Estimation of the level of Salivary Interleukin 6 (IL-6) and its' correlation with the clinical parameters in patients with periodontal diseases

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Abstract:

Background: There is wide diversity of biochemical components of the human saliva this makes it suitable to be used as a biological of diagnostic value. Any change of biochemicals of the saliva could be related to periodontal diseases. Saliva can provide different advantages, including easy collection and non-invasive sampling method when it used as a diagnostic material. Cytokines are pivotal to the pathogenesis of periodontal disease and may be used as markers in diagnosis. Proinflammatory cytokines are mediators closely associated with the pathogenesis of periodontitis. In saliva of the patients with chronic periodontitis , the levels of several interleukins were increased .Numerous cytokines have been identified at sites of chronic inflammation such as periodontitis. One of these is interleukin-6 (IL-6), which is a major mediator of host response to infection and tissue injury. IL-6 plays a major role in B cell differentiation and also promotes T cell proliferation and bone resorption.

Subjects and methods: In the present study one hundred and one (101) males patients were enrolled with an age ranging from (35-55) years. The sample were divided into three main groups (30) healthy control patient, (30) patients with gingivitis, and (41) patients have chronic periodontitis All were from attendants to Department of Periodontics, School of Dentistry, University Sulaimani. All subjects were in good general health and had not received previous periodontal therapy or taken antibiotics, or anti-inflammatory drugs in the three months before the study .The Clinical Periodontal Parameters include Plaque index(PLI) ,Gingival Index (GI) , Bleeding on Probing(BOP), Probing Pocket Depth(PPD)and Clinical attachment level (CAL) .Saliva was collected for five minutes (5-10 ml) from each patient into a sterile tube. Saliva was immediately centrifuged at 3500 rpm for ten minutes and stored at −20°C, until analysis by Cobas E analyzer (Roche made in Germany) for detection of Interleukin 6 (IL-6)). Concentrations of the Interleukin 6 (IL-6) in saliva measured in (pg/μl.)

Results: The concentration of interleukin-6 (IL-6) (pg/μl) in saliva was higher in chronic periodontitis group (13.4pg/μl))than in gingivitis(7.5pg/μl) and in control group(5.27 pg/μl).There was high significant differences among concentration of IL-6(pg/μl) among the three groups P-value(0.000). The mean pocket depth in chronic periodontitis group (5.48. ±1.64) and the mean of clinical attachment loss (3.26, ± 1.49). There were high significant difference between chronic periodontitis , gingivitis and control group in clinical parameters [Plaque index(PLI) ,Gingival Index(GI) , Bleeding on Probing(BOP), Probing Pocket depth (PPD)] p-value (0.000).

Conclusion: The concentration of interleukin-6 (IL-6) (pg/μl) in saliva was higher in chronic periodontitis group than in gingivitis group and in control group and IL-6 can be regard as diagnostic marker which give information about progression of periodontal disease.

Keywords: periodontitis, cytokine, gingivitis, Interleukin 6, saliva

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I. Introduction

Periodontal disease is a chronic microbial and inflammatory process characterized by the presence of pathogenic bacteria, impaired host immune response and destruction of supporting structures of the teeth, including gingiva, periodontal ligament and supporting alveolar bone. Periodontal diseases broadly categorized into gingivitis and periodontitis (1).Gingivitis is an early inflammation of the gingiva together with formation of

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dental plaque . When it untreated, this will progress to periodontitis which is classified in three important categories, including aggressive and chronic periodontitis (CP) and periodontitis associated with systemic disease (2). Chronic periodontitis (CP) is found to be the most prevalent periodontal disease in adults (3). According to World Health Organization（WHO）periodontal disease affects between 10% and15% of the world's population (4, 5). It is estimated that about 15% of adults aged (21 to 50 years) and about 30% of subjects > 50 years of age have severe periodontitis (6).

Cytokines are water-soluble glycoproteins secreted by hematopoietic and non-hematopoietic cells in response to infection. The primary function of cytokines is intercellular signaling (7).An inflammatory cytokine is induced during an inflammatory response and is associated with the onset and/or progression of the disease. of periodontal diseases ,therefore, researches have paid attention to the roles of inflammatory cytokines in saliva (8).Cytokines play an important role in the host response to the periodontal film (9), and they are pivotal to the pathogenesis of periodontal disease and may be used as diagnostic markers (10).Interleukin (IL)-6 may promote the degeneration of inflamed periodontal tissues(11).IL-6 is an inflammatory cytokine that is released from monocytes, lymphocytes, or endothelial cells at sites of tissue injury and it provokes the release of neutrophils and platelets from the bone marrow into blood circulation, a part of acute and chronic inflammatory reactions (12).

