THE EFFECT OF NON-SURGICAL PERIODONTAL THERAPY ON INTERLEUKIN-8 (IL-8) LEVELS IN GINGIVAL CREVICULAR FLUID IN PERIODONTITIS PATIENTS - AN INTERVENTIONAL STUDY

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ABSTRACT:

INTRODUCTION: Interleukin 8 (IL-8) an important pro-inflammatory α-chemokine in inflammatory conditions like Periodontitis; it has been detected in gingival crevice / Gingival Crevicular Fluid (GCF). Monitoring of IL-8 levels in GCF may be an important marker for progression of periodontal disease activity. Thus, this study was conducted with an aim of determining GCF levels of IL-8 in periodontitis patients before and after non surgical periodontal therapy.
METHOD: 27 Stage II, III, IV Grade A, B, C Periodontitis patients were included in this non-randomized interventional study. Clinical Parameters recorded were Probing Depth (PD) and Clinical Attachment Level (CAL) using pressure sensitive probe and GCF was collected at baseline, 1 and 3 months after non-surgical treatment. Sandwich ELISA technique was used in order to determine levels (Pg/ml) of IL-8 in GCF. Analysis of Variance (ANOVA) and post hoc tests were used in statistical analysis to determine the change in PD, CAL, and IL-8 levels over time.
RESULTS: There was statistically significant reduction in Probing depth and gain in Clinical Attachment Level (CAL) after non-surgical treatment ($P<0.001$). IL-8 levels in GCF showed that increase in levels of IL-8 at 3 months following non-surgical periodontal treatment ($p>0.05$).
CONCLUSION: Based on the findings of the study, non-surgical periodontal treatment raises GCF concentrations of IL-8, which may imply that IL-8 plays a role in healing or represents the host's defense against periodontal infections.

Key Words: Clinical Attachment Level, Gingival Crevicular Fluid, Interleukin-8, Periodontitis, Probing Depth
Introduction

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Cytokines (Greek "Cyto"- cells and "Kinos"- movement) are low molecular weight polypeptides which are secreted by lymphocytes, effector cells and Antigen producing cells (APC). The term cytokine was coined by Dinerallo C. in 1971. He also stated that cytokine-mediated effects are seen in Inflammation, Immunology, Cancer and Atherosclerosis. These Cytokines carry signals locally between cells and hence, have an effect on other cells. They are soluble proteins that cell function in health and disease states. [1] Cytokines are classified into interleukins, chemokines, monokines, interferons and lymphokines based on their source and target cells. Interleukins primarily act on leukocytes, lymphokines produced by lymphocytes, monokines produced by monocytes, interferons have antiviral response, and chemokines mediate chemotaxis. [2] Cytokines act by attaching themselves to certain receptors on target cells. Based on their actions they can be anti-inflammatory, pro-inflammatory antiviral, macrophage, B-cell, T-cell or mast cell activating.

IL 8 is a pro-inflammatory cytokine and plays an important role in pathogenesis of periodontitis. IL-8 attracts polymorphonuclear leukocytes (PMNs), induces attachment of PMNs to endothelial cells and their trans-endothelial migration as well as propagation of myeloperoxidase, elastase, glucorinidase and formation of superoxide and hydrogen peroxide. IL-8 has been reported in GCF in periodontitis patients. [3], [4] and is a chemical mediator in periodontitis.

Association of Interleukin-8 with periodontitis is still controversial whether it increases or decreases with disease activity and if even after periodontal treatment. [5] Hence, this research was undertaken to determine the effect of non-surgical treatment on GCF IL-8 levels in periodontitis patients.

Materials and Method

Source of Data

A non-randomized interventional study was carried out in the outpatient Department of Periodontology. Institutional Ethics Committee approval was obtained before the start of the study. Sample size calculated was 23 patients where Confidence Interval was set at 95% and power of the study was 80% to account for drop outs sample size was increased to 27.

