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Original Article

The comparison of the salivary concentration of interleukin-17 and interleukin-18 in patients with chronic periodontitis and healthy individuals

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ABSTRACT

Background: Regarding the prevalence and importance of periodontal disease and the potential of salivary biomarkers for the early diagnosis of these diseases, this study was conducted to compare salivary concentrations of Interleukin-17 (IL-17) and Interleukin-18 (IL-18) in patients with chronic periodontitis and healthy individuals.

Materials and Methods: The present research was a descriptive–analytical and also a cross-sectional study. Unstimulated saliva with full-mouth clinical periodontal recordings were obtained from 20 healthy individuals and 20 individuals with chronic periodontitis. The concentrations of salivary IL-17 and IL-18 were determined using the enzyme-linked immunosorbent assays. The nonparametric Mann–Whitney U-test was used for statistical analysis of the findings. Alpha level was set at 0.05.

Results: The mean salivary concentration of IL-18 in patients with chronic periodontitis was 143.10 pg/mL, which was higher than the same concentration in healthy controls (78.33 pg/mL), (P = 0.035). The mean salivary concentration of IL-17 in patients with chronic periodontitis and healthy controls was 3.51 and 4.57 pg/mL, respectively, and there was no difference between the two groups (P = 0.283).

Conclusion: Within the limitations of the present study, it may be suggested that an elevated salivary IL-18 level in chronic periodontitis patients has the potential to be a biomarker for periodontal tissue destruction.

Key Words: Interleukin-17, interleukin-18, periodontitis, saliva

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INTRODUCTION

Periodontitis is a multi-functional, chronic, and inflammatory disease, which have influences on the protective structure of the teeth, and begins and expands as a result of a complex interaction between the pathogens and the host defense system.[1,2] Chronic periodontitis is a type of periodontitis and is characterized by numbers of factors, including probing pocket depth (PPD), clinical attachment level (CAL), loose tooth, furcation involvement, bleeding on probing (BOP), plaque index (PI), and radiographic evaluations of marginal bone resorption.[3] According to Eke's study, the prevalence

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and severity of periodontal disease were reported to be approximately >47% among the adult population of the United States in 2009.[4] The outcome of periodontal diseases is periodontium inflammation,[5] which ultimately results in bone resorption, loose tooth, and early tooth loss.[6] Accordingly, primary and usual treatment is scaling and root planning, which involves the mechanical debridement of periodontal pockets, and disrupts existing biofilms. This treatment is performed using sonic and ultrasonic scalers or hand-held instruments[7] however, the use of this mechanical device in reducing the bacterial etiologic agents is difficult, time-consuming, and also ineffective in some cases.[8] Therefore, with respect to the importance and prevalence of periodontal diseases, it is required to recognize methods for these diseases early diagnosis. There are numerous biomarkers in saliva, plasma, gingival crevicular fluid (GCF), and blood that are used to screen and predict the initial changes in periodontal tissues and determine the efficacy of the treatment. Saliva is extensively used as a biomarker to determine the activity of periodontal diseases, since it allows rapid screening and access to accurate information, and provides a reliable assessment of the periodontal disease condition.[9] Regarding, the advantage of using saliva is the ease of collection and diverse content of microorganisms and mediators of the host response.[10] Although many studies were conducted in this field, the crucial role and pathogenesis of only a limited number of biomarkers have been proved. The result of a systematic review article, conducted by de Lima *et al*. who reviewed 905 studies in Brazil, indicated that only MIP-1a, IL-1B, and IL-6 had acceptable diagnostic accuracy, and there is limited evidence on the diagnostic capability of salivary biomarkers.[11] This result proposes that further studies on the cytokines that were referred in this research are required for achieving a better understanding of their potential association with periodontal diseases. Interleukin 17 (IL-17) is a T-lymphocyte-derived cytokine produced by macrophages, dendritic cells, mast cells and natural killer cells.[12] IL-17, in collaboration with other cytokines, including IL-1B, tumor necrosis factor-alpha (TNF-α), ONKOSTATIN-M, and interferon (IFN-c), produces more potent intracellular biologic effects.[13] IL-17 increases the RANKLE gene expression and reduces the expression of the osteoprotegerin gene in osteoblasts both in *in vivo* and *in vitro*, along with increasing the production of osteoclasts and bone resorption in a mouse model of arthritis.[14] Furthermore, IL-17 plays a controversial role.[15] On the one hand, it causes bone remodeling, similar to many inflammatory cytokines, and contributes to bone resorption,[16] on the other hand, it plays a protective role in bone against pathogens such as PG.[17] Consequently, the role of IL-17 is still unclear.[15,18] Interleukin 18 (IL-18) is a pro-inflammatory cytokine belonging to the IL-1 family and is originally called the causative agent of IFNs-c. IL-18 is produced by active macrophages, keratinocytes, dendritic cells, intestinal epithelial cells, osteoblasts, and adrenal cortex cells.[19] IL-18 plays a pro-inflammatory role along with IL1-B and strengthens immune responses by inducing other cytokines (IL1-B, TNF-a, and IL-8), and induces the response of both T helper 1 (Thl) and T helper 2 (Th2) type cells.[19-21] It can also result in neutrophil migration and osteoclast activity and is considered as important for the cleansing of intracellular pathogens and viruses.[22,23] Previous studies suggest that IL-18 has relationship with periodontal diseases, due to its elevated concentration in GCF, serum and gingival tissue samples that has been reported in this disease.[24-29] Considering the importance of finding, diagnostic markers for periodontal diseases and the lack of information on the exact relationship between IL-17 and IL-18 with these diseases, the present study was conducted at Faculty of Dentistry, Shahid Beheshti University of Medical Sciences in 2017. It is hoped that the results of this study help researchers to take a small but effective step in the early diagnosis of periodontal diseases.