Interleukin-6 (IL-6) is an important mediator of host response to tissue injury and infection .It plays a major role in differentiation of B cell and also enhances proliferation of T cell and bone resorption. The correlation between tissue levels of IL-6 and the severity of the coincident inflammation is significant. In mononuclear cells isolated from inflamed gingival tissues of patients with periodontitis spontaneous production of IL-6 has been reported. The levels of IL-6 may correlate with the severity of periodontal disease. IL-6 is increased in sites of refractory periodontitis compared to stable sites which indicate that IL-6 could be a diagnostic marker for sites of active periodontal disease (13).

Systemic Saliva is said to be a "mirror of the body". It is an indicator of health not just in the oral cavity but throughout the body (14) .About 99.5 % of its total volume is water and the rest of 0.5% includes different ions, biochemical and many other important biomarkers (3). Salivary analysis has become an important resource for monitoring general health and the state of disease due to its origin, composition equivalent to serum, and interactions with other organs (15) so ,the use of saliva has many advantages, including the simple and non-invasive method of collection and low-cost storage (16). During the last few decade analysis of saliva has attracted periodontitis 'attention ,saliva as an important laboratory test for the evaluation of many salivary conditions ,including periodontitis (17) .Saliva contains both locally and systemically derived biochemicals with relatively important diagnostic value, which could be used for detecting periodontal diseases. They are used for identification of the type and severity of periodontitis, in addition, monitoring the treatment success. Today a number of advanced methods use saliva for diagnosis of some oral and systemic diseases (18). Saliva used as a biofluid for early detection of diseases' risk factor ,prognosis, and monitoring treatment response (14). Detection of salivary biomarkers is a non-invasive laboratory examination for early diagnosis of periodontitis (3). The present study was conducted to estimate the levels of interleukin-6 and to correlate its' level with clinical parameters in patients with gingivitis, chronic periodontitis when compared to periodontally healthy individuals.

II. Aims Of The Study
To estimate the levels of interleukin-6 in patients with gingivitis, chronic periodontitis when compared to subject with normal periodontium.
To correlate the levels of interleukin-6 with the clinical parameters [plaque index (PLI), gingival index (GI), probing pocket depth (PPD) and clinical attachment loss (CAL) in patients with gingivitis, chronic periodontitis.

III. Materials And Methods
Subjects
One hundred and one (101) males patients were enrolled with an age ranging from (35-55) years All subjects were from attendants to department of periodontics, School of Dentistry, University of Sulaimani. All individuals were in good general health and had not received previous periodontal therapy or taken antibiotics, immunomodulatory or anti-inflammatory drugs in the 3 months before the study. A written explanation of the aim of the study was provided for each patient a designed consent according to the Helsinki Declaration was obtained .Institution alethic review committee approval for the study was obtained. The participants were divided into three main groups (30) healthy control patient, (30) patients with gingivitis, and (41) patient shave chronic periodontitis.

Exclusion Criteria

Smoking ,alcohol drinking, Systemic antibiotic therapy or anti-inflammatory therapy within the last three months ,those who were on long-term medication that could affect the expression of gingivitis or periodontitis, patients with any systemic disease including diabetes, viral, fungal, or bacterial infections ,and

Patients with < 20 teeth were excluded from the study**.**

Design of the Study

All the individuals were informed about the purposes of the investigation and consented to its protocol and they were free to accept or refuse to be participated in the study .They were subjected to a questionnaire including questions relating to their name, age ,full medical history ,dental history ,medications and   if they smoked or drank alcohol. Following this full examinations saliva collection and registration of clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) were carried out.