Selection criteria
Patients of age 30-65 years, systemically healthy with Stage II, III, IV Grade A, B, C periodontitis [5] were included in the study and site of collection for gingival crevicular fluid was maxillary teeth (as gravity affects GCF flow). Patients with systemic disease, receiving antibiotic, anticoagulant, immunosuppressant, anti inflammatory or steroids since last 6 months, Patients who received any periodontal treatment preceding 6 months, Women who were pregnant, lactating, Post menopausal females or on oestrogen therapy and Patients not willing for participation in the study and further follow up were excluded from the study.

Clinical parameters (Probing depth (PD) and Clinical attachment loss (CAL)) were checked and recorded before, one month and three months after non-surgical periodontal therapy (i.e. SRP). PD and CAL were measured at 6 sites Mesio-buccal, Mid-buccal, Disto-buccal, Mesio-palatal, Mid-palatal and Disto-palatal areas using a pressure sensitive probe. [Axe pressure sensitive

probe, Bluedent India, Tamil Nadu, India] Sites with deepest PD and CAL [Figure 1] were taken into consideration for each maxillary tooth and mean for the same was calculated. After measurement of clinical parameters GCF collection was done. [Figure 2]

GCF collection

GCF was collected from the deepest part of buccal surfaces of maxillary molars (GCF collection was done only from maxillary teeth as gravity promotes capillary action) before, 1 month and 3 months after non surgical treatment. Patients were instructed not to eat anything 1 hour prior to sample collection. Patients seated in upright position on dental chair. The sites meant for sample collection were dried and isolated properly with cotton rolls to prevent contamination. Collection of GCF was done using micro-capillaries of 1 mm diameter and 5ul capacity.

Preservation of samples

After GCF collection, Micro-capillaries containing GCF sample were emptied into Eeppendorf tubes containing 50 ul phosphate buffer saline with 0.05% Tween-20. Samples were stored at 4`C initially and then were frozen at -40`c to -80`c until cytokine analysis.

Cytokine Investigation

Before the investigations, the samples were placed in centrifuge tubes to which 1 ml of 9% sodium chloride solution was added. Thereafter samples were stored at +4`C for further 18 hours, and the level of IL-8 in samples collected at baseline, 1 and 3 months was determined by Sandwich ELISA (KRISHGEN BioSystems, USA) after centrifugation. [Figure 3] A specific IL-8 monoclonal antibody was coated on to a microplate. IL-8 antibody was bound to this antibody and immobilized. Then the microplate was washed in order to remove unbound proteins and horseradish peroxidase specific for IL-8 was added to each well. 200 ul of substrate was added to each well; any color developed was considered to be proportional to concentration of IL-8 (pg/ml). The intensity of color (optical density) was measured on a microplate reader 450 nm. A standard curve was generated by computer using optical density and concentration of IL-8 (pg/ml). [Figure 4] The ELISA test was triplicated for determining IL-8 concentration in samples at the three timelines.

Statistical analysis

To determine the change in PD, CAL, and IL-8 levels at various periods, statistical analysis was utilized. Data normality was examined using the one-sample Kolmogorov-Smirnov test. When the data exhibited a normal distribution, the Analysis of Variance (ANOVA) and Post hoc tests were employed. Data was subjected to the statistical analysis with the Statistical Package for the Social Science (SPSS software version 16.0, SPSS Inc, IBM Corp. New York, USA).

Results

27 patients were included and there was no fall out of patients in this study. Age range of the patients included in the study was from 30-60 years with the mean age being 44.47 ± 7.385 years with 15 male and 12 female participants. Data on pocket depth, clinical attachment level and interleukin 8 were in the normal distribution.

There is a statistically significant difference in PD measurements between the various timelines in the study (P< 0.001) [Figure 5]. Post Hoc Tukey HSD test show significant decrease from baseline to one month and baseline to three months. [Table 1]
There is a statistically significant decrease in CAL measurements from baseline to 1 month and 3 months after non-surgical treatment (P< 0.001). [Figure 6]. In terms of clinical attachment level, there was a highly significant decrease from baseline, one and three months which was revealed by Tukey HSD Post Hoc test. [Table 2].