MATERIALS AND METHODS

The present research was a descriptive-analytical and also a cross-sectional study. In accordance with the research conditions, sampling was performed on a continuous and nonrandom basis form, among individuals who were referred to the Periodontology Department of Faculty of Dentistry, Shahid Beheshti University of Medical Sciences in summer and autumn of 2017. Unstimulated saliva samples were later collected from 20 healthy individuals and 20 individuals with chronic periodontitis.

Exclusion criteria were as follows: history of smoking, periodontal treatment during the past 6 months, systemic diseases (such as diabetes mellitus, immunodeficiency, and hepatitis), and antibiotic use during the past 6 months. Salivary samples were taken from 20 nonsmoker and systemically

healthy patients with chronic periodontitis, and 20 nonsmoker and periodontally and systemically healthy patients. Patients were requested to drink water before the start of treatment (to reduce the salivary viscosity); however, they were also requested to avoid drinking water or eating for at least 30 min before the treatment. Before the completion of medical records and treatment, while they were sitting straight and with the open eyes, had their heads bent forward and rested in this head posture 5 min.[30] Then, the saliva was emptied in the same posture from the bottom lip into 15 ml sterile falcon tubes to collect samples. All tubes were labeled, coded, and immediately transferred to the laboratory for subsequent steps. PI, BOP, PPD, and CAL at 6 points of each tooth (mesiobuccul, mid-buccal distobuccal, mesiolingual, mid-lingual, distolingual) was measured and documented by the Probe "O" of the University of Michigan. The healthy group should have at least five teeth per quadrant; all teeth must have PPD <3 mm and had no history of periodontitis, CAL, BOP, and bone loss. PI also must be <40%. The group of patients with moderate-severe periodontitis should have at least five teeth per quadrant, at least four teeth per jaw with PPD ≥5 mm along with CAL ≥4 mm, PI >40%, and also showed BOP in 80% of the regions.[31] The samples were transferred to the laboratory with 15 ml Falcon tubes, and after that were immediately placed in a centrifuge (Eppendorf Germany), and they were centrifuged at 4000 g for 15 min at 4°C and the suspended particles and cells inside were deposited. The debris-free saliva (a minimum of 150 µl) was poured into four 1.5 ml microtubes, and then immediately frozen at −70°C after being encoded. In this study, no specific intervention was performed on individuals, and only their saliva was collected to determine the levels of IL-18 and IL-17. However, the study was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki. The study conditions were explained to the participants, and the written consent were obtained from them. In this study, IL-I8 and IL-I7 salivary concentrations were measured by applying Human ILI8 enzyme-linked immunosorbent assay (ELISA) kit (Biovendor, Brono, Czech Republic) and Human ILI7 ELISA kit (IBL International, Hamburg, Germany), respectively. ILI7 and ILI8 salivary concentrations were measured in terms of the kit instruction and ELISA standard method, and the results were reported in pg/mL. Data on the concentration and optical absorption rate of standard solutions were imported into Excel software, and also for each of the cytokines, the standard concentration curves were plotted. Concentrations of IL-18 and IL-17 were calculated for unknown samples based on the obtained curves. Finally, the concentrations obtained from the curve were doubled when 50% of the samples were diluted according to the kit instruction.[32,33]