Method
Collection of the saliva

To avoid the possibility of contamination of saliva the following instructions were given to the patients: Do not eat, drink, and use oral hygiene measures for 2 hours before collection   of saliva,   rinse mouth with water to remove food residue before sample collection, swallow to increase hydration and wait at least (10 minutes) after rinsing before collecting saliva to avoid sample dilution. After rinsing, patients were seated comfortably in the dental chair with eyes open and head tilted   slightly forward. Saliva was collected in the mouth while patient kept his lips closed saliva was collected for five minutes (5-10 ml) from each patient into a sterile vessel according to the method described by (19). Saliva was immediately centrifuged at (3500 rpm) for 10 minutes and stored   at (−20°C) until subsequent analysis by Cobas E analyzer (Roche made in Germany).

Clinical Periodontal Parameters
Plaque index (PLI) The assessment of dental plaque at   four surfaces of each tooth except 3 rd. molar was done by using plaque index system according to the plaque index (20).

Gingival Index (GI)

The gingival inflammation at the four surfaces of each tooth except 3rd molar was assessed using the criteria of the GI (21).

Bleeding on Probing (BOP)

William periodontal probe markings at(1,2,3,5,7,8,9 and10mm) was inserted to the bottom of the periodontal pocket or sulcus for four surfaces of each tooth except 3rd molar and was moved gently along the tooth(root) surface. If bleeding occurs within 30 seconds after probing, the site was given as positive (+) score (1) and a negative (-) score (0) for the non-bleeding site (22).

Probing Pocket Depth (PPD)

The distance in millimeters from the gingival margin to the base of gingival sulcus or pocket was recorded   by using William periodontal probe which was inserted into gingival crevice or pocket as close as possible to the long axis of the tooth at four surfaces of each tooth except 3rd molar, and the site for measurement were   labial/ buccal, lingual/palatal, mesial and distal surfaces, no pressure was used to insert periodontal probe (23).

Clinical attachment level (CAL)

By using William s graduated periodontal probe will insert into the buccal (labial), lingual (palatal) ,mesial and distal tooth surfaces the distance between the cemento-enamel junction (CEJ) and the base of pocket will measure to the nearest millimeter.

Detection of Interleukin 6 (IL-6)

The presence of cytokine in the saliva was determined by using Cobas E analyzer (Roche made in Germany). Concentrations of the   Interleukin 6 (IL-6) in s a l i v a measured in (pg/μl.).

Statistical analyses

All the data of the sample were subjected to computerized statistical analysis using SPSS version 20 computer program. The statistical analysis included:

1. **Descriptive Statistics:** Medians, means, standard deviations, minimum and maximum values.
2. **Inferential Statistics:** Kruskal-Wallis H test for the comparison among the groups, Mann-Whitney U test used if Kruskal-Wallis H test give significant groups difference to detect the difference between each two groups, Spearman's rank test to demonstrate the relation among variables in each group. In the statistical evaluation ,the following levels of significance were used:

P > 0.05	NS	Non-significant
0.05 ≥ P > 0.01	S	Significant
P ≤ 0.01	HS	Highly significant

IV. Results

The descriptive statistical results of clinical parameters were found that the mean of PLI was higher in (1.03) than gingivitis group (0.62)), and control group (0.27). In addition the mean values of GI were higher in CP group (1.24) than in gingivitis group (0.61) and in control group (0.27). Moreover, the mean percentages of bleeding sites in CP group were higher than in gingivitis and control group (37.57 ,20.62,1.17),   respectively as shown in table (1).Furthermore, in CP group the mean of pocket depth (5.48 ±1.64) and the mean of clinical attachment loss (3.26 ±1.49) as shown in table (2). By using Mann-Whitney U test there were high significant difference between CP, gingivitis and control group in PLI,GI PPD and BOP% p-value (0.000) as shown in table (3).

The median of concentration of IL-6 (pg/µl) in saliva was higher in CP group (13.4pg/µl) than in gingivitis group (7.5pg/µl) and in control group (5.27 pg/µl) as shown in table (1). By using Mann-Whitney U test , there were high significant difference p-value(0.000) between CP, gingivitis and control group in concentration of interleukin 6 (IL-6)(pg./µl) in saliva .While there was no significant difference between gingivitis and control groups p-value (0.093) as shown in table (3).

Table (4)illustrates the correlation among variables in control group, there was weak positive non-significant correlation between PLI and GI r (0.282) p-value(0.131).Also, there was positive weak none significant correlation between PLI and BOP% r (0.044)   p-value (0.819) .In addition the correlation between GI and BOP% is weak positive none significant   r (0.170) p-value (0.368).Moreover there was weak positive none significant correlation   between GI and the level of IL6 in saliva r (0.084) p-value (0.658). The correlation between BOP% and   the level of IL6 in saliva   was   positive weak none significant r (0.121) p-value (0.526). While there was a negative correlation   between PLI and the level of IL6 in saliva r (- 0.037) p-value (0.84).

