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# Comparision of Salivary CXCL10 Levels In Smokers and Non-Smokers With Stage III / IV Generalized Periodontal Disease

Research Article

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## Abstract

**Aim:** The aim of this study was to compare salivary CXCL10 levels with stage III and IV generalized periodontitis in smokers and non-smokers.

**Patients and Methods:** Subjects were classified as stage III and/or IV generalized periodontitis subjects, if they had pocket probing depth (PD) of ≥6 mm and/or clinical attachment level (CAL) of ≥5 mm in >30% of the teeth, tooth loss of ≤4 teeth, radiological bone loss extending to the middle third of root and beyond and vertical bone loss of ≥3mm. 84 subjects were recruited in this study, Group I consists of 42 non-smoker subjects with stage III and/ or stage IV generalized periodontitis and Group II consists of 42 smoker subjects with stage III and/ or stage IV generalized periodontitis. ELISA analysis was performed to determine the salivary CXCL10 levels.

**Statistics analysis:** A student t-test was used to compare continuous variables between two groups and Pearson's correlation test was used to correlate CXCL10 with clinical parameters for both the groups. A p-value of $< 0.05$ was considered statistically significant.

**Results:** Clinical parameters in both the groups were positively correlated with CXCL10 levels and higher concentrations of CXCL10 were found in group II subjects as compared with the group I subjects ($255 \pm 193.7$ and $84 \pm 35.8$, $P \leq 0.005$).

**Conclusion:** Smoking influences salivary CXCL10 levels in stage III and/or IV generalized periodontitis subjects.

**Keywords:** CXCL10; Periodontal Disease; Smoking; Biomarker And Saliva.

## Introduction

Periodontitis is a chronic irreversible inflammatory disease of supporting tissues of teeth caused by microorganisms [1], systemic diseases like coronary heart disease [2], diabetes mellitus [3], atherosclerosis and other risk factors like obesity [3], smoking [4], poor oral hygiene, stress, and depression [5] destroying alveolar bone loss with increased pocket probing depth, clinical attachment loss, and gingival recession. Smoking is considered as a significant risk factor for periodontitis as smokers are five times more prone to develop periodontitis [6]. smoking cessation has been shown to slow the progression of periodontal disease [7-9]. Smoking increases the risk of periodontitis by elevating colonization of periodontal pathogens [10], alters immune-inflammatory response elevating inflammatory mediators like NFκβ, PGE2, neutrophil elastase, and MMP-8 [11, 12]. In periodontitis elevated levels of inflammatory cytokines, chemokines, and other mediators like interleukin (IL)-1β, IL-6, IL-10, IL-12, interferon (IFN)-γ induced protein (IP)-10 or CXCL10 are elevated.

Based on chemical structure, chemokines are divided into two families: CXC and CC and their receptors are CXCR and CCR [13], they are further classified into C, CC, CX3C, and CXC subgroups. CXCL10 is a pro-inflammatory cytokine that is secreted by various cells like PMN, keratinocytes, fibroblasts, endothelial cells, monocytes [14-18]. IFN-γ binds to the IFN-γ receptor and activates the JAK-STAT pathway which leads to activation of CXCL10 transcription [19]. CXCL10 binds to the CXCR3 receptor which is present on Th1 cells, cytotoxic cells, and natural killer cells and triggers immune response at the site of infection as well as increases lymphocyte chemotaxis, thus elevating inflammatory response [20-22]. CXCL10 is an established biomarker in cardiac diseases like left ventricular dysfunction [23, 24]. Studies confirmed that CXCL10 contributes to bone resorption in dis-

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eases like rheumatoid arthritis [25-27]. Recently CXCL10 has received considerable attention as a biomarker for periodontal diseases owing to its influence on osteoclastogenesis [28]. Studies in CXCL10 in serum, saliva, and GCF have shown that CXCL10 levels are higher in periodontal diseased compared to healthy and concluded that CXCL10 can serve as a biomarker in periodontitis [29-31]. Further CXCL10 was studied in the saliva of smokers compared with non-smokers and it was found that smokers had higher levels than non-smokers. However, this finding was observed in a limited sample size, the findings cannot be conclusive. Therefore, the effects of smoking on CXCL10 chemokine levels in periodontal disease needs further clarification. This will help to understand the effect of smoking on CXCL10 levels. Although, CXCL10 levels have been evaluated in smokers and non-smokers of the periodontitis group but owing to the small smoker sample size definitive results couldn't be obtained. Thus, the aim of the present study was to detect and quantify the salivary levels of CXCL10 in smokers and non-smokers and also to check the correlation of the clinical parameters with CXCL10 in stage III or IV generalized periodontitis subjects.

## Materials and Methods

A total of 84 individuals were recruited by convenience sampling from the outpatient clinic of SRM Dental College and Hospital, Ramapuram, Chennai. They were categorized into two groups. Group I comprising of 42 stage III/IV Generalized periodontitis non-smokers subjects, Group II comprising of stage III/IV Generalized periodontitis smokers subjects. The study period for recruitment of people and sample collection was from December 2019 till February 2020. Before sample collection, Informed consent was obtained from all the subjects.