Data analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., USA). The nonparametric Mann–Whitney U-test was used for statistical analysis of the findings, due to the abnormal distribution of the concentrations obtained for both IL-18 and IL-17 groups [Figure 1].

RESULTS

This research was performed on 40 samples, including 20 patients with chronic periodontitis and 20 healthy individuals. The women–men ratio among the periodontitis and healthy groups was 7:13 and 12:8, respectively. The mean age in the periodontitis and healthy groups was 49.9 and 25.75 years, respectively. The healthy controls were significantly younger in comparison with the periodontitis group ($P < 0.05$). Table 1 shows a summary of the characteristics of the population studied in terms of age and periodontal criteria. All periodontal criteria measured, including PPD, CAL, PI, and BOP were significantly higher in the periodontitis group compared to the healthy group ($P < 0.05$).

The mean salivary concentration of IL-18 was 143.10 and 78.33 pg/mL in the patients with chronic periodontitis and the healthy group, respectively. Regarding, this difference was statistically significant ($P = 0.035$).

The mean salivary concentration of IL-17 was 3.51 and 4.57 pg/mL in patients with chronic periodontitis and the healthy group, respectively. Regarding, this difference was not statistically significant ($P = 0.283$).

The statistical indices related to the IL-18 and IL-17 concentrations are indicated in Table 2.

DISCUSSION

In the present study, IL-18 and IL-17 concentrations were measured in patients with chronic periodontitis

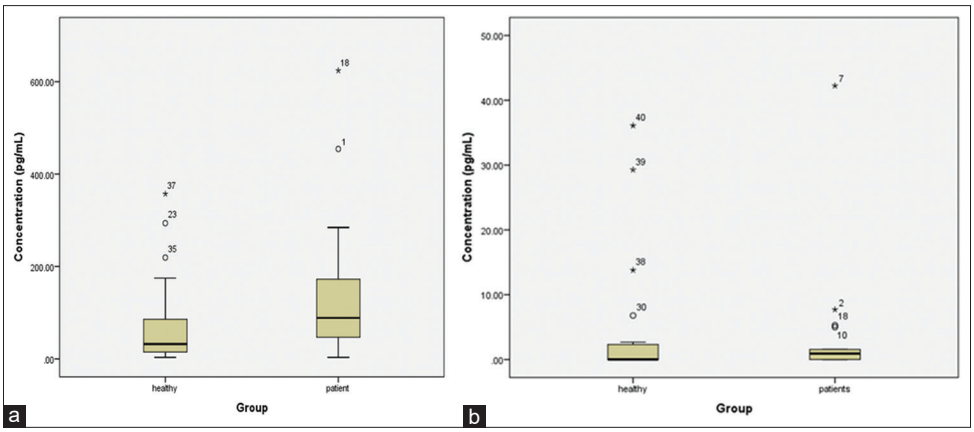

Figure 1: (a and b) Box plot for salivary concentrations of IL-18, IL-17 in patients with chronic periodontitis and healthy subjects.