Table (5) demonstrates the correlation among variables in gingivitis   group. There was strong positive high   significant correlation between PLI and GI   r (0.697) p-value (0.000).Also there was strong positive high significant correlation between PLI and BOP% r (0.624) , p-value (0.000). In addition, there was strong positive high   significant correlation between GI and BOP%   r (0.806) p-value (0.000)   . Moreover, there was weak none significant   between GI and the level of IL6 in saliva   r (0.040) p-value (0.833).Furthermore the correlation between BOP% and the level of IL6 in saliva r (-0.046) p-value (0.811) weak positive non-significant .While there was a negative correlation   between PLI and the level of IL6 in saliva r (-0.037) p-value (0.847).

Table (6) clarifies the   correlation among variables in CP group**.** There was strong positive high significant correlation between PLI and GI r (0.412) p-value (0.007). Also, there was positive weak none significant correlation   between PLI and BOP% r (0.141),   p-value (0.381). In addition, , there was a weak positive correlation between PLI and PPD r (0.011) ,p-value (0.946). Moreover, There was a positive weak non-significant correlation   between PLI and the level of IL6 in saliva r (0.096) p-value (0.552). While   there was weak negative non-significant correlation   between PLI and CAL r (-0.066) p-value (0.684).

The correlation between GI and BOP% is positive high significant r (0.752) p-value (0.000) .In addition there was weak none significant correlation   between GI and PPD r (0.094) ,p-value (0.557) .While there was a weak non-significant correlation   between GI and CAL r (0.091), p-value (0.571). There was weak negative none significant correlation   between GI and the level of IL6 in saliva   r (-0.095) p-value (0.553) as shown in table (6).

There was weak positive non significant correlation between and BOP% and PPD r (0.248) , p-value (0.118) .In addition, there was a weak positive non -significant correlation   between BOP% and CAL r (0.246) p-value (0.121). The correlation between PPD   and   CAL   was positive high significant   r (0.966), p-value (0.000). The correlation   between PPD   and the level of IL6 in saliva was weak positive non- significant

r (0.248), p-value (0.118). There was a weak positive significant correlation between CAL and the level of IL6 in saliva r (0.321), p-value (0.041). The correlation between BOP% and the level of IL6 in saliva was weak negative non-significant correlation r (-0.070) p-value (0.664) as shown in table (6).

Table (1) descriptive statistics and groups' difference

Variables	Groups	Descriptive						Comparison (d.f.=2)	
		N	Median	Mean	S.D.	Min.	Max.	X^2	p-value
PLI	Control	30	0.3	0.27	0.10	0.05	0.44	81.954	0.000 (HS)
	Gingivitis	30	0.6	0.62	0.12	0.44	0.93		
	C.P	41	1.03	1.06	0.34	0.61	2.12		
GI	Control	30	0.3	0.27	0.15	0.01	0.71	83.520	0.000 (HS)
	Gingivitis	30	0.6	0.61	0.12	0.39	1.05		
	C.P	41	1.24	1.24	0.35	0.61	2.04		
BOP%	Control	30	0.45	1.17	1.61	0	5	68.232	0.000 (HS)
	Gingivitis	30	14.69	20.62	14.83	5	68.75		
	C.P	41	34	37.57	24.04	5.88	90.79		
LI-6	Control	30	5.27	7.43	6.13	1.19	29.91	21.704	0.000 (HS)
	Gingivitis	30	7.5	11.13	12.15	1.74	67.34		
	C.P	41	13.4	21.11	23.09	4.57	119.6		

Comparison was done using Kruskal-Wallis H test

Table (2) descriptive statistics of pocket depth and clinical attachment loss in C.P. group

Variables	N	Median	Mean	S.D.	Min.	Max.
PPD	41	4.5	5.48	1.64	4	10.5
CAL	41	2.5	3.26	1.49	1.5	7

