the host response include recruitment of neutrophils, production of
protective antibodies, and possibly the release of anti-inflammatory cytokines including transforming growth factor-β, interleukin-4 (IL-4), IL-10, and IL-11. The pro-inflammatory cytokines including IL-1α, IL-1β, tumor necrosis factor-α, prostaglandins, IL-6, and/or IL-8 may induce connective tissue and alveolar bone destruction.

Cigarette smoking is one of the most important environmental risk factors in periodontitis, as more clinical attachment loss and bone loss have been observed in smoking than in nonsmoking patients. The effect of smoking on the periodontal tissue depends on daily consumption quantity and duration of smoking. Smokers are more likely to harbor potential periodontal pathogens and smoking impairs various aspects of innate and acquired immune responses.

Smoking may be responsible for less-favorable periodontal treatment outcome and may cause disease progression despite a strict periodontal maintenance care program. The negative effects of smoking involved in periodontitis can be managed by nonsurgical periodontal therapy, including scaling and root planing (SRP), systemic and locally delivered antibiotics in combination with regular maintenance care. A meta-analysis evaluated the impact of smoking on nonsurgical therapy and found that probing depth reduction in sites where probing depth was initially ≥5 mm was significantly greater (0.433 mm) in nonsmokers than in smokers. Thus, smoking is one of the factors that impact the stability of treatment results.

Cytokines are important host response to infection, tissue degradation, and repair. IL-1β and IL-8 are pro-inflammatory cytokines, which are involved in periodontal pathogenesis. IL-1β stimulates the synthesis of prostaglandin E2, platelet-activating factor, and nitric oxide that results in vascular changes associated with inflammation, increasing site of infection and tissue injury. IL-8 is a polymorphonuclear chemoattractant and has direct actions on osteoclast differentiation by signaling through chemokine receptor. This study attempts to evaluate and compare the serum levels of IL-1β and IL-8 in smokers and nonsmokers with chronic periodontitis before and 1 month after nonsurgical periodontal therapy.

**MATERIALS AND METHODS**

This study was a comparative interventional study. The subjects of this study were selected from the outpatients of Department of Periodontics, Sree Mookambika Institute of Dental Sciences, Kulasekaram. Ethical clearance for the study was obtained from the Research Ethics Committee of Sree Mookambika Institute of Medical Sciences, Kulasekaram. A written informed consent was obtained from the subjects who participated in the study. The age of the subjects ranged between 20 and 65 years; all were men.

The patients were questioned regarding their smoking status and systemic diseases. The systemic diseases such as cardiovascular diseases, diabetes mellitus, malignancies, and rheumatoid arthritis were excluded.

Patients with chronic periodontitis was diagnosed according to the criteria of 1999 American Academy of Periodontology (AAP) including attachment loss of ≥5 mm at more than 30% of the sites, bleeding on probing, and patients with ≥20 functional teeth.

Clinical examination included the recording of fullmouth plaque index (PI) by Loe (1967), gingival index (GI) by Löe and Silness (1963), gingival recession, probing pocket depth (PPD), and clinical attachment level (CAL). The clinical parameters were assessed at six sites of all teeth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) using William’s graduated periodontal probe. Clinical parameters were assessed at baseline and 4 weeks after initial periodontal therapy.

The total number of patients taken in this study was 52, of this 26 were smokers with chronic periodontitis and 26 were nonsmokers with chronic periodontitis.

**Sample preparation**

Under sterile condition from each patient of two groups, 5 mL venous blood sample was collected before SRP from the antecubital fossa venipuncture using the 23-gauge needle with 5-mL syringe [Figure 1]. The blood was placed in the clot activator tube for 30 min [Figure 1] and then centrifuged at 3000 rpm for 10 min. Then the supernatant serum is separated from the clot activator tube [Figure 2]. It was then transferred to a plastic vial and stored at –80°C until the time of assay. After 1 month, 5 mL blood is collected from the same patients to assess the levels of IL-1β and IL-8. The serum IL-1β and IL-8 were estimated by enzyme-linked immunosorbent assay (ELISA) sandwich technique.

