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Interleukin-17 and interleukin-23 levels in gingival crevicular fluid of patients with chronic and aggressive periodontitis

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Abstract

Introduction: *Interleukin-17 is a pro-inflammatory cytokine with a wide range of protective and destructive effects in periodontitis. The role of IL-23 is stabilisation and expansion of Th-17. The aim of this study was to assess whether patients with aggressive and chronic periodontitis exhibit different gingival crevicular fluid (GCF) concentrations of IL-17 and IL-23 compared with clinically healthy subjects.*

Material and methods: *GCF samples were obtained from 32 patients: 10 with chronic periodontitis (CP), 12 with aggressive periodontitis (AgP), and 10 healthy controls (HC). IL-23 and IL-17 concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Comparison of study groups was performed with ANOVA and Tukey HSD tests. Spearman's correlation coefficient was used to assess correlations between the variables.*

Results: *IL-17 concentration was significantly higher in the healthy group compared to the AgP and CP groups ($p < 0.001$), but there were no significant differences between the CP and AgP groups. IL-23 levels in the healthy group were significantly higher than that in the AgP group ($p < 0.001$). Cytokine concentrations did not correlate significantly with probing depths and clinical attachment levels.*

Conclusions: *Gingival crevicular fluid concentrations of IL-17 and IL-23 were significantly higher in the healthy group compared to periodontitis groups.*

Key words: *interleukin-23, interleukin-17, chronic periodontitis, gingival crevicular fluid, aggressive periodontitis.*

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Introduction

Periodontitis is defined as an inflammatory disease of tooth supporting tissues and is classified into different types based on clinical, radiographic, laboratory and historical features of the disease. Chronic periodontitis is the most common form of the disease. In this type of disease, the amount of periodontal tissue destruction is consistent with local factors and the disease progression rate is slow to moderate. Aggressive periodontitis is another subgroup of periodontal disease that is characterized by a rapid rate of attachment loss in systemically healthy individuals. The disease shows a familial aggregation. The amount of tissue destruction seems to be inconsistent with the amount of plaque and calculus [1].

Periodontal tissue destruction that occurs in periodontitis is a result of the interaction between periodontopathic pathogens and the host immune system [2]. Although exposure to pathogens is the prerequisite for disease initiation, subsequent progression in large part seems to be characterized by the host immune response and cytokine profile [3, 4].

Some hypotheses were proposed to explain the dissimilarities in the clinical manifestation of chronic periodontitis and aggressive periodontitis. Several investigations have focused on aspects responsible for increased susceptibility to periodontal destruction in aggressive periodontitis, including a particular microbial profile [5], functional defects of neutrophils and monocytes [6, 7] and the periodontal levels of inflammatory cytokines/chemokines [8].

It has been suggested that T-cell have a determinant role in the individual's susceptibility to advanced and probable aggressive periodontal destruction. Accordingly, studies have focused on the roles of different types of

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T-cells in the pathogenesis of periodontitis [9-15]. Th-17 is a novel subset of CD4+ (T helper) that is differentiated in the presence of the transforming growth factor (TGF-β), IL-6, IL-1β, IL-21 and was expanded specifically by IL-23. These cells predominantly secrete IL-17 and their name comes from this cytokine [16].

Interleukin-17 is a newly introduced pro-inflammatory cytokine, with a wide range of protective and destructive activities [11, 16-18]. IL-17 has a crucial role in neutrophil homeostasis and can induce epithelial cells, fibroblasts and osteoblasts to release a variety of inflammatory cytokines/chemokines [17]. Interleukin-17 was shown to have a protective function against extracellular pathogens [19], with a central role in innate immunity [20]. Furthermore, it is suggested that this interleukin has a high capacity to induce osteoclastogenesis through promotion of the receptor activator of NF-Kb ligand (RANKL) on osteoclastic cells [21]. Therefore, according to both the protective and destructive effects of IL-17, it appears it could act as a double-edged sword in periodontal diseases. IL-23 secreted by macrophages and dendritic cells after encountering microorganisms and their products plays a dominant role in IL-17 production [16, 22]. There is increasing evidence about the correlation between IL-23 and IL-17 pathway and periodontitis [9, 10, 18, 22–27]. The amount of IL-17 and IL-23 in tissue samples, saliva as well as GCF of patients with different types of periodontal diseases has been investigated [9, 10, 22, 24, 26, 28] but there are not enough data on the relationship between IL-17 and IL-23 levels and severity of periodontal diseases. Gingival crevicular fluid is an exudate from periodontal tissues and the assessment of such proteins and biomarkers in gingival crevicular fluid (GCF) has been introduced as a non-invasive method to monitor the progression of periodontal diseases [29-31]. So, the present study was designed to assess IL-17 and IL-23 levels in the GCF of patients with aggressive periodontitis and compare it with chronic periodontitis and periodontally healthy subjects.

