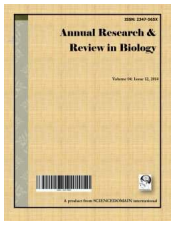

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Levels of Interleukin-18 in Saliva and Gingival Crevicular Fluid in Patients with Chronic Periodontitis and Healthy Subjects

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Authors' contributions

This work was carried out in collaboration between all authors. Author ZRE designed the study, wrote the protocol and interpreted the data. Authors JZR and FR anchored the field study, gathered the initial data and performed preliminary data analysis. Author DZ managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Cytokines play a key role in the initiation and progression of chronic periodontitis. This study aimed to measure and compare the levels of Interleukin (IL)-18 in both saliva and gingival cervicular fluid (GCF) of patients with chronic periodontitis and healthy controls.
Study Design: In this descriptive study we assessed the levels of IL-18 in unstimulated whole saliva and GCF samples among patients with chronic periodontitis and individuals with healthy periodontium.

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Place and Duration of Study: Department of periodontology and Department of biochemistry, Services Institute of Shahid Sadughi University of Medical Science, between May 2015 and Jan 2016.
Methodology: Thirty two subjects were divided into two groups; 16 individuals with healthy periodontium and 16 patients with chronic periodontitis. GCF and saliva samples were obtained. The concentrations of IL-18 in GCF and saliva were determined using ELISAs.
Results: There was no significant difference between the levels of IL-18 in saliva and GCF samples among the study groups (Respectively *P*= 0.44 and *P*= 0.38). Also, significantly different was not observed while comparing the IL-18 level of saliva with the IL-18 level of GCF in both healthy subjects (*P*= 0.8) and patients with chronic periodontitis (*P*= 0.61).
Conclusion: Similar levels of IL-18 among study groups suggested that levels of IL-18 in saliva and GCF cannot be used as a predictable biomarker for early diagnosis of periodontal disease.

Keywords: Gingival crevicular fluid; interleukin-18; periodontitis; saliva.

1. INTRODUCTION

Periodontal disease is an infectious disease, which occur mainly as a result of activation immune-inflammatory defense mechanism against dental biofilms. It has been postulated that proinflammatory cytokines production leads to tissue destruction and disease progression [1,2]. Cytokines play a critical role in the initiation and progression of chronic periodontitis [3]. IL-18 with proinflammatory and tumor-suppressive properties was first discovered in 1995 by Okamura et al. [4] and formerly termed interferon-γ-inducing factor [5]. IL-18 is a member of the IL-1 superfamily that is produced mainly by activated macrophages, dendritic cells, Kupffer cells, keratinocytes, intestinal epithelial cells, osteoblasts and adrenal cortex cells [1,5-7]. It is unique to having a capacity to induce both T helper 1 (Th1) and Th2 cytokines according to the immunological context [8]. Furthermore, the control of Th1 and/or Th2 expression is fundamental to the immuneregulation of periodontal disease [1,9].

IL-18 is described as being upregulated in various chronic diseases [10]. It consist in promoting activation of neutrophils [11] and modulation of IL-1β production [12]. In some studies a direct correlation between the severity of periodontal disease and level of IL-18 has been found [13,14-18]. However, in another study, a significant difference in the concentration of IL-18 in GCF of periodontitis and healthy control group was not observed [19]. Due to discrepancies among studies with regard to association between IL-18 and periodontal disease, and since seldom studies have been conducted to assess the concentrations of IL-18 in saliva, this study aimed to measure and compare the concentration of IL-18 in both saliva and GCF of patients with chronic periodontitis and healthy subjects.

2. MATERIALS AND METHODS

2.1 Subjects

Thirty two individuals, 17 males and 15 females, in the age range of 25-50 years were enrolled in the study. All individuals gave their written informed consent. Institutional ethics review committee approval for the study was obtained. The healthy control group contained 16 subjects with healthy periodontium having a gingival index (GI) <1 mm and probing pocket depth (PPD) <3 mm with no attachment loss. The test group comprised 16 patients with chronic periodontitis, according to American Academy of Periodontology (AAP) criteria 1999, having PPD ≥5 mm with CAL 4 mm at least in two teeth. The exclusion criteria were pregnancy, smoking, systemic disease and history of periodontal therapy as well as antibiotics or anti-inflammatory drug treatment within past 6 months.

