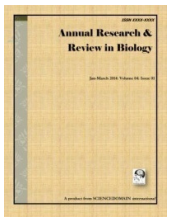

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Gingival Crevicular Fluid Levels of Interleukin – 18 in Chronic Periodontitis and Aggressive Periodontitis – A Comparative Study

S. Thirumalai[1*] and Sheeja Varghese[2]

[1]Department of Periodontics, KSR Institute of Dental Science and Research, Tiruchengode, Tamil Nadu, India.
[2]Department of Periodontics, Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to estimate and compare the levels of IL-18 in gingival crevicular fluid of chronic periodontitis patients, aggressive periodontitis patients and healthy controls and to correlate them with clinical parameters of the disease.
Materials and Methods: The study populations include chronic periodontitis (20), aggressive periodontitis (20) and healthy subjects (20). The clinical parameters such as PI, GI, PPD, and CAL were assessed. GCF samples were collected from the site with the deepest probing depth. The levels of IL-18 were analyzed by enzyme linked immunosorbent assay.
Results: The mean levels of IL-18 in GCF for healthy controls were 441.5±1434.2, for chronic periodontitis group it was 1160.4±3096.9, and for aggressive periodontitis group it was 784.3±1834.3. There was no statistically significant difference observed between the three groups (p=0.205). The correlations obtained between clinical parameters and IL-18 levels were found to be statistically insignificant.
Conclusion: There was no significant difference in the levels of IL-18 in GCF between healthy

**Corresponding author: Email: thirumalai.ney84@gmail.com;*

controls, chronic periodontitis and aggressive periodontitis groups. There is no significant correlation between the levels of IL-18 and clinical parameters such as PI, GI, PPD, and CAL in chronic periodontitis and aggressive periodontitis.

Keywords: Chronic periodontitis; aggressive periodontitis; Interleukin-18, GCF.

1. INTRODUCTION

IL-18 was first discovered in 1995 by Okamura et al. and was originally identified as interferon γ inducing factor. It is a pro- inflammatory cytokine and tumor –suppressive cytokine which due to its structure, receptor family and signaling pathways belongs to IL-1 cytokine family. It is produced by Kupffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts, and adrenal cortex cells. IL-18 is produced as a 24-kDa inactive precursor and is cleaved by IL-1β-converting enzyme (ICE, caspase-1) to generate a biologically active, mature 18-kDa peptide.

The primary functions of IL-18 include the induction of IFN-γ and TNF-α in T cells and natural killer cells and the up- regulation of T helper type 1 (Th1), cytokines including IL-2, granulocyte–macrophage colony-stimulating factor, and IFN-γ [1]. It plays an important role in the innate immunity and has been shown to induce not only Th1 cytokines but also Th2 cytokines, such as IL-4, IL- 5, IL-10, and IL-13 [2]. IL-18 alone can stimulate Th2 cytokine production as well as allergic inflammation [3].

It has been suggested that IL-18 might play an important role in gingival inflammation because the relatively high expression of IL-18 was found in gingival samples with increasing sulcular depth [4]. High gingival crevicular fluid (GCF) levels of IL-18 were found in inflamed sites from periodontitis patients regardless of severity of the disease when compared with gingivitis patients only. Moreover, shallow pockets in periodontitis patients had a significantly higher level and concentration of IL-18 when compared with shallow pockets in patients with gingivitis [5].

The aim of the present study was to evaluate and compare the levels of IL-18 in GCF in patients with chronic periodontitis and aggressive periodontitis and correlate them with the clinical parameters.

2. MATERIALS AND METHODS

2.1 Sample Size

The study population divided into 3 groups patients with chronic periodontitis (n=20), aggressive periodontitis (n=20), and healthy subjects (n=20).

2.2 Study Design and Inclusion Criteria

Subjects within the age group of 20-50 years both males and females with atleast twenty natural teeth were included in the study. Patients with healthy periodontium having a GI<1mm and PPD<3mm with no attachment loss were included in the healthy control group. Patients were categorized into chronic periodontitis having PPD $\geq$5mm with CAL 4mm were included according to AAP criteria 1999. Patients were categorized into aggressive periodontitis having PPD $\geq$5mm with CAL4mm were included according to AAP criteria 1999.