Interleukin-8 (IL-8) levels did not significantly change across the time frames according to an ANOVA test [Figure 7]. A post-hoc test was not carried out because there was no difference in the timelines. Mean IL8 scores decreased from baseline to 4.45 (10.12%) after 1 month, while mean IL8 scores increased from baseline to 6.22 (14.14%) after 3 months. [Table 3]

Discussion

Periodontitis is an inflammatory disease of the tooth and its surrounding tissue. In periodontitis gram-negative anaerobes and other micro aerophilic bacteria like Porphyromonas gingivalis, Tannerella forsythia and Aggregatibacter actinomycetemcomitans that colonize tooth structure are responsible for disease initiation and progression. Page et al. [76] described that these microorganisms and various endotoxins produced by them, converts junctional epithelium to pocket epithelium which ultimately leads to tooth loss. The host responds with an immediate inflammatory and immune response against this constant microbial challenge. The initial response comprises of recognition of microbial components like Lipopolysaccharides (LPS) and bacterial DNA followed by production of inflammatory mediators, Reactive oxygen species (ROS), matrix metalloproteinases (MMPs), chemokines and cytokines. In addition, there is activation of antigen-specific lymphocytes and initiation of an adaptive response characterized by migration of other inflammatory cells at the site. One of the important cytokines which plays an important role in host defense is IL-8. [7]6]
Chand found inconsistent data about IL-8 levels in patients with Periodontitis in a systematic review and meta-analysis, which was explained by variations in collection methods (stimulated or unstimulated), processing (speed and time of centrifugation), preservation (temperature, time, and presence/absence of protease inhibitors), and biomarkers detection techniques could count for the differences in the results of the studies. [5]

IL-8 is a pro-inflammatory cytokine released by macrophages, lymphocytes, epithelial cells and endothelial cells and acts on neutrophils. IL-8 has an important role in neutrophil chemotaxis, phagocytosis and osteoclast differentiation and activity. For this reason, IL-8 is also known as 'Neutrophil Activating Factor'. It also up regulates expression of adhesion molecules on the surface of neutrophils enhancing leukotriene B4 production promoting neutrophil adherence to endothelial and epithelial cells. IL-8 is found to have a higher concentration of IL-8 in GCF before clinical signs of inflammation are apparent. Hence, it was inferred that IL-8 is an important chemical mediator in periodontitis. [3]

For this reason, IL-8 levels before and after periodontal treatment will be a good predictor of periodontal disease activity. This study was done with an aim of determining various clinical parameters and GCF levels of Interleukin-8 (IL-8) in periodontitis patients before and after non surgical periodontal therapy.

In this study micropipettes were used for GCF collection as suggested by Egelberg et al. [8] GCF collection in micropipettes occurs by capillary action. PD and CAL are recorded to determine extent of periodontitis because according to the American Academy of Periodontology these are most important parameters for diagnosing periodontitis. [9] Maxillary teeth were selected for GCF collection as gravity effects capillary action.

The results of this study show statistically significant reduction in Probing depth and gain in Clinical attachment level after non-surgical treatment. ($P<0.001$) Reduction in Probing depth and gain in Clinical attachment level both are not clinically significant at 1, 3 months after non-surgical treatment (approximately >1 mm). IL-8 concentration in GCF showed that results were not statistically significant for both timelines. Similar results were observed in a randomized control trial done by Chung et al. [10] They compared IL-8 levels in healthy and periodontitis patients and found higher levels of IL-8 concentration in healthy. Thus, we can conclude from present study that IL-8 levels increase after treatment due to improvement in gingival health. On the contrary, Mathur et al. [11] came to a conclusion in a case-control study that, IL-8 concentration is higher in diseased individuals as compared to non-diseased.

It has been observed that IL-8 concentration in GCF is influenced by activity of various enzymes in GCF. In periodontitis, during interaction with microorganisms azurophilic granules present in neutrophils release various lysosomal enzymes such as β-glucoronidase, elastase, myeloperoxidase and many more which may aggravate periodontal destruction. [12] Brandolini et al. [13] observed that when primed by IL-1β, IL-8 triggers neutrophil to release elastase. Finoti LS et al. [14] conducted a systematic review and meta-analysis determining connection of IL-8 levels in periodontitis and periodontally healthy patients. 31 studies were included which showed lower levels of IL-8 in GCF of periodontitis patients as weighed against periodontally healthy individuals but were variable.
It has also been observed that higher of α-Chemokine concentration is found at wound healing sites. Nanney et al. [15] showed that members of α-Chemokine family have specific affinity for IL-8 receptors on endothelial cells and this binding of IL-8 to IL-8R is responsible for healing effect in skin wounds. It can be said that IL-8 has an effective antibacterial response by the host, with both protective and destructive effects. But further research is required in order to establish this fact.