2.2 Sampling

The Saliva and GCF samples were collected between 10 and 11:30 am. All participants were requested not to eat or drink for at least two hours before sampling. Seated patients were instructed to rinse the mouth thoroughly with water. After 15 minutes they asked to allow saliva to pool in the bottom of the mouth and spit it into sterile collection tubes every minutes for 5 minutes called non-stimulated spitting method.

Subsequent to saliva sampling, GCF samples were obtained from the site with the deepest probing depth. The selected teeth were isolated with cotton rolls and air dried. Supragingival plaque was removed carefully with a sterile

scaler. GCF was collected by placing paper points at the entrance of the gingival pocket, and gently touching the marginal gingiva. It was ensured that the samples were not contaminated by saliva or blood. Samples were immediately transferred to sterile microtubes containing 250 µl phosphate-buffered saline (PBS). All samples were immediately frozen at 70ºC until subsequent cytokine analysis.

2.3 Detection of IL-18

The samples were centrifuged at 3000 g. The ELISA development kits [Bender MedSystems, Vienna, Austria) were used to analyze the concentrations of IL-18 in both saliva and GCF samples. All procedures were carried out in duplicate according to the manufacturer's instructions. Optical densities at 405 nm were measured (reference wavelength 630 nm). The concentrations of IL-18 in the saliva and GCF were then determined by comparing the average absorbance readings of each sample with the concentrations in the assay standard curve.

2.4 Statistical Analysis

All statistical analyses were carried out using SPSS software (version 16). Comparisons of IL-18 levels between two groups were made with Mann-Whitney Test for saliva and Independent T-Test for GCF. Furthermore, pairwise comparisons were performed using paired t-test for detect possible correlation between IL-18 levels in saliva and GCF of same subjects. Probability values <0.05 were considered statistically significant.

3. RESULTS

Median and mean values of Salivary and GCF concentrations are outlined in Table 1. In this study we have assessed IL-18 levels in GCF and saliva of 30 subjects (16 healthy controls and 16 in chronic periodontitis group).

There was no significant difference between the levels of IL-18 in saliva samples of patients with chronic periodontitis and healthy subjects (*P*= 0.44) (Fig. 1). Similarly, there was an insignificant difference between IL-18 levels of GCF in patients with chronic periodontitis and healthy subjects (*P*= 0.38) (Fig. 2). While comparing the IL-18 level of saliva with the IL-18 level of GCF, significantly different was not observed in both healthy subjects (*P*= 0.8) and patients with chronic periodontitis (*P*= 0.61).