2.3 Exclusion Criteria

Smokers and former smokers, patients having systemic diseases such as diabetes mellitus, hepatitis, hypertension, heart disease, rheumatoid arthritis, patients taking medication such as phenytoin, cyclosporine, calcium channel blockers, patients who underwent periodontal treatment within six months before the study were excluded from the study.

The clinical parameters such as PI, GI, PPD, & CAL were assessed. PI and GI were measured for the six Ramfjord teeth (16, 12, 24, 44, 32, and 36) as well as for the site from which the sample was obtained. Probing pocket depth (PPD) and Clinical attachment loss (CAL) were measured using a Williams' periodontal probe for the entire dentition. The mean PPD and CAL were calculated for the entire dentition as well as the site from which the sample was obtained.

2.4 Collection of GCF Samples

GCF samples were collected from the site with the deepest probing depth. All the clinical examinations and sample site selections were performed on day 1 and GCF was collected on

the day 2. After gently drying the area, supragingival plaque was removed without touching the marginal gingiva and the area was isolated with cotton rolls to avoid saliva contamination. GCF was collected by placing a micropipette at the entrance of gingival sulcus gently touching the gingival margin. 1-5µl of GCF was collected using a 1to 5µl calibrated volumetric micro capillary pipette (Sigma–Aldrich, St. Louis, MO). Each sample was collected for a minimum of 10 minutes and the sites that did not express any GCF were excluded from the study. The collected GCF samples were immediately transferred to airtight plastic vial and stored at-70ºC until assayed.

2.5 Analysis of GCF Levels of IL-18

GCF samples were analyzed by enzyme-linked immuno- sorbent assay (ELISA). GCF levels of IL-18 were assayed with commercially available ELISA kit (Cusabio Biotech). The kit included assay plate, standard, biotin antibody diluents, sample diluents, HRP (Horseradish Peroxidase)-avidin diluent, biotin-antibody, HRP-avidin, wash buffer, TMB (tetra methyl-benzidine) substrate and stop solution. All procedures were performed according to the manufacturer's instructions.

2.6 Statistical Analysis

All statistical analyses were carried out using SPSS software version 17. The overall comparisons of IL-18 levels between the three groups were performed using Kruskal Wallis test and the pairwise comparison was performed using Mann Whitney U test. The correlation between IL-18 ation. $p<0.05$ was considered statistically significant. And the clinical parameters such as PPD, CAL, PI and GI were carried out using Karl-Pearson's correlation. $p<0.05$ was considered statistically significant.

3. RESULTS

A total of 60 subjects were recruited for the study (20 healthy controls, 20 in chronic periodontitis and 20 in aggressive periodontitis group) and IL-18 levels were assessed.IL-18 levels between the three groups were compared.

The mean levels of IL-18 for healthy controls was 441.5$\pm$1434.2, for chronic periodontitis group it was 1160.4$\pm$3096.9, and for aggressive periodontitis group it was 784.3$\pm$1834.3. The median values 70, 102 and 117 respectively. There was no statistically significant difference observed between the three groups (p=0.205) (Table 1 and Graph 1). Mann Whitney U test was used to compare the IL-18 levels between healthy controls and chronic periodontitis. The results reveals that there was no significant difference observed between the two groups (p=0.068) (Table 2). There was an insignificant difference observed between the healthy controls and aggressive periodontitis (p=0.289) (Table 3). We observed no significant difference between the levels of IL-18 in chronic periodontitis and aggressive periodontitis (p=0.679) (Table 4).

Table 5 represents the correlation of IL-18 levels with the full mouth clinical parameters (GI, PI, PPD and CAL) for both chronic periodontitis and aggressive periodontitis groups.