Presence of various systemic and local factors like diabetes mellitus, neutrophil disorders, pulmonary diseases, stress, smoking etc. may also influence host response to periodontal inflammation and hence, IL-8 levels. Engebretson S et al. [16] observed that GCF levels of IL-8 and β-glucoronidase were higher in adult patients with Type II diabetes mellitus as compared to those without Type II diabetes mellitus with similar amount of periodontal destruction.

Results of study illustrate a significant increase in IL-8 concentration in GCF along with reduction in probing depth and gain in clinical attachment levels after non-surgical periodontal treatment; this may indicates that IL-8 has a positive role and that it has a protective rather than destructive outcome on the periodontium.

Goutoudi P. et al. [17] concluded that the total amount of IL-8 in GCF may be considered more precise as compared to concentration of IL-8 in GCF. Hence, a higher level of study (Randomized Control Trial) could be carried out which also considers Total amount of IL-8 in GCF rather than only concentration of IL-8 in GCF in order to determine effect of non-surgical and surgical periodontal treatment on IL-8 levels in GCF with a longer follow up period. Further study determining the total amount and healing effect of IL-8 in GCF should be considered to determine the effect of non-surgical treatment on IL-8 levels.

Conclusion:

IL-8 has an important role to play in wound healing and inflammation. [18] It prompts deposition of type I collagen, fibronectin and tenascin, expedites the movement of fibroblasts and is a chemotactic to fibroblasts at the time of wound healing. IL-8 possesses anti-inflammatory activity where they impede the adherence of leukocytes to activated endothelial cells. [19] As evidence in this study shows that increased presence of IL-8 after 3 months of therapy may suggest the anti-inflammatory and defensive role of the chemokines on periodontium.

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Figure 1: Measurement of clinical parameters