Table 1: Specifications of clinical criteria in patients with chronic periodontitis and healthy subjects

Clinical criteria	Chronic periodontitis		Healthy		
	Mean±SD	Median (minimum-maximum)	Mean±SD	Median (minimum-maximum)	*P*
Age (year)	49.90±8.31	51.50 (32-63)	25.75±2.67	25 (22-31)	<0.05
PPD (mm)	6.95±1.57	7 (5-10)	1.80±0.61	2 (1-3)	<0.05
CAL (mm)	5.85±1.63	5 (4-9)	0	0	<0.05
BOP (%)	88.5±6.7	90 (80-100)	0	0	<0.05

SD: Standard deviation; PPD: Probing pocket depth; CAL: Clinical attachment level; BOP: Bleeding on probing

Table 2: Comparison of the indices of interleukin-18 and interleukin-17 concentrations in pg/mL in both healthy and periodontitis groups using Mann-Whitney U

Biomarker	Chronic periodontitis		Healthy		
	Mean±SD	Median (minimum-maximum)	Mean±SD	Median (minimum-maximum)	*P*
IL-18	143.10±155.30	88.60 (3.40-623.96)	78.33±101.96	32.15 (3.40-357.01)	0.035
IL-17	3.51±9.35	0.92 (0-42)	4.57±10.22	0.01 (0-36)	0.383

SD: Standard deviation; IL: Interleukin

and healthy controls, respectively. The mean salivary concentration of IL-18 in patients with chronic periodontitis was 143.10 pg/mL, which was higher than the same concentration in healthy controls (78.33 pg/mL). The mean salivary concentration of IL-17 in patients with chronic periodontitis and healthy controls was 3.51 and 4.57 pg/mL, respectively. Accordingly, there was no difference between these two groups. The higher salivary concentrations of IL-18 in the group of patients with chronic periodontitis obtained in the present study are in agreement with the study performed by Ozcaka *et al*. and Banu *et al*.[31,34] The results of the present study demonstrated a higher concentration of IL-18 in GCF, which are consistent with the study of Orozco *et al*., Figueredo *et al*., Nair *et al*., and Pradeep *et al*.[24,27,29,35] Johnson and Serio reported a higher concentration of IL-18 in gingival tissue samples in the points with a PPD of more than 6 mm compared to healthy individuals, which is also in agreement with the findings of the present study.[25]

Pradeep *et al*. found a positive relationship among the GCF concentration of IL-18, PPD, and CAL.[29] Banu *et al*. also indicated that periodontal indices (such as PPD, CAL, and PI) in the periodontitis group were higher than the healthy group.[34] Ozcaka *et al*. also reported that all periodontal criteria in the periodontitis group were higher in comparison with the healthy group.[31] In the current study, as it was expected, all periodontal criteria (e.g., PPD, CAL, PI) in the periodontitis group were higher than the healthy group. It appears that this cytokine is locally produced in periodontal tissues; therefore, it is possible to support the hypothesis that IL-18 is a biomarker involved in periodontal tissues destruction. However, Chitrapriya *et al*. reported different concentrations for IL-18, which is inconsistent with the findings of the current study and also above mentioned studies. They found higher concentrations of IL-18 in gingival tissue samples of patient with gingivitis, healthy controls, and periodontitis group, respectively. They