Table (3) Mann-WhitneyU test after Kruskal-Wallis H test

Variables	Groups		Mann-Whitney U test	p-value
PLI	Control	Gingivitis	0.5	0.000 (HS)
	Control	C.P	0	0.000 (HS)
	Gingivitis	C.P	79	0.000 (HS)
GI	Control	Gingivitis	33	0.000 (HS)
	Control	C.P	1	0.000 (HS)
	Gingivitis	C.P	26.5	0.000 (HS)
BOP%	Control	Gingivitis	0.5	0.000 (HS)
	Control	C.P	0	0.000 (HS)
	Gingivitis	C.P	331.5	0.001 (HS)
IL-6	Control	Gingivitis	336.5	0.093 (NS)
	Control	C.P	234.5	0.000 (HS)
	Gingivitis	C.P	360.5	0.003 (HS)

Table (4) correlation among variables in control group

Variables		GI	BOP%	IL-6
PLI	r	0.282	0.044	-0.037
	p-value	0.131	0.819	0.847
GI	r		0.170	0.084
	p-value		0.368	0.658
BOP%	r			0.121
	p-value			0.526

Table (5) correlation among variables in gingivitis group

Variables		GI	BOP%	IL-6
PLI	r	0.697	0.624	0.051
	p-value	0.000	0.000	0.791
GI	r		0.806	0.040
	p-value		0.000	0.833
BOP%	r			-0.046
	p-value			0.811

Table (6) Correlation) among variables in C.P. group

Variables		GI	BOP%	PD	CAL	IL-6
PLI	r	0.412	0.141	0.011	-0.066	0.096
	p-value	0.007	0.381	0.946	0.684	0.552
GI	r		0.752	0.094	0.091	-0.095
	p-value		0.000	0.557	0.571	0.553
BOP%	r			0.248	0.246	-0.070
	p-value			0.118	0.121	0.664
PPD	r				0.966	0.248
	p-value				0.000	0.118
CAL	r					0.321
	p-value					0.041

V. Discussion

Periodontal disease cause by release of inflammatory mediators, which lead to a significant breakdown of periodontium and finally lead to tooth loss (Kornman etal., 1997) (25).

Salivary biomarkers are being explored as methods of monitoring general health and in the early diagnosis of disease ((10, 24). Saliva is an inexpensive, noninvasive, an of salivary sample, (26). The local action of cytokines which is dependent on recruitment, activation and interaction of immune competent cells can explain the site specific nature of cytokine expression (27).

The present study examined the levels of IL-6 in saliva of three main groups healthy control patient(30)Thirty patients with gingivitis., and forty one patients have chronic periodontitis .

The results of the present study showed that the median level of IL-6 in the chronic periodontitis group (13.4 pg/mL) was higher than in the gingivitis, (7.5pg/mL) and control (5.27 pg/mL) groups this difference was statistically high significant. These findings were in agreement with results of (Javed etal. 2014) (28), who reported that salivary IL-6 levels are elevated in patients with chronic periodontitis (CP). Also in agreement with (Gurban and Drugarin, 2008) (29), who showed that a local increase of Proinflammatory

cytokines (IL-6) in the patients with different stages gingivitis, comparing to healthy subjects·. These results indicated that shifting in the balance between the activities of pro-inflammatory and anti-inflammatory cytokines during the periodontal inflammation could affect the intensity and duration of inflammation. The intensity, duration and resolution of inflammation depend on shifting the balance between the activities of Proinflammatory and anti-inflammatory cytokines during the periodontal inflammation (Honda etal. , 2006) (30). On contrast to the present study (Telesetal. , 2014) (31) demonstrated that there was no difference in the levels of, interleukin-6 in saliva, between periodontal health and periodontal diseases. The range of IL-concentration in saliva was often quite variable .This variability in many studies may reflect the complex multifactorial nature of the disease and differences in sampling techniques and assays used for analysis.

VI. Conclusion

It can be concluded from the study that there was high significant difference in clinical parameters [Plaque index(PLI) ,Gingival Index(GI) , Bleeding on Probing (BOP), Probing Pocket Depth (PPD)] among chronic periodontitis , gingivitis and control group . There were high significant difference p-value (0.000) between CP, gingivitis and control group in concentration of interleukin 6 (IL-6) (pg. /µl) in saliva and it was suggested that the increased levels of these inflammatory cytokines in periodontitis may have diagnostic and prognostic potentials for the monitoring of periodontal disease.

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