For the chronic periodontitis group, the correlation coefficients for gingival index with IL-18 level was 0.304 and for plaque index with IL-18 levels it was -0.122. There was a positive correlation between gingival index and IL-18 levels and negative correlation obtained between plaque index and IL-18 levels. The results showed no significant statistical analysis (p=0.193 and 0.607 respectively). The correlation coefficient for mean probing pocket depth with IL-18 levels was 0.272 and for mean clinical attachment loss with IL-18 levels it was 0.265. There was a positive correlation obtained between mean probing pocket depth, mean clinical attachment loss and IL-18 levels. The results revealed that there was no significant differences observed (p=0.246 and 0.260 respectively).

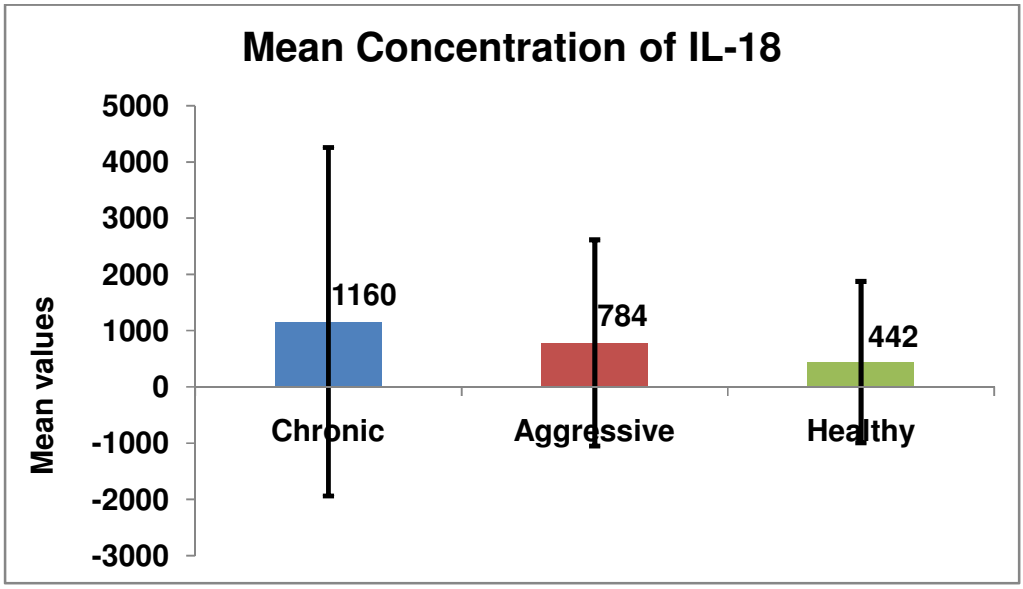
For the aggressive periodontitis group, the correlation coefficient for gingival index with IL-18 levels was -0.307 and for plaque index with IL-18 levels it was -0.209. There was a negative correlation obtained between gingival index, plaque index and IL-18 levels. These were no significant differences observed (p=0.188 and 0.377 respectively). The correlation coefficient for mean probing pocket depth with IL-18 levels was -0.186 and for mean clinical attachment loss with IL-18 levels it was -0.227. There was a negative correlation obtained between mean probing pocket depth, mean clinical attachment loss and IL-18 levels. The results were found to be not significant (p=0.432 and 0.336 respectively).

Table 6 represents the correlation of IL-18 levels with the clinical parameters (GI,PI, PPD and CAL), at the test site, for both chronic periodontitis and aggressive periodontitis groups.

