Estimation of soluble CD14 level in saliva of patients with different periodontal conditions and its correlation with periodontal health status

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ABSTRACT

Background: Cluster of differentiation 14 (CD14) is a serum/cell surface glycoprotein; and it is a pattern recognition receptor. CD14 expressed on the surface of various cells, or it found soluble in saliva and other body fluids. It has been proposed that soluble CD14 (sCD14) may play a protective role by controlling Gram negative bacterial infections through its capacity to bind lipopolysaccharide. This study was conducted to assess the level of soluble CD14 in saliva of patients with different periodontal diseases and healthy subjects and determine its correlation with clinical periodontal parameters.

Materials & Methods: A total of 80 subjects, age ranged (25-50) years old, divided into three main groups, group I consisted of 45 chronic periodontitis patients, group II consisted of 20 gingivitis patients, lastly group III comprised 15 apparently- healthy volunteers. Unstimulated whole saliva samples were collected to determine levels of soluble CD14 in saliva by enzyme-linked immune–sorbent assay (ELISA). Clinical periodontal parameters were recorded at four sites per tooth including plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level.

Results: A highly significant difference (P<0.01) was found for salivary sCD14 levels among the three groups, also it was greater in chronic periodontitis group than those detected for gingivitis and healthy controls with a highly significant difference (P<0.01). Furthermore, Spearman's correlation analysis showed statistically highly significant strong correlations (P < 0.05) between salivary sCD14 levels and each of (probing pocket depth, clinical attachment level). And non-significant correlation between salivary sCD14 level with plaque, gingival & bleeding on probing indices.

Conclusion: The findings of the present study reemphasize the importance of whole saliva as sampling method in terms of immunological purposes in periodontal disease and suggest that the elevated sCD14 concentration may be one of the host-response components associated with the clinical manifestations of periodontal disease.

Key words: soluble CD14, periodontal diseases, saliva. **(J Bagh Coll Dentistry 2014; 26(1):138-143).**

الخلاصة

الخلفية: كتلة التمايز 14 هي بروتين سكري يوجد في مصل الدم او على سطح الخلايا. وهو احد مستقبلات نمط التعرف. يتواجد على سطح مختلف الخلايا, منها الخلايا الاحادية الدموية, خلايا البلعمة النسيجية, الخلايا العدلة وكذلك الخلايا الليفية اللثوية. او يوجد ذائب في اللعاب او سوائل الجسم الاخرى. وقد اقُترح ان sCD14 ربما يلعب دور وقائياً عبر السيطرة على الاتهابات البكتيرية السالبة من خلال قدرته على الارتباط مع السكريات المتعددة البروتينات. ان المعلومات المتوفرة عن مستوى تركيز مستقبلات CD14 الذائبة في اللعاب عند مرضى النساغ المزمن قليلة جدا, لذا اعدت هذه الدراسة لتكون الاولى في العراق لاكتشاف مستوى هذه المستقبلات الذائبة في لعاب مرضى النساغ المزمن ومرضى التهاب اللثة وكذلك تحديد ارتباط هذه المستقبلات مع مؤشرات ما حول الاسنان السريرية.

المواد والطرق: اجمالي 80 شخص تتراوح اعمارهم بين (25 – 50) سنة , موزعون على 3 مجاميع , المجموعة 1 تتالف من 45 مريض لديهم مرض النساغ المزمن,اما المجموعة الثانية فتتالف من 20 مريض بالتهاب اللثة . واخيرا مجموعة المتطوعين الاصحاء وعددهم 15 شخص. وقد تم اخذ عينات اللعاب لتحديد مستوى المستقبلات وتحليلها بواسطة نظام الايلايزا(مقايسة الانزيم الممتز المناعي) وكذلك قياس مؤشرات ما حول الاسنان السريرية مثل مؤشر الصفيحة الجرثومية, مؤشر التهاب اللثة, مؤشر النزف عند التسبير, عمق جيوب اللثة وفقدان الانسجة الرابطة سريرياً.

النتائج: اظهرت هذه الدراسة أن هناك فرق معنوي بين مرضى النساغ المزمن ومرضى التهاب اللثة والاصحاء بالنسبة الى مستوى الـ CD14 الذائبة في اللعاب علاوة على ذلك، أظهر تحليل الارتباط سبيرمان وجود ارتباط بين البيانات من مستويات CD14 الذائبة في اللعاب وسبر عمق جيب اللثة وفقدان الانسجة الرابطة سريرياً .(P <0.01)

الاستنتاج: إن نتائج هذه الدراسة تؤكد مجدداً على أهمية اللعاب و طريقة أخذ العينات من اجل ألاغراض المناعية في مرضى النساغ المزمن ومرضى التهاب اللثة وتشير إلى أن ارتفاع تركيز مستقبلات CD14 الذائبة في اللعاب قد تكون واحدة من مكونات استجابة المضيف المرتبطة للمظاهر السريرية لمرضى النساغ المزمن ومرضى اللثة.

INTRODUCTION

Periodontal diseases are complex bacteria-induced infections characterized by an inflammatory host response to plaque microbiota and their by-products. Most of these microorganisms have virulence factors capable of causing massive tissue destruction both directly, or indirectly. In response to the aggression, host defense mechanisms activate innate and adaptive immune responses [1].

Periodontal disease is initiated and maintained in the first line by not only Gram negative (-ve) but also Gram positive (-ve) bacterial infection of the gingival sulcus [2].

Recognition of Gram -ve bacteria involves membrane-associated positive (-ve) bacterial infection of the gingival sulcus [2]. Recognition of Gram -ve bacteria involves membrane-associated lipopolysaccharide (LPS) activation of a series of proinflammatory cytokines and inflammatory mediators from various host cells through a key pathway of cell stimulation: LPS/ Lipopolysaccharide binding protein (LBP)/ cluster of differentiation 14 (CD14) [3]. Host recognition pathways for both Gram -ve and +ve bacteria comprise pattern recognition [2, 4].

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Cluster of differentiation 14 (CD14) is a serum/cell surface glycoprotein; is considered to be an important receptor for initial bacterial recognition [5, 7]. It is predominantly expressed on the surface of various cells, including peripheral blood monocytes, tissue macrophages [8], neutrophils, and chondrocytes [9], as well as gingival fibroblasts [10]. CD14 can be found in two forms, a membrane-bound (mCD14) protein [5, 11] and a circulating soluble (sCD14) form found in saliva, GCF and other body fluids [11, 12].

While limited information is available on the exact contribution of sCD14 to, and its mechanism of action in the pathogenesis of periodontal disease, it can be speculated that sCD14 plays a significant role because it is detected in elevated amounts in the GCF of patients with periodontitis [13].

It has been proposed that sCD14 may also play a protective role by controlling Gram -ve bacterial infections through its capacity to bind LPS [14]. Then concentrate LPS on the host cell surface for further recognition by the innate host response system [15, 16]. The signal transduction of the LPS/LBP/CD14 ternary complex on effectors cells is then transferred via the toll-like receptor 4 (TLR4)/MD-2 complex [17, 18]. Upon stimulation, the TLR4/MD-2 complex leads to expression of inflammatory mediators, [19] i.e. tumor necrosis factor-α (TNF-α), (IL-1 β), IL-6, and (IFN-γ) [20]. Besides its function in LPS/cell-wall products signaling, sCD14 might play a