Inclusion criteria include 84 systematically healthy subjects of both genders with an age range of 20 to 50 years and patients with no drug history of immunosuppressants, anti-inflammatory, antibiotics also patients who didn't undergo any periodontal treatment during the past 3 months were included during this study. Sample size calculation was calculated from Aldahlawi s et al study with 80 percent power and 5% alpha error.

Age, gender, smoking habits, and clinical periodontal examination were assessed by one skilled examiner. Clinical parameters like pocket probing depth (PD) and clinical attachment loss (CAL) were calculated on all 6 sites per tooth (mesiobuccal, mid buccal, distobuccal, distolingual, mid lingual, and mesiolingual) with a UNC-15 probe and also Plaque index [32] and Gingival index [33] were recorded for 4 surfaces of all the teeth (mesial, distal, lingual or palatal, and labial or buccal). The diagnosis was made consistent with clinical parameters and radiographic evaluation proposed by the American Academy of Periodontology (AAP) in 2017 [34]. Both the groups include stage III and IV generalized periodontitis subjects. In group 2 current smokers were recruited as per CDC classification. Subjects with a history of periodontal therapy within the last 3 months, intake of antibiotics, immunosuppressants, and anti inflammatory drugs for the past 3 months, pregnant or lactating females, former smokers, and presence of any systemic disease were excluded from participating during this study. Patients were instructed to not take any food or liquids 2 hours before saliva sample collection. Before collection of saliva, patients were instructed to rinse their mouth with water, after which unstimulated whole mixed saliva was collected during a sterile container by spitting method [35]. After collection, saliva samples were centrifuged at 3000 rpm for 10 min and therefore the supernatant was stored at -80°C until further analysis. ELISA analysis - the amount of CXCL10 was analyzed in saliva employing a commercial ELISA kit (Raybiotech, GA, US). Standard solutions were prepared and diluted as per the manufacturer's instruction. Samples were added to CXCL10 coated wells and analysis was performed as per manufacturers instruction and absorbance values were determined by an ELISA Reader in picogram/milliliter values. All statistical analysis was performed using SPSS version 17 for Microsoft Windows. the data were expressed as Mean ± SD. An Independent sample student t-test was used to compare continuous variables between two groups. Pearson's correlation test was used to correlate CXCL10 with clinical parameters for both groups. A p-value of<0.05 was considered statistically significant.

## Results

A total of 84 subjects including both males and females were recruited in the study with a mean of 35 years ± 7.1 (range 20-50 years). In group I there was female predominance with 24 females and 16 male subjects and group II consists of 42 male smokers with mean of 34.7 ± 6.8 years.

Comparison of clinical parameters and CXCL10 between groups are shown in table 1, allperiodontal clinical parameters were highly significant. Comparison of CXCL10 levels in both the groups are shown in graph 1.

Spearman's rank correlation was performed to analyze the associations between the CXCL10 levels with clinical parameters (PD, CAL, PI and CI) in both the groups are shown in table 2 and table 3.

## Discussion

CXCL10, an inflammatory chemokine plays a role in periodontal bone remodelling under pathological conditions [13]. In osteoclast precursors, CXCL10 induces osteoclast differentiation, which helps to bind RANK to RANKL and contributes to bone resorption [36]. Further, it also upregulates alkaline phosphatase

**Graph 1. Represents CXCL10 or IP-10 levels in both the groups.**

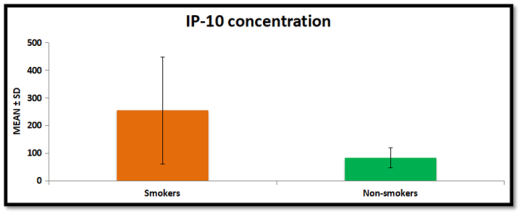

Table 1. Comparison of clinical parameters and CXCL10 between group I and group II.

| Clinical parameters and CXCL10 values | Group I (generalized periodontitis non-smokers) n=42 Mean and SD | Group II (generalized periodontitis smokers) n=42 Mean and SD | p value |
|---|---|---|---|
| GI | 2.2 ± 0.1 | 2.1 ± 0.2 | 0.127 |
| PI | 2 ± 0.1 | 2.2 ± 0.1 | .000** |
| PPD | 4.2 ± 0.4 | 4.5 ± 0.5 | 0.015 |
| CAL | 4.3 ± 0.4 | 4.7 ± 0.7 | .004** |
| CXCL10 | 84 ± 35.8 | 255 ± 193.7 | .000** |

**p < 0.05 is significant ,**p < 0.005 is very significant, GI= Gingival index, PI= Plaque index, CAL= Clinical attachment loss, PPD= Pocket probing depth, cxcl10