Table 1. Comparison of IL-18 levels between the three groups

Groups	IL-18 levels (Mean±SD)	IL-18 levels (Median)	p-value
Healthy	441.5±1434.2	70	0.205
Chronic Periodontitis	1160.4±3096.9	102	
Aggressive Periodontitis	784.3±1834.3	117	

Kruskal Wallis test



Graph 1. Comparison of IL-18 levels between the three groups

Table 2. Comparison of IL-18 levels between healthy controls and chronic periodontitis

Groups	IL-18 levels (Mean ± SD)	IL-18 levels (Median)	p-value
Healthy	441.5±1434.2	70	0.068
Chronic Periodontitis	1160.4±3096.9	102	

Mann Whitney U test

Table 3. Comparison of IL-18 levels between healthy controls and aggressive periodontitis

Groups	IL-18 levels (Mean ± SD)	Il-18 levels (Median)	p-value
Healthy	441.5±1434.2	70	0.289
Aggressive Periodontitis	784.3 ±1834.3	117	

Mann Whitney U test

Table 4. Comparison of IL-18 levels between chronic periodontitis and aggressive periodontitis

Groups	IL-18 levels (Mean ± SD)	IL-18 levels (Median)	p-value
Chronic Periodontitis	1160.4±3096.9	102	0.678
Aggressive Periodontitis	784.3±1834.3	117	

Mann Whitney U test

For the chronic periodontitis group, the correlation coefficients for gingival index with IL-18 levels was -0.358 and for plaque index with IL-18 levels it was -0.241. There was a negative correlation obtained between gingival index, plaque index and IL-18 levels. The results revealed that there were no significant differences observed (p=0.121 and 0.307 respectively). The correlation coefficient for mean probing pocket depth with IL-18 levels was 0.008 and for mean clinical attachment loss with IL-18 levels it was 0.063. There was a positive

Table 5. Correlation of the full mouth clinical parameters with IL-18 levels in chronic periodontitis and aggressive periodontitis

Groups	Variables	Correlation coefficient	p-value
Chronic Periodontitis	GI	0.304	0.193
	PI	-0.122	0.607
	PPD	0.272	0.246
	CAL	0.265	0.260
Aggressive Periodontitis	GI	-0.307	0.188
	PI	-0.209	0.377
	PPD	-0.186	0.432
	CAL	-0.307	0.188

Table 6. Correlation of the clinical parameters at the test site with IL-18 levels in chronic periodontitis and aggressive periodontitis

Groups	Variables	Correlation coefficient	p-value
Chronic Periodontitis	GI	-0.358	0.121
	PI	-0.241	0.307
	PPD	0.008	0.974
	CAL	0.063	0.792
Aggressive Periodontitis	GI	-0.414	0.069
	PI	-0.420	0.065
	PPD	-0.321	0.168
	CAL	-0.228	0.334

Karl Pearson's correlation

GI: Gingival index; PI: Plaque index; PPD: Probing pocket depth; CAL: Clinical attachment loss

correlation obtained between mean probing pocket depth, mean clinical attachment loss and IL-18 levels. The results were found to be insignificant (p=0.974 and 0.792 respectively).

For the aggressive periodontitis group, the correlation coefficients for gingival index with IL-18 levels was -0.414 and for plaque index with IL-18 levels it was -0.420. There was a negative correlation obtained between gingival index, plaque index and IL-18 levels. These was no statistically significant differences were observed (p=0.069 and 0.065 respectively). The correlation coefficients for mean probing pocket depth with IL-18 levels were -0.321 and for mean clinical attachment loss with IL-18 levels it were -0.228. There was a negative correlation obtained between mean probing pocket depth, mean clinical attachment loss and IL-18 levels. The results reveals that there was an in significant differences observed (p=0.168 and 0.334 respectively).

4. DISCUSSION

Periodontitis is an inflammatory disorder characterized by the interaction between pathogenic bacteria and the immune and inflammatory responses of the host. A complex network of cytokines are involved in the inflammatory and immune responses in the inflamed periodontal tissues during the progression of periodontal disease [6,7]. These cytokines affect activation of macrophages and differentiation of $CD4^+$or $CD8^+$cells towards either a Th1 or Th2 phenotype. The balance between Th1 and Th2 phenotypes determines the successful resolution of inflammation.