Material and methods

Study population

The study population was selected from individuals referred to the Department of Periodontics, Faculty of Dentistry, Shahed University from October 2013 to June 2014. Thirty-two systematically healthy individuals were divided into three groups according to the periodontal status: 12 patients with aggressive periodontitis (AgP), 10 patients with moderate to severe chronic periodontitis (CP) and 10 periodontally healthy subjects (HP). Chronic periodontitis was defined as having at least 5 teeth with a probing depth of ≥ 5 mm and a clinical attachment loss of ≥ 3 mm and presence of BOP. Generalized aggressive periodontitis was diagnosed as < 35 years of age, at least three permanent teeth other than first molars/incisors with probing depth (PD) and clinical attachment level (CAL) > 5 mm, and familial aggregation [1, 2]. The control healthy group had no signs of bleeding on probing (BOP), gingival inflammation, attachment loss and increased pocket depth. Subjects with a history of periodontal treatment, including scaling and root planing and/or receiving antibiotic or anti-inflammatory drugs during the last 6 months, were not included in the study. Also, subjects who had any allergic, inflammatory and autoimmune diseases were excluded from the study. The Clinical Ethics Committee of Shahed University, Tehran, Iran, approved the study protocol (approval code: IR.Shahed.REC.1394.147). The aims of the study were fully explained and informed consent was obtained from all the participants.

Full-mouth periodontal PD and CAL were recorded and full-mouth periapical radiography were performed for each patient.

Site selection and GCF collection

After periodontal examination and supra gingival prophylaxis, GCF samples were collected on the subsequent day to prevent contamination of samples with blood. In the periodontitis groups, GCF was collected from two deepest and nonadjacent pockets that had BOP. In the control group, the samples were obtained from mesiobuccal sulci of the upper first molars. On the day of sampling, the selected area was isolated with cotton rolls to prevent saliva contamination and dried with a blast of air. Supragingival plaque was removed gently with a periodontal curette, without touching the gingival margin. Then a paper strip (Periopaper, Oraflow Inc., USA) was placed in the pocket/sulcus and left there for 30 seconds. Any paper strips that were contaminated with blood or saliva were excluded from the study. Following GCF collection, the samples were transferred to an air-tight microtube and stored at –20°C.

IL-17 and IL-23 assay

After collection of samples, the paper strips were placed in 150 µL of the phosphate-buffered saline (PBS) solution. Then the samples were centrifuged at 10,000 rpm for 30 minutes. These samples were assayed for IL-23, using a human IL-23 ELISA kit (Bender Med Systems GmbH, Austria (catalog no. BMS2017)) and for IL-17 using a human IL-17A ELISA kit (Bender Med Systems GmbH, Austria (catalog no. BMS2023)) based on the manufacturer's instructions.

Statistical analyses

The Shapiro-Wilk test was applied to evaluate normal distribution of data. Levene's test was used for assessing the equality of variances in different groups. The significance of difference between groups was assessed by one-

way ANONA and Tukey tests. Spearman's correlation coefficient was used to assess any correlation between cytokine concentrations and clinical parameters. Statistical significance was set at $p < 0.05$.

Results

Our study population consisted of 32 patients (11 males and 21 females). The demographic and periodontal parameters of the study population are presented in Table 1. The concentrations of IL-17 and IL-23 in GCF of the three study groups are shown in Figure 1.

The mean concentration of IL-17 in GCF samples in AgP, CP and H controls was 0.89 ±0.58, 1.46 ±1.20 and 22.81 ±23.63, respectively and was significantly different between the three groups ($p < 0.001$). Pairwise comparisons (Tukey tests) indicated that IL-17 concentration was significantly higher in the healthy group than the AgP and CP groups ($p < 0.001$) but there was no significant difference between the CP and AgP groups.

The IL-23 level in the healthy group (mean ±SD: 186.55 ±183.51) was significantly higher than that in the AgP group (mean ±SD: 34.34 ±47.59) ($p < 0.001$) but the concentration of this cytokine was not significantly different between the CP and healthy, and CP and AgP groups ($p > 0.1$). Spearman's rho correlation analysis indicated a significant correlation between the concentrations of IL-17 and IL-23 in all groups but there was no significant correlation between cytokine concentrations and periodontal clinical parameters, including probing depths (PD) and clinical attachment levels (CAL).

Table 1. Patients' demographic and clinical characteristics

Clinical parameters	AgP patients ($n = 12$)	CP patients ($n = 10$)	H controls ($n = 10$)
Gender	2 male/ 10 female	5 male/ 5 female	4 male/ 6 female
Age (years)	28.4 ±5.9	45.8 ±10.48	30 ±5.2
CAL (mm; mean ±SD)	6.57 ±3.66	5.16 ±1.82	0
PD (mm; mean ±SD)	6.4 ±0.97	6.4 ±1.06	2.01 ±0.61

