Interleukine-6 Level in Saliva of Patients with Chronic Periodontitis: A Case-Control Study

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ABSTRACT

Background:Periodontal diseases are infectious diseases in which periodontalpathogens trigger chronic inflammatory and immune responses. Interleukine-6 is a multifunctional cytokine playing a central role in inflammation and tissue injury.The aim of the study IS to determine the level of Interleukin-6(IL-6) in saliva of patients with chronic periodontitis compared to healthy subjects.

Materials and Methods:The total subjects of the present study is 60, divided into 3 groups; 20 patients with chronic periodontitis with pocket depth(PD ≥4 mm)(group I), 20 patients with pocket depth(PD <4 mm) with clinical attachment loss (group II), and 20 healthy controls with pocket probing depth (PPD ≤ 3 mm) without clinical attachment loss (group III). Un-stimulated salivary sample was taken from each subject and was investigated for the presence of Interleukine-6by using Enzyme-linked Immunosorbent Assay (ELISA) technology.

Results:Mean IL-6 levels in saliva in patients with chronic periodontitis (98.40 ± 18.44 ng/L)was significantly higher than in controls (11.67 ± 3.32; p=0.001). Also a significant difference in IL-6 levels in saliva was observed between the PPD ≥ 4 mm and PPD < 4 mm groups and between PPD ≥ 4 mm and control groups, as well as statistically significant differences were observed between PPD < 4 mm and control groups (P < 0.001).

Conclusion:The Interleukine-6 level in saliva can be considered as one of inflammatory biomarker indicators of severity of periodontitis.

Keywords:Chronic periodontitis, saliva, Interleukine-6. (J Bagh Coll Dentistry 2016; 28(1):103-108).

INTRODUCTION

Saliva is one of the most important body fluids, which contains a large number of proteins and peptides that are easily accessible and may serve as a potential source to measure biomarkers released during disease initiation and progression, it has significant association with inflammatory, connective tissue destruction and bone remodeling phases of periodontal disease. Subsequently, saliva has been used in the diagnosis of periodontal disease and monitor response to treatment[(1,2).]

Periodontitis is an inflammatory disorder affecting supporting tissues of the teeth, primarily initiated by a small group of gram-negative anaerobic bacteria within periodontal pockets. The stimulation of host defense system against bacterial pathogens result in connective tissue breakdown and alveolar bone destruction [(3)]. During periodontitis, inflammatory cytokines (IL-1β, IL-6, and TNF-α) and reactive oxygen species (ROS) are released from immune cells to fight the periodontal pathogens; the activation of these proinflammatory molecules leads to tissue destruction as a result of complex interactions between the pathogenic bacteria and the host's immune response. Role of host immune response is furthermost important factor in periodontitis as it determines both disease progression and severity [(4)].

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Cytokines are water-soluble glycoproteins, secretedby hematopoietic and non-hematopoieticcells in response to infection. Their primary function is intercellular signalling[(5)],which are central to the pathogenesis of an ever-increasing number of diseases, including periodontal disease[(6)].Cytokines play a key role in a number of biological activities including, development, proliferation, regeneration, repair and inflammation [(7)]. An inflammatory cytokine may be described as a cytokine which is induced during an inflammatory response and is related with the onset and/or progression of the insult. So far, interleukin (IL)-l alpha (α) IL-1beta (β) IL-6, IL-8, and tumour necrosis factor (TNF)- alpha (α) have been categorized as inflammatory cytokines[(8,9)]. Contributing inflammatory mediators and tissue destructive molecules have been detected in the gingival tissues, gingival crevicular fluid (GCF) and saliva of patients affected by periodontitis. Qualitative and quantitative changes in the composition of these biomarkers could have diagnostic and therapeutic significance [(10,11).]