IL 18, a proinflammatory cytokine in the IL-1 superfamily, has a unique capacity to induce either Th1 or Th2 differentiation depending on the immunological context. There is considerable evidence suggesting a defective transition from a Th1 to a Th2 cytokine profile within the gingival tissues and defective B cell activation by Th2 cells at sites with periodontitis [8,9]. Moreover, the role ofTh1 ⁄ Th2 responses in both chronic periodontitis [10,11,12] and aggressive periodontitis, remains unresolved.

IL-18 is up regulated in chronic diseases; also, the levels of IL-18 have been evaluated in gingivitis and periodontitis. The clinical parameters such as PI (Loe and Silness), GI (Silness and Loe), PPD, & CAL assessed to correlate the levels of IL-18 with the severity of the disease. The mean PPD and CAL were

calculated for the entire dentition as well as for the site from which the sample was obtained.

GCF composition reflects the nature and amplitude of the host response to the microbial plaque challenges. The collection of GCF is a minimally invasive procedure and the analysis of specific constituents in the GCF provides a quantitative biochemical indicator for the evaluation of the local cellular metabolism that reflects a person's periodontal health status [13].

GCF levels of IL-18 were assayed with commercially available ELISA kits. ELISA assays are sensitive and directly measure the quantity of antibody capable of reacting with a specific antigen without the possibility of interference by non-immunoglobulin binding material [14].

The overall comparisons of IL-18 levels between the three groups were performed and the pairwise comparison was performed. The correlation between IL-18 and the clinical parameters such as pocket depth, clinical attachment level, plaque index and gingival index were carried. $p<0.05$ was considered statistically significant.

In the present study, the mean level of IL-18 was highest in chronic periodontitis group (1160.4 ± 3096.9), followed by aggressive periodontitis group (784.3 ± 1834.3) and least in healthy subjects (441.5 ± 1434.2) whereas the median values were highest for aggressive periodontitis group (117), followed by chronic periodontitis (102) and least in the healthy group (70). When chronic periodontitis group was statistically compared with healthy group, the difference was not significant ($p=0.068$). This result is inconsistent with a previous study of Johnson et al. [4], which observed a significantly higher concentration of IL-18 in periodontitis sites compared to healthy sites. In their study gingival tissue samples were used whereas in the present study GCF was used to assess IL-18 levels. Figueredo et al. [5] founded significantly higher IL-18 in periodontitis sites compared with gingivitis sites in periodontitis patients and gingivitis patients. In their study the GCF samples were pooled from multiple sites, whereas in the present study GCF sample was collected from a single site. On the contrary, Turkoglu et al. [15] founded significantly higher IL-18 concentration in healthy group as compared to gingivitis and chronic periodontitis groups. Moreover they founded similar level of IL-18 concentration in periodontitis and gingivitis. In their study GCF was collected from a single site similar to the present study. Conflicting results among these studies as well as with our study could be explained by the differences in the methodology, and samples used, as well as inter individual variability in host response.

In the present study when IL-18 levels were compared between aggressive periodontitis and healthy patients, the difference was statistically insignificant ($p=0.289$). Similarly, while comparing the levels of IL-18 between chronic periodontitis patients and aggressive periodontitis patients, the difference was statistically insignificant ($p=0.678$).

In the present study, we hypothesized an increased level of IL-18 in chronic periodontitis and aggressive periodontitis as compared to healthy subjects. Also, a significant difference in IL-18 levels between aggressive periodontitis and chronic periodontitis was hypothesized.

These assumptions were made based on the fact that Th1 cytokines are involved in the strong innate response, leading to a gingival lesion whereas Th2 response leads to poor immune response and progressive periodontal lesion [16]. On the contrary, recent studies have stated that Th2 response favours periodontal attachment apparatus whereas Th1 response favours loss of periodontal attachment level [17]. These conflicting results point towards importance of both Th1 and Th2 cytokines and their imbalance in the pathogenesis of periodontal diseases. Moreover IgG2 is a predominant antibody present in aggressive periodontitis and is distinct in its production which is promoted by Th1 cytokines [18].

IL-18 is an important cytokine which regulates both Th1 and Th2 balance. So we expected a significant difference between chronic periodontitis and aggressive periodontitis patients, but the results did not support the hypothesis.