attributed this higher concentration of IL-18 to the sampling of regions with lower inflammation rates in the periodontitis group, because the mean GI in the periodontitis and gingivitis groups was 2.059 and 2.203, respectively.[36] This difference may be also associated with different periodontal criteria used in the Chitrapriya *et al*., for the reason that they only sent CAL ≥1 in 30% of the regions as inclusion criteria for choosing patients in the periodontitis group. However, the present study set more stringent periodontal criteria for selecting subjects in the periodontitis group. Although they also considered radiographic evidence of bone resorption in their assessments, the CAL at the level of 1 mm does not appear to be evident in radiography. The present study indicated lower salivary IL-17 concentration in patients with periodontitis that is consistent with Ozcaka's study. Ozçaka *et al*. state that there is an emerging understanding of the role of Thelper17 and IL-17 cytokines in periodontal diseases, and little is known about its main role in the disease pathogenesis and host conservation.[31] Furthermore, Pradeep *et al*. reported in their study that GCF-concentration of IL-17 is near to zero. Since the results of their research indicated a lack of IL-17 in GCF, they recognized that it cannot be considered as a biomarker in periodontal disease development.[37] Isaza-Guzmán *et al*. also find no relationship between IL-17 salivary concentrations and chronic periodontitis, and stated that it is futile to decide on the role of this cytokine in periodontal disease or its severity detecting.[38] Johnson and Serio reported equal concentrations of IL-17 in tissue samples with PPD ≥6 mm and a sulcus ≤3 mm.[25] Yetkin Ay *et al*. found a lower concentration of IL-17 in patients with chronic periodontitis with PPD ≥5 mm and stated that this finding could be due to the high GCF volume in the patient's pockets. They reported lower IL-11/IL-17 concentration in the GCF samples of patients with chronic periodontitis in comparison with healthy subjects in their study that may also be indicator of an imbalance of cytokines in deeper pockets.[39] However, Chitrapriya *et al*. observed higher gingival concentrations of IL-17 in the group of patients with chronic periodontitis compared to the healthy group. The discrepancy between this result and that of the current study is probably due to the difference in the sample, in which investigated the IL-17 level.[36] Further interactions seem to occur in the gingival tissue samples than salivary samples. Furthermore, the lower mean age of patients with periodontitis in their study may indicate different host defense responses and possibly higher cytokine IL-17concentrations. Awang *et al*. found higher salivary, gingival serum concentrations of IL-17 in the group of patients with chronic periodontitis compared to the healthy controls. Furthermore, this study demonstrated a positive relationship between IL-17 concentration and clinical criteria, including CAL, PPD, and BOP.[40] Consequently, this difference could be caused by the large sample size and the possibility of matching intervening factors to identify this relationship more accurately in the present study. In addition, the patients studied in this experiment were requested to fast from the night before the sampling. This different sampling process is also effective on the outcome. Yang *et al*. reported in their study that salivary concentrations of IL-17 in the group with chronic periodontitis were higher than the healthy group. A positive relationship was also documented with all the clinical criteria and the number of *T. dent* cola and *Tannerella forsythia* bacteria. They found that after nonsurgical periodontal treatment, IL-17 concentrations were under the initial concentration during 1 and 3 months follow-ups. Therefore, this study demonstrated a strong relationship between IL-17 salivary concentrations and chronic periodontitis.[41] In the present study, the racial difference between the Chinese population of this study and the Iranian population may justify the inconsistency of the results of these two studies. Mitani *et al*. reported higher GCF concentrations of IL-17 in patients with chronic periodontitis compared to the healthy participants. This study also showed a positive relationship between IL-17 concentration and CAL.[42] This study reports high levels of IL-17 in GCF samples, which may indicate a local event in the inflamed regions vicinity without any systemic symptoms.

It is true that the individuals of control group in this study had lower age ranges, but due to the limitations in matching the control group with the test group in terms of all periodontal criteria and because of the lower mean age of healthy controls compared with chronic periodontitis patients, inevitably the individuals of control group was selected from the younger people.

Finally, although little is known about the definite role of IL-17 in the pathogenesis of the periodontal disease, it is suggested that this cytokine, considering that in addition to increasing inflammation, has pro-osteoclastogenic effects, and plays a major role

in the pathogenesis of periodontitis, rheumatoid arthritis, and other diseases associated with bone immunopathology. It is hoped that future clinical trials, reveal definite effects of IL-17 in periodontitis using topical interleukin 17-blockers, and more importantly, provide an effective treatment for this inflammatory disease.[43]

CONCLUSION

Dental plaque biofilm cannot be eliminated. However, the pathogenic nature of the dental plaque biofilm can be reduced by reducing the bioburden (total microbial load and different pathogenic isolates within that dental plaque biofilm) and maintaining a normal flora with appropriate oral hygiene methods that include daily brushing, flossing and rinsing with antimicrobial mouth rinse.[44] In conclusion, IL-18 salivary concentrations were higher in patients with chronic periodontitis than healthy controls so that IL-18 may be considered as a biomarker for the early diagnosis of periodontal disease.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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