Table 2. Correlation of clinical parameters with CXCL10 levels in group I generalized periodontitis non-smokers. GI= Gingival index, PI= Plaque index, CAL= Clinical attachment loss, PPD= Pocket probing depth.

| Correlation of CXCL10 | GI | PI | PD | CAL |
|---|---|---|---|---|
| CXCL10 Pearson coefficient (r) | .376* | .414** | .815** | .824** |
| p value | 0.05 | 0.01 | 0.01 | .01* |

**Correlation is significant at 0.01 level
* Correlation is significant at 0.05 level

Table 3. Correlation of clinical parameters with CXCL10 levels in group II generalized periodontitis smokers. GI= Gingival index, PI= Plaque index, CAL= Clinical attachment loss, PPD= Pocket probing depth.

| Correlation of CXCL10 | GI | PI | PD | CAL |
|---|---|---|---|---|
| CXCL10 Pearson coefficient (r) | .761** | .749** | .962** | .954** |
| p value | 0.01 | 0.01 | 0.01 | .01* |

** Correlation is significant at 0.01 level
* Correlation is significant at 0.05 level

and beta N acetyl hexosaminidase enzymes that lead to bone remodelling by binding to CXCR3, a chemokine receptor produced by endothelial cells and expressed on osteoblasts [37].

The role of CXCL10 as a biomarker in periodontitis has been studied and it was found to be elevated in periodontitis [29, 30, 38, 40] and sometimes decreased in periodontal inflammation [39]. Therefore, the role of CXCL10 in periodontitis needs to be further studied. The influence of smoking on CXCL10 levels also needs further validation.

In the present study, periodontal parameters like pocket probing depth and clinical attachment loss were significantly correlating with CXCL10 levels in both the groups. This suggests that, in periodontitis there was an elevated level of CXCL10. Similar findings were observed by Shimada Y et al [29] in 2012, who observed 40 cytokines and chemokines in GCF of 11 periodontitis subjects and found that CXCL10 levels were elevated in sites with higher bleeding scores.

In a multiplex proximity extension assay Panezai et al [30] analysed 92 cytokines in serum samples of both rheumatoid arthritis subjects and healthy subjects, and observed chemokines like CXCL10 were positively associated with periodontitis.

In addition, Rath-Deschner et al [38] year 2020 found that chemokines like CXCL5, CXCL8 and CXCL10 levels were elevated in inflamed gingival sites compared to healthy sites in both humans and rats.

In the current study, between groups, the CXCL10 marker was found to be higher in smoking subjects with periodontitis which was statistically significant. This finding suggests that, despite similar clinical parameters, smoking influences CXCL10 levels.

Cigarette smoking is a modifiable risk factor and induces PMN respiratory burst and oxidative stress in periodontal tissues. In addition, it induces oxygen depletion in periodontal tissues and alters the response of PMN to periodontal bacteria. Smoking also influences chemokine levels in periodontal disease. Li Nie et al in 2008 observed that CXCL10 levels were elevated in mice exposed to cigarette smoking. Cigarette smoke elevates PMN and chemokines such as CXCL10, CXCL9, CXCL10 binds to receptor CXCR3 and attracts T lymphocyte at the inflammation site [40]. In CD4 T cells, CXL10 activates the ERK and Akt signalling pathways, which suggests that these signalling pathways may be involved in the RANKL and TNF induction of CXL10 in CD4 T cells. Kwak et al, 2008 concluded that CXCL10 induces osteoclast differentiation by inducing RANKL in CD4 T cells in a co-culture of osteoclast precursors and CD4 T cells [25]. In the present study, CXCL10 levels were detected in both the groups, clinical parameters like gingival index, plaque index, pocket probing depth and clinical attachment loss were statistically significant in both the groups. However, CXCL10 levels were elevated in group II compared to group I. Similarly, Souto G R et al [41] evaluated chemokines levels in gingival tissue samples of generalized periodontitis in smokers and non-smokers and observed elevated chemokine levels in smokers. Aldahlawi et al [31] also observed elevated levels of CXCL10 in saliva, serum and GCF of chronic periodontitis compared to gingivitis subjects and CXCL10 levels were elevated in smokers. However, statistical significance could not be obtained due to very less smoker subjects.

Results of the present study indicate that, CXCL10 is elevated in periodontitis and smoking causes elevation of this biomarker by altering PMN response and therefore causing chemokine increase. This exaggerates the periodontal inflammation as evidenced by high CXCL10 levels in this study. Smoking is a modifiable risk factor, and decreasing or modifying this risk can significantly reduce these chemokines. In future, CXCL10 levels before and after phase I nonsurgical therapy in periodontitis subjects might help us to assess if inflammation is related to CXCL10 levels.

There is no literature evidence showing elevated levels of CXCL10 in stage III or IV generalized periodontitis in smokers compared to stage III or IV generalized periodontitis in non-smokers, correlating systemic and local CXCL10 levels with clinical parameters. A larger sample size and duration of smoking status could have improved the results of the present study.

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