**Assay procedure of interleukin-8**

100 mL of sample was added in each well. Add 50 mL of diluted biotinylated anti-IL-8 to all wells. Incubated
at room temperature (18°C–25°C) for 1 h. After it wash each well and add 100 μL of streptavidin-horseradish peroxidase (HRP) solution into all wells. Then incubated at room temperature (18°C–25°C) for 30 min and repeat wash. Add 100 μL of ready-to-use 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution into all wells and incubate in the dark for 12–15 min at room temperature. Add 100 μL of H2SO4 stop reagent into all wells. Read the absorbance value of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wavelength.

**Assay procedure of interleukin-1β**

Add 100 μL of sample and 50 mL biotinylated anti-IL-1β to all wells. Incubated for 3 h at room temperature and washed three times. Add 100 μL of streptavidin-HRP and incubated 30 min at room temperature. Washed three times and 100 μL of ready-to-use TMB added. Protect it from light, let the color develop for 12–15 min. On it add 100 μL H2SO4 and absorbance was read at 450 nm.

**Statistical analysis**

The data were analyzed by Statistical Package for the Social Sciences (SPSS) software program, version 22.0. Paired t test was applied to find the statistical significance before and after treatment within and between the groups. Analysis of variance (ANOVA) (post hoc) followed by Dunnett’s test was applied to find statistical significance between the groups. A value of P < 0.05 was considered statistically significant at 95% confidence interval.

**Results**

The purpose of this study was to compare the effect of nonsurgical periodontal therapy on serum levels of IL-1β and IL-8 in smokers and nonsmokers with chronic periodontitis. This study included a total number of 52 male patients. The patients were categorized into two groups of 26 patients each group. The nonsmokers

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**Figure 1:** Blood collection and blood sample transferred to clot activator tube and kept for 30 min

**Figure 2:** Blood sample placed in centrifuge and serum transferred to cryotube
with chronic periodontitis group showed reduction in clinical parameters; IL-1β and IL-8 after nonsurgical periodontal therapy were statistically significant ($P < 0.05$) [Table 1]. The smokers with chronic periodontitis group showed reduction in clinical parameters, and IL-1β after treatment was statistically significant ($P < 0.05$) and showed statistically significant increase in IL-8 after treatment.

**DISCUSSION**

Smoking is the strongest modifiable risk factor for periodontitis. Smokers are less healthy with increased risk of infections due to impaired immune system. Smoking may negatively affect the results of both nonsurgical and surgical periodontal therapy. The response of periodontal treatment is difficult to predict accurately in case the patient is exposed to the continued effects of smoking.

Smoking may have negative periodontal effects through vascular alterations, altered neutrophil function, decreased immunoglobulin G production, decreased lymphocyte proliferation, increased prevalence of periodontopathogens, bone resorption, and altered fibroblast attachment and functions. These in turn show negative effect in eliminating pathogens by mechanical therapy on the periodontium. Furthermore, the inflammatory cytokines alter the ratio of the receptor activator of the nuclear factor kappa-B ligand/osteoprotegerin (RANKL/OPG) (important factors for bone resorption and modeling) and lead to greater bone loss in smokers.