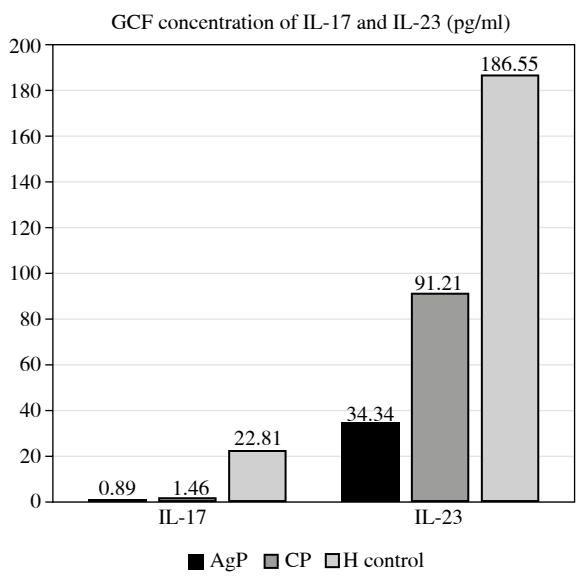

Fig. 1. The concentration of IL-17 and IL-23 in the study groups. AgP – aggressive periodontitis, CP – chronic periodontitis, H control – healthy control

Discussion

The periodontal activity is mainly mediated by immune responses and a complex network of inflammatory mediators. Gingival crevicular fluid is an exudate from periodontal tissues and the assessment of biomarkers in GCF has been introduced as a non-invasive method to monitor the progression of periodontal diseases [29, 30]. In a systematic review, a chemokine/cytokine profile in the GCF of aggressive and chronic periodontitis was compared. It was concluded that although in some studies a higher pro-inflammatory crevicular cytokine/chemokine level has been observed in aggressive periodontitis than chronic periodontitis, the data are conflicting [8]. In this study the concentrations of IL-17 and IL-23 in the GCF were examined and compared in aggressive periodontitis, advanced chronic periodontitis and periodontally healthy subjects.

Our data demonstrated that IL-17 concentrations in GCF of healthy samples were significantly higher than those in periodontitis groups ($p < 0.001$) and between periodontitis groups, the concentrations of IL-17 in CP subjects were higher than those in GAP patients. Similar results have been reported in previous studies [9, 24, 28, 32]. Ay *et al.* in two separate investigations found that the concentrations of IL-17 were significantly lower in the CP group and in aggressive periodontitis subjects than healthy controls; furthermore, the total amount of IL-17 decreased in deeper pockets [24, 32]. Johnson *et al*. evaluated the gingival concentrations of IL-17 in different pocket depths. They showed lower concentrations of IL-17 in the gingiva adjacent to ≥ 6 mm pockets [2]. Both chronic and aggressive periodontitis patients in our study suffered from the advanced disease, and the mean probing depth in both groups was about 6.5 mm.

Shaker and Gallab evaluated IL-17 GCF levels in chronic and aggressive periodontitis. Similarly to our results, the concentration of IL-17 was higher in healthy subjects than GAP, but given the higher total amount of IL-17 in GAP patients and lower levels of IL-17 after non-surgical periodontal therapy, they concluded that IL-17 may

have a potential role in the etiopathogenesis of periodontal diseases [9].

In this study population, the concentration of IL-23 was also higher in the healthy group compared to the GAP ($p < 0.001$), and a positive correlation was found between the concentrations of IL-17 and IL-23 in all the groups. Lester *et al*. evaluated the IL-17 and IL-23 concentrations in the gingiva from normal and inflamed sites. Similarly to the present study, they found that the concentration of these interleukins was positively correlated with each other [33]. Since it is known that IL-23 can induce clonal expansion of Th-17 and stimulate IL-17 production, this positive correlation between the two interleukins can be explained.

In the present study, no significant correlation was found between crevicular concentrations of IL-17 and IL-23 and clinical attachment loss even though a positive correlation has been shown in other studies between serum [34] and gingival [33] concentration of IL-17 and clinical attachment loss.

In contrast, some investigations have revealed higher levels of IL-17 in the GCF of periodontitis patients [9, 10, 22, 34]. Cifcibasi *et al*. reported more elevated total amounts of IL-17 and IL-23 in the GCF of generalized aggressive patients than healthy controls and attributed the difference to the possible role of these pro-inflammatory cytokines in the periodontal pathogenesis [10]. In a study by Vernal *et al*., the total amount of crevicular IL-17 was higher in patients with periodontitis than in healthy participants. But the IL-17 concentration exhibited a reverse pattern and was higher in the healthy group [22]. It seems the discrepancies in the results of the reports are somehow related to the way the outcomes are presented (i.e. the concentration or total amount). Therefore, it is known that the crevicular concentration of the biomarkers may decrease because of the higher volume of GCF in inflamed sulci and/or pockets in inflamed sites than healthy gingival sites. Furthermore, since IL-17 is a potent cytokine in bone resorption, its lower concentration in periodontally affected sites may be due to its consumption. So, there will not be a sufficient concentration of IL-17 remaining in GCF of periodontal diseases. In the case of IL-23, as it is a potent inducer of IL-17, it actively participates in IL-17 synthesis in order to cause more tissue destruction [24, 25].

In conclusion, based on our results, the crevicular concentrations of IL-17 and IL-23 were lower in patients with aggressive and chronic periodontitis than in healthy subjects. But this may not necessarily mean less interleukin production in periodontal diseases.

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The authors declare no conflict of interests.

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