Interleukin-6 (IL-6), is a pleiotropic cytokine that acts as both a pro-inflammatory and anti-inflammatory activity.It exerts anti-inflammatory properties through enhancement of tissue inhibitor of metalloproteinase (TIMP) production and suppression of pro-inflammatory cytokines IL-1β and TNF-α. In addition, down-regulation of pro-inflammatory cytokines and up-regulation of anti-inflammatory molecules (e.g. IL-1 receptor antagonist, TNF soluble receptor) in acute inflammatory processes [(12,13)]. It has a profound

effect on B cells, promoting plasma cell differentiation and immunoglobulin (Ig) secretion, also enhances T cell proliferation[14].Recent evidence has suggested that IL-6 may play important roles in the development of specific immune response, as described mice lacking IL-6 or TNF-α gene expression are deficient in T and B cell function[15]

IL-6 is known as one of the key cytokines of host response to inflammation and tissue injury such as that seen in chronic periodontitis [16] and induces bone resorption by itself and in conjunction with other bone-resorbing agents.There is a significant correlation between tissue levels of IL-6 and the severity of the coincident inflammation.Elevated levels of IL-1β and IL-6 have been shown to be induced by periodontal pathogens and are correlated with the continuous tissue destruction observed in periodontitis [17,18]. Furthermore, sspontaneous production of IL-6 has been reported in mononuclear cells isolated from inflamed gingival tissues of patients with periodontitis.On the one hand, IL-6 levels may correlate with the severity of periodontal disease. In addition, higher IL-6 levels have been observed in sites ofrefractory periodontitis compared to sites of stable, advanced periodontitis, which suggests that it could be a diagnostic marker for sites of active periodontal disease [19].

The hypothesis was to testthe relationship between salivary IL-6 levels and clinical findings in patients with chronic periodontitis and individuals with healthy periodontium and to assess usefulness of IL-6 for diagnosis of periodontal severity.

The present study has the following objectives:

1. To assess IL-6 levels in the saliva in patients with chronic periodontitis in comparison to individuals with healthy periodontium.
2. Tocompare the salivarylevel of IL-6 with the severity of the periodontal disease.

MATERIALS AND METHODS

Study Groups

A total of 60 subjects (32 females and 28 males; mean age of 37.40 ±3.84 years) were included in the present study who attended the Department Periodontal Surgery, College of Dentistry, Hawler Medical University in Erbil during the period of 15th November 2014 to 5th February 2015.

Out of the 60 subjects, 40 were diagnosed with chronic periodontitis (experimental group)[20]. Within the experimental group two subgroups were formed according to periodontal pocket occurrence: one considered as group of subjects with more severity of periodontal disease pocket depth (PD≥ 4 mm) (20 patients) and the other subjects with less severity of disease (PD< 4 mm) with clinical attachment loss (20 patients). The control group was compromised of 20periodontally healthy individuals without clinical attachment loss, without pocket depth >3 mm and with bleeding index (BOP<10%).

All subject had more than 20 teeth and none of the subjects reported any coexisting systemic disease particularly systemic neurological disorder (e.g., epilepsy or schizophrenia), long-term pharmacological treatment, pregnant, lactating women, smoking and alcoholism, individuals treated with anti-inflammatory and antibiotics within the previous 3 months, were excluded from the study. Informed consent was obtained from all the patients before participation in the study, which was approved by the Ethical Committee of College of dentistry/ Hawler Medical University.

Saliva Sampling

Unstimulated whole saliva were collected from all participants in a given time between (9:00a.m. and 11:00 a.m.). The subjects (cases and controls) refrained from eating, drinking, and practicing oral hygiene habits (flossing, brushing, and mouth rinses) within at least 2 hours prior to saliva collection. Subjects were asked to rinse their mouth with distilled water, following which they expectorated at least 3mL of un-stimulated whole salivainto a 5mL sterile tubes according to the method described by Navazesh [21]

All samples were immediatelycentrifuged at 3000 rpm for 20 minutes and clear supernatant was stored at-20ºC pending analysis. The IL-6 levels in saliva samples were measured with a Human Interleukine-6 (IL-6) ELISA Kit provided by MyBiosource International Inc., USA (Catalog # MBS164590). The analytical sensitivity for the test was 1.03ng/L, and the detection range was between 2 ng/L→600 ng/L.

Statistical Analysis

Statistical analysis was performed by using the SPSSPC/ Windows version 21 software packages (SPSS Inc., Chicago, IL, USA). Paired *t*-test was used to compare IL-6 concentrations in saliva between 2 given groups. One-way analysis of variance was used to determine the differences between the groups and the significance of mean difference between the groups was done by Tukey's multiple comparison test. All data were expressed as means±SD (a value of $p<0.05$ was considered significant).