Figure 2: Collection of Gingival Crevicular fluid

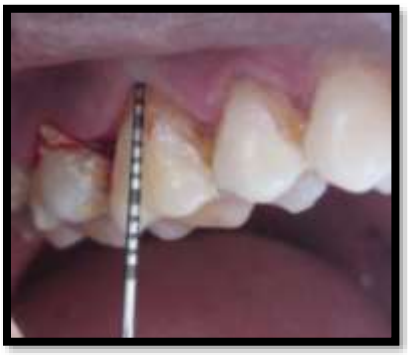

Figure 3: ELISA Kit

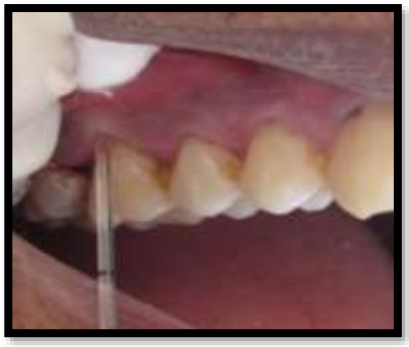

Figure 4: ELISA Reader

Figure 5: Probing Depth values at different timelines using ANOVA test

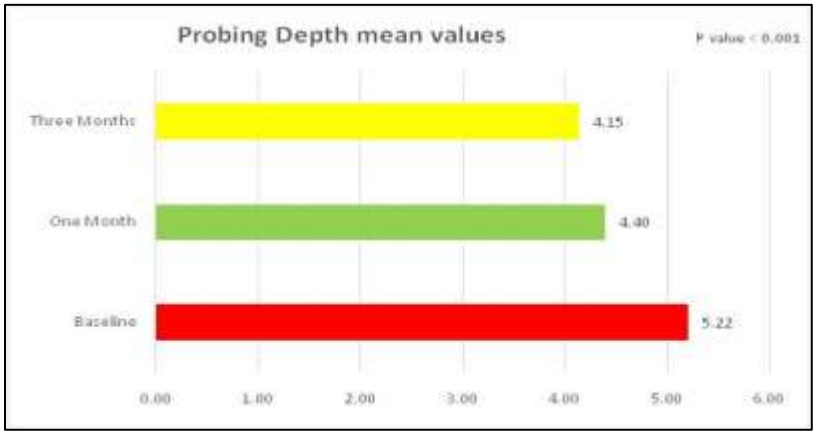

Table 1: Probing Depth at different timelines using Post Hoc Test

Probing Depth		Mean Difference	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Baseline	One Month	0.82 ± 0.19	0.001*	0.37	1.27
	Three Months	1.07 ± 0.19	0.001*	0.61	1.52

* Statistically Significant

Figure 7: Clinical Attachment Level at different timelines using ANOVA test

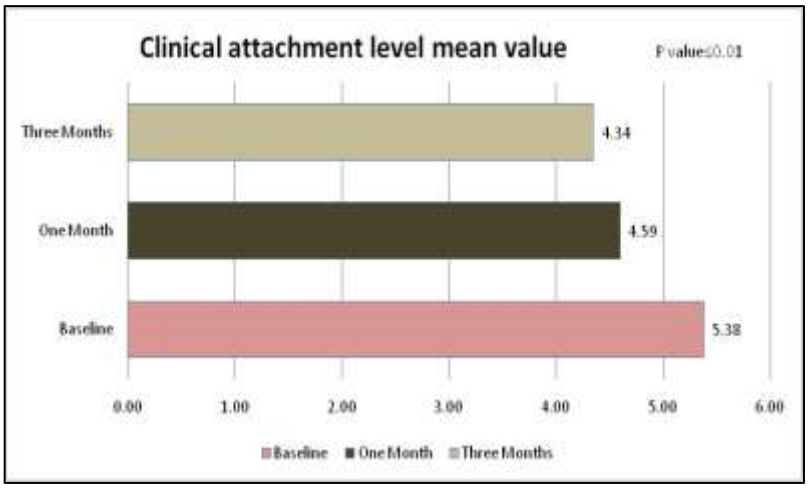

Table 2: Clinical Attachment Level at different timelines using ANOVA test

Clinical Attachment Level		Mean Difference	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Baseline	One Month	0.78 ± 0.19	0.001*	0.33	1.24
	Three Months	1.03 ± 0.19	0.001*	0.58	1.49

* Statistically Significant

Figure 8: Interleukin 8 levels using ANOVA test

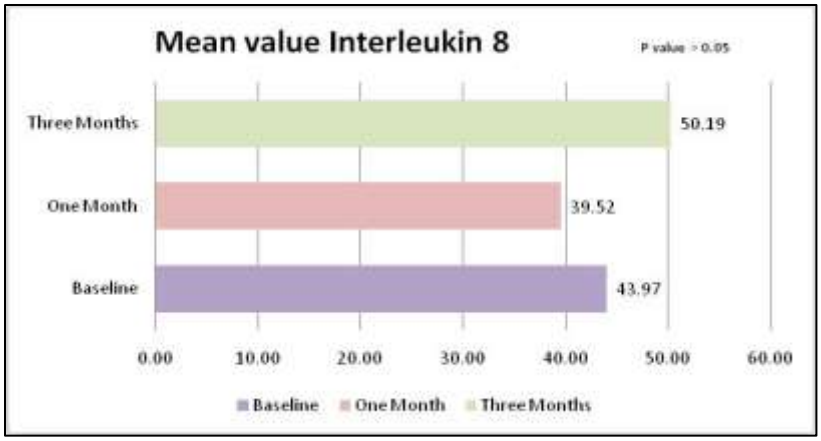

Table 3: Interleukin 8 levels at different timelines using Post Hoc Test

Interleukin 8		Mean Difference	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Baseline	One Month	4.45 ± 6.20	0.75	-10.37	19.27
	Three Months	-6.22 ± 6.20	0.58	-21.04	8.60