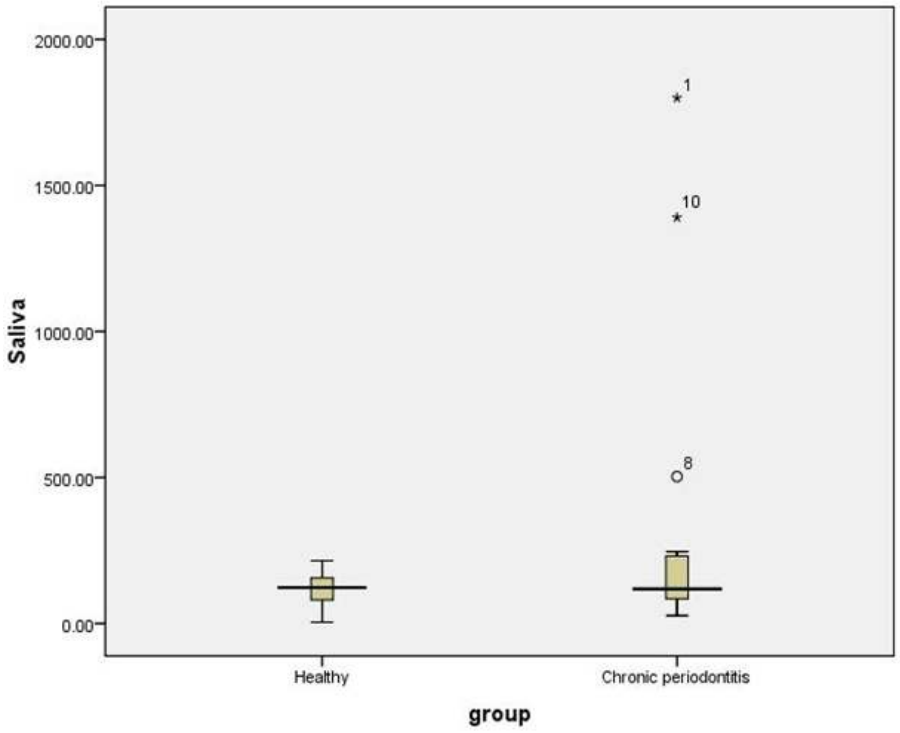

Fig. 1. Box plot of mean levels of IL-18 for saliva in healthy subjects and chronic periodontium

Table 1. Salivary and GCF concentrations of IL-18 in the study groups

	Healthy (n=16)		Chronic periodontitis (n=16)		P. value
	Median (pg/ml)	Mean (SD)	Median (pg/ml)	Mean (SD)	
GCF	130.09	139.17 (58)	0.38	123.96 (30.9)	0.38*
Saliva	122.53	117.61 (60)	0.44	340.63 (527.6)	0.44*
P. value	0.8*		0.61*		

SD, Standard deviation, insignificant*

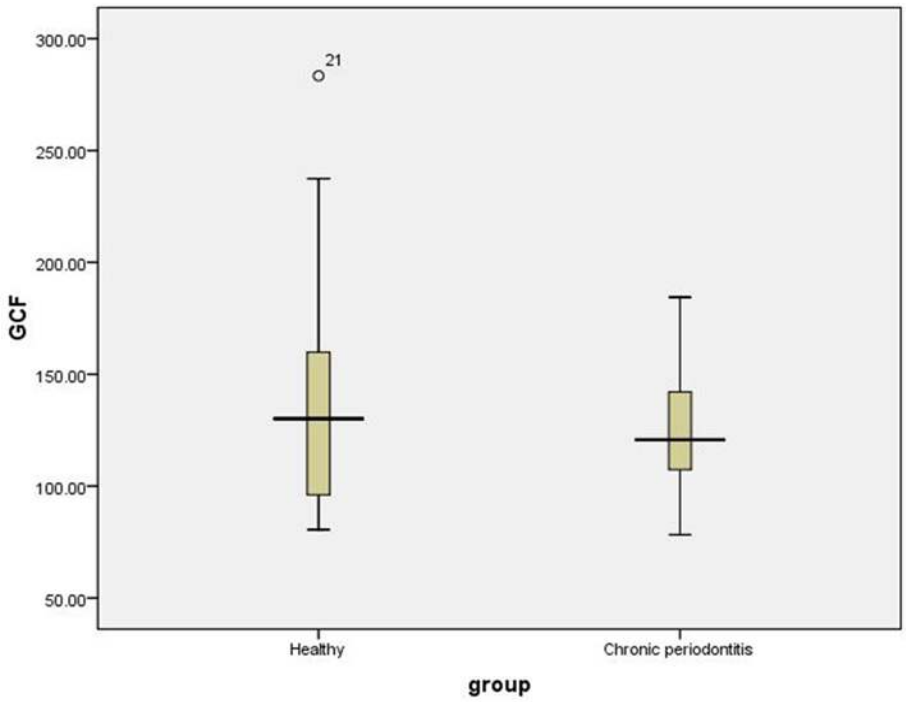

Fig. 2. Box plot of mean levels of IL-18 for GCF in healthy subjects and chronic periodontium