RESULTS

The mean concentrationof IL-6 in saliva of patients with chronic periodontitis was (98.40 ± 18.44 ng/mL), whereas in the control group was (11.67 ± 3.32 ng/mL). The difference in the IL-6 level was statistically highly significant (p< 0.01) (Table 1).

Table 2 showed a comparison of mean concentration of the salivary IL-6 of three groups. ANOVA revealed highly significant difference inIL-6 level among the groups (F= 990.80 P < 0.001). In Tukey's multiple comparison test a highly significant differences were observed in IL-6 concentration in saliva between PPD ≥ 4 mm and PPD < 4 mm groups(114.43 ± 9.74 vs. 82.36 ± 7.85, q=12.45 ; p < 0.001), as well as between PPD ≥ 4 mm and control groups (p < 0.001).Further, the mean concentration of IL-6 level in saliva of PPD < 4 mm group was also found to be significantly different as compared to control group (82.36 ± 7.85 vs.11.67 ± 3.32, q=36.28; p < 0.001) (Table 3).

Table 1: Comparison of Salivary IL-6 level (ng/L) between Chronic Periodontitis Patients and Control Groups(by *t*-test)

Groups tested	Number	Concentrations of IL-6 (ng/L) (Mean ±SD)	t-value	P-value
Case (Chronic periodontitis)	40	98.40 ± 18.44	33.75	P<0.001 **
Control (healthy patients)	20	11.67 ± 3.32	15.75	

IL-6: Interleukine-6; SD-Standard deviation; ** Paired sample t- test: p < 0.001, Highly significant

Table 2: ANOVA Test for Detection ofSalivary IL-6 (ng/L) Level in Different Study Groups.

Study Groups	Salivary IL-6 Level (ng/L) (Mean ±SD)	F value	P value ANOVA
Group I: PPD ≥ 4 mm (n=20)	114.43 ± 9.74	990.80	P < 0.001 **
Group II: PPD < 4 mm(n=20)	82.36 ± 7.85		
Group III: Control(n=20)	11.67 ± 3.32		

**** indicates 5% level of significance (p < 0.05).**
The F value is based on one-way ANOVA

Table 3: Tukey's Test Results Multiple Comparison of Salivary IL-6 Level between the Groups.

Study Groups	Dependent variable Salivary IL-6 Level (ng/L)	Groups Compared	Significance		
			Mean difference	q-	p-value
Group I: PPD ≥ 4 mm	114.43 ± 9.74	I Vs III	102.77 ± 11.13	41.31	P < 0.001
Group II: PPD < 4 mm	82.36 ± 7.85	II Vs III	70.70 ± 8.77	36.28	P < 0.001
Group III: Control	11.67 ± 3.32	I Vs II	32.07 ± 11.52	12.45	P < 0.001

DISCUSSION

Periodontal disease consequences from release of inflammatory mediators, and the result is a significant breakdown of tooth supporting tissues, finally leading to tooth loss[22]. The intensity, duration and resolution of inflammation depend on shifting balance between the activities of proinflammatory and anti-inflammatory cytokines during periodontal inflammation [23,24].IL-6 has multiple biological activities. Early production of IL-6 during infection and through differential control of leukocyte recruitment, activation and apoptosis – has emerged as one of a network of mediators directing this shift from innate immune response to an adaptive immune response[18].

Considering the above mentioned functions of IL-6 and its role in various chronic diseases, including periodontal disease, we tried to evaluate and compare the levels of IL-6 in saliva in healthy and chronic periodontitis patients. Periodontal disease and PPD were taken into consideration to assess the connective tissue destruction and bone loss.

Results of the present study showed that the mean concentration of salivary IL-6 was significantly elevated in chronic periodontitis subjects as compared to controls (p < 0.001).

These data are consistent with previous findings of Costa *et al.*,[8]and Ebersole *et al.*,[25]which shows significantly higher salivary

IL-6 levels in patients with chronic periodontitis as compared to subjects with healthy periodontium. Also Geng *et al.,*[26]reported significantly higher salivary IL-6 concentrations in chronic periodontitis in comparison with healthy control group.With regard to periodontal diseases, a reverse transcription polymerase chain reaction (RT-PCR) and ELISA studiesobservedthat the mRNA expression, as well as protein expression, of IL-6 was increased in patients with periodontal diseases than in healthy control subjects[27,28]. The results of the present study for the IL-6 levels, obtained using ELISA, were consistent with previous findings.