When individual values of IL-18 were analyzed we could find a wide variation among the patients within all the three groups. The concentration of IL-18 in chronic periodontitis group ranged from a maximum of 12000pg/ml to a minimum of 40pg/ml, for aggressive periodontitis it ranged from 7000pg/ml to 35pg/ml, and in healthy subjects from 6500pg/ml to 50pg/ml. This wide range of IL-18 concentration within each group was due to the outlander values of IL-18 in only a

few patients (1 patient in healthy, 2 in chronic and 2 in aggressive). These outlander values of IL-18 resulted in higher standard deviation and statistical insignificance inspite of higher mean as well as median values in the periodontitis group compared to healthy subjects.

The variation in individual values could have been either due to any undetected systemic conditions even though we have excluded all the possible confounding systemic diseases, or due to individual variation in host response.

Offenbacher et al. [19] analyzed multiple GCF biomarkers (33 markers) in experimental gingivitis in humans and found that the pattern of biomarker expression during induction and resolution of inflammation varied considerably among subjects with similar clinical responses. They also pointed out that cellular and molecular pathways are tightly integrated and coordinated with feedback that serves to reinforce specific response while dampening others.

Thus the influence of one cytokine depends upon the other cytokines and mediators in the local environment. This may explain the wide variability seen between the individuals in the same group.

When correlated with the clinical parameters of the disease, in the chronic periodontitis group, there was a positive correlation between the PPD, CAL and IL-18 levels at the test site as well as full mouth PPD, CAL and IL-18 levels. Also, there was a positive correlation between full mouth gingival index and IL-18 levels. There was a negative correlation between the full mouth plaque index and IL-18 levels, and between plaque index, gingival index at the test site and IL-18 levels. However, all these correlations were found to be statistically insignificant.

In the aggressive periodontitis group, there was a negative correlation between the full mouth clinical parameters as well as the test site clinical parameters with IL-18 levels. However, it was statistically insignificant.

These results were not consistent with a previous study by Schallhorn et al. [20]. They correlated the periodontal parameters (probing depth, attachment loss and bleeding on probing) with systemic levels of IL-18 and found that the extent of bleeding on probing exhibited a significant positive correlation with systemic IL-18 levels. The extent of clinical attachment loss also exhibited a correlation with IL-18 levels [20]. In their study, the clinical parameters were assessed as the percentage of sites whereas in our study we have assessed a mean full mouth PPD and CAL for each patient. Another study by Pradeep et al. also found a positive correlation between the levels of IL-18 in GCF and PPD and CAL [21].

In the present study GCF volume collected was not standardized, but to overcome this, for each sample a separate dilution factor was calculated and the concentration was calculated based on adjusted dilution factor individually.

Thus in the present study we could not find a significant difference in the IL-18 concentration between chronic periodontitis, aggressive periodontitis, and healthy subjects. Also, there was no correlation between GCF IL-18 levels and clinical parameters of the disease.

Evaluation of the levels of a single cytokine cannot predict the inflammatory status of the periodontal diseases as the immune and inflammatory course of periodontitis is governed by the involvement of a complex network of cytokines.

To clearly understand the role IL-18, cluster analysis of all the cytokine involved in the network is needed. Moreover, cross-sectional study, like this, cannot predict the exact role of this cytokine in the progression of the disease. Further, longitudinal studies analyzing multiple cytokines should be carried out to determine the role of IL-18 in the pathogenesis of periodontal diseases.

5. CONCLUSION

From this study it can be concluded that, IL-18 is present in GCF samples of healthy subjects, chronic periodontitis patients and aggressive periodontitis patients. There is no significant difference in the levels of IL-18 in GCF between healthy controls, chronic periodontitis and aggressive periodontitis. There is no significant correlation between the levels of IL-18 and clinical parameters such as gingival index, plaque index, probing pocket depth and clinical attachment loss in chronic periodontitis and aggressive periodontitis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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