4. DISCUSSION

GCF composition reflects the periodontal inflammatory status as a result of the interplay between the bacterial biofilm and periodontal tissues. The collection of GCF is a minimally invasive procedure and the analysis of specific components of GCF provides a quantitative biochemical marker for the assessment of periodontal health condition [20]. Saliva could be used in the diagnosis of oral and systemic diseases. Salivary biomarkers such as cytokines could be used to monitor periodontal health status or periodontal disease initiation and progression. Saliva could not only be used to help diagnose oral diseases, but it would also be used in the diagnosis of systemic disorders. This has been considered as the possible alternative to classic examination for early disease diagnosis [21,22].

Cytokines play crucial roles in the inflammatory and immune responses during periodontal disease progression [23]. Monitoring cytokine production may be particularly useful to diagnose an individual's periodontal disease initiation and progression [24]. IL-18, a proinflammatory cytokine in the IL-1 superfamily, induce interferon γ (INF-γ) production in T cells and promotes Th1 and Th2 cytokines upregulation [10].

Therefore, we assumed that the levels of IL-18 were increased in chronic periodontitis as compared to healthy subjects. Contrary to this assumption, in the present study, no significant increase was found in the level of IL-18 in saliva and GCF of chronic periodontitis compared with healthy subjects. The wide range of IL-18 concentration in saliva of chronic periodontitis group, was observed as follows: a maximum of 1799.72 pg/ml to a minimum of 26.56 pg/ml. It

was due to the outlander values of IL-18 in only 3 subjects and resulted in statistical insignificance because of high standard deviation of 527.56 in this group despite the higher mean values in the periodontitis group compared to healthy group. This variation in the level of IL-18 of samples could be because of any unknown systemic conditions in spite of excluding all the detected effective systemic disease or due to diversity in host immune response of individuals.

A similar result in a recent study demonstrated no significant difference in GCF level of IL-18 in healthy group as compared to gingivitis and chronic periodontitis groups [25]. Recently, Thirumalai et al. demonstrated no significant difference in the level of IL-18 in GCF of periodontal disease and healthy subjects which is in accordance with our findings [19].

Contrary to the result of these studies, Johnson et al. found significantly higher IL-18 concentration in periodontitis sites as compared to healthy sites. In their study gingival biopsies were used, whereas in the present study GCF was used to assess IL-18 level. In the study carried out by Orozco et al. GCF level of IL-18 in shallow inflamed sites in periodontitis patients were increased when compared with gingivitis sites in control gingivitis patients. In contrast to our study, they assess the correlation between severity of periodontal disease and levels of IL-18 [13].

Figueredo et al. [14] found GCF level of IL-18 to be increased in periodontitis patients compared with gingivitis patients. In their study, the GCF samples were pooled from a subset of multiple sites, whereas in the present study GCF sample was collected from a single site. Conversely, their emphasis was on the progression of gingivitis to periodontitis, without studying in healthy subjects. Also, finding of Pradeep et al. in detecting the role of GCF IL-18 in periodontal health and disease was against our result [15].

In the researches conducted by Banu et al. and also by Ozçaka et al. salivary IL-18 levels were measured. The results from these studies demonstrated increased salivary IL-18 levels in patients with chronic periodontitis compared with healthy subjects. These findings are inconsistent with our results; however, it could be explained by the differences in the methodology, and samples used, as well as inter individual variability in host response [16,26].

Evaluation of the levels of multiple biomarkers might be more useful to determine the inflammatory status of periodontal diseases due to involvement of a complex network of cytokines in disease initiation and progression. Additional, to clearly understand the role of IL-18 in the pathogenesis of periodontal diseases, longitudinal studies assessing multiple cytokines should be carried out.

5. CONCLUSION

Based on the results of this study, IL-18 is present in saliva and GCF samples of both healthy and chronic periodontitis groups. However, there was no significant correlation between IL-18 concentration and periodontal status. Consequently, levels of IL-18 in saliva and GCF cannot be used as a predictable biomarker for early diagnosis of periodontal disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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