This study has observed that smokers with chronic periodontitis group showed significantly ($P < 0.05$) lower GI and higher PI, gingival recession, PPD, and CAL as compared to nonsmokers with chronic periodontitis at baseline [Table 2]. The obtained results are in accordance with the previous study Clarke et al.\textsuperscript{15} found that nicotine may cause vasoconstriction in the peripheral blood vessels and thus may reduce the clinical signs of gingivitis. Cell attachment was significantly less on root surfaces obtained from heavy smokers compared with nonsmokers and healthy controls.\textsuperscript{16} This study also found that after nonsurgical periodontal therapy smokers with chronic periodontitis and nonsmokers with chronic periodontitis showed a statistically significant improvement in GI, PI, gingival recession, PPD, and CAL [Tables 1 and 2]. Patients with chronic periodontitis showed significantly higher levels of inflammatory cytokines such as IL-1β and IL-8 in periodontal tissues than healthy individuals; furthermore, depressed IL-10 level was reported in smokers with periodontitis than in nonsmokers.\textsuperscript{17} The serum IL-1β and serum IL-8 are derived initially from gingival tissues as response to bacterial stimulation, especially by lipopolysaccharide (LPS). This study assessed the serum IL-8 and serum IL-1β before and after treatment. The smokers with chronic periodontitis showed lower IL-8 compared to nonsmokers with chronic periodontitis at baseline, which was not statistically significant [Tables 1 and 2]. The study conducted by Fredriksson et al.\textsuperscript{18} showed that smoking reduced the sensitivity of peripheral neutrophils to stimulate IL-8. Smokers with chronic periodontitis showed significant increase in IL-8 compared to nonsmokers with chronic periodontitis after SRP [Graph 1]. This finding is in accordance with the study conducted by Goutoudi et al.\textsuperscript{19} in gingival crevicular fluid (GCF), where IL-8 concentration in smokers increased following therapy. This study observes that serum IL-1β showed increase in both smokers and nonsmokers with chronic periodontitis at baseline. The study conducted by Talvan et al.\textsuperscript{20} showed that plasmatic levels of IL-1β and IL-8 were significantly higher in chronic periodontitis. After SRP, both groups showed statistically significant ($<0.05$) decrease in IL-1β [Tables 1 and 2 and Graph 2]. Overall, the inflammatory markers were lower in smokers with chronic periodontitis group showed reduction in clinical parameters; IL-1β and IL-8 after nonsurgical periodontal therapy were statistically significant ($P < 0.05$) [Table 1]. The smokers with chronic periodontitis group showed reduction in clinical parameters, and IL-1β after treatment was statistically significant ($P < 0.05$) and showed statistically significant increase in IL-8 after treatment.

**Table 1: Comparison of mean periodontal parameters and serum levels of interleukin-8 and interleukin-1β within the control group**

| Observations                        | Control group (mean ± SD) | $P$ Value |
|-------------------------------------|---------------------------|-----------|
|                                     | Pretreatment              | Posttreatment |         |
| Plaque index                        | 1.23 ± 0.42               | 0.36 ± 0.16 | 0.001*   |
| Gingival index                      | 1.26 ± 0.42               | 0.42 ± 0.22 | 0.001*   |
| Recession (mm)                      | 0.49 ± 0.63               | 0.36 ± 0.58 | 0.001*   |
| Clinical attachment level (mm)      | 3.31 ± 1.03               | 2.76 ± 0.58 | 0.001*   |
| Probing pocket depth (mm)           | 2.91 ± 0.67               | 2.40 ± 0.57 | 0.001*   |
| Interleukin-8 (pg/mL)               | 0.10 ± 0.03               | 0.09 ± 0.02 | 0.05     |
| Interleukin-1β (pg/mL)              | 0.26 ± 0.11               | 0.14 ± 0.06 | 0.001*   |

SD = standard deviation  
* $P < 0.05$ significant compared pre- and posttreatment within the control group
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Chronic periodontitis than nonsmokers with chronic periodontitis, and SRP was effective in lowering IL-1β but smokers showed increase in levels of IL-8 after treatment. It is found that the level of IL-1β was lower in smokers when compared to nonsmokers, suggesting that production of pro-inflammatory biomarkers is depressed in smokers, but these mediators are still present at concentrations capable of pathogenesis. This study is contrast with one study which observed no influence of smoking on the levels of IL-1β.

The results of the clinical parameters such as gingival recession, PPD, and CAL are in expected line with systematic review conducted by Labriola et al. The limitations of this study were small sample size and limited duration of time.

**Conclusion**

Smokers with chronic periodontitis showed more periodontal destruction and systemic inflammatory markers compared to nonsmokers with chronic periodontitis. After periodontal therapy, both groups showed statistically significant improvement in clinical parameters and biochemical parameters excluding IL-8. Future studies are necessary to validate the observations of this study and to evaluate other factors influencing the cytokine levels in serum.

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**Conflicts of interest**

There are no conflicts of interest.

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