On the contrary Teles *et al.,* [29)]. In their study on 118 subjects; 74 chronic periodontitis and 44 periodontally healthy individuals, measured the salivary levels of different cytokines, including IL-6 by ELISA method. They stated that the levels of IL-6 were higher in patients with chronic periodontitis than the healthy individuals but statistically not significant. Higher concentrations of salivary IL-6 in periodontitis patients compared to healthy controls were reported by Ramseier *et al.,*[30]although differences did not reach statistical significance.

Results obtained in current study indicated that mean concentration of salivary IL-6 was significantly elevated in patients with PPD ≥ 4mm group as compared to control group (114.43 ± 9.74 vs 11.67 ± 3.32, q=41.31; P< 0.001). Even after comparing patients with PPD ≥ 4 mm group with PPD < 4 mm group (114.43 ± 9.74 vs 82.36 ± 7.85, q=36.28; P< 0.001) a significant increase was noticed. Moreover, when comparing among all three groups, salivary IL-6 level increased significantly from the control to the sever periodontitis group (P< 0.001).

This clearly proves that chronic periodontitis patients with PPD ≥ 4mm group alone contributed much to the difference found in salivary IL-6 level as compared to the chronic periodontitis as seen with PPD < 4 mm group as well as with control groups. Thus, the presence of elevated levels of IL-6 in the saliva of patients with chronic periodontitis PPD ≥ 4 mm group , along with the significant association with extent of probing pocket depth or clinical assessments of periodontal destruction, strongly suggests an important role for this mediator in the pathogenesis of periodontal disease.

Similar results were obtained by Javed *et al.,*[31] they proved that there is a significant correlation between the level of IL-6 in saliva and the clinical parameters such as PPD, CAL and BOP, where they found an increase in the salivary IL-6 levels as the severity of the periodontal disease increased. Research by Guillot *et al.,*[19]observed a significant correlation between tissue levels of IL-6 and the severity of the coincident inflammation and demonstrated that IL-6 in gingival crevicular fluid impacts the severity of periodontal disease and is associated with clinical attachment loss. In addition, higher IL-6 levels have been observed in sites of refractory periodontitis compared to sites of stable, advanced periodontitis, which suggests that it could be a diagnostic marker for sites of active periodontal disease[19].This may be explained by the fact that the increased levels of IL-6, with other factors seen in patients with periodontitis may be associated with the destruction of periodontal tissue and resorption of bones[32]. Kurihara[33],proved that IL-6 which is locally produced by osteoclasts, which is very important factor regulating differentiation of these cells and takes part in the processes of resorption. This result also comes in agreement with and extend the overall findings that IL-6 seem to be key biomarkers that are elevated in the oral fluids of periodontal patients[4,34]. Altogether, these studies suggested that the increased levels of inflammatory cytokines, particularly IL-6 in periodontitis may have diagnostic and prognostic potentials for the monitoring of the disease and therapeutic decision.

Further, findings in a recent study believed that salivary IL-6 reflects the response of mucosal immune system [35].A study by Seymour and colleagues [36]demonstrated that inflammatory cytokines in whole saliva might be derived from gingival crevicular fluid (GCF). This demonstrates the importance of considering local concentrations of inflammatory cytokines in periodontal diseases. It has been stated that bacteria penetration and particularly lipopolysaccharide (LPS) into the tissues results in the recruitment and activation of the monocyte/T lymphocyte axis. This leads in turn to increase secretion of inflammatory cytokines including (IL-1β, IL-6, and TNF-α) by tissue inflammatory cells, which have been associated with periodontal tissue destruction [37]. In conclusion, it could be hypothesized that IL-6 is produced locally by tissue cells in response to an inflammatory stimulus and by salivary gland cells, reflecting the response of the mucosal immune system. Despite the source of the IL-6, it is appropriate to assess immunologic patterns relevant to systemic or local disease conditions[38].

Finally,conclusionof the present study is that the salivary level of IL-6 was directly proportional with the extent of probing pocket depth, suggesting that IL-6 in saliva can be considered as

one of inflammatory biomarkers of severity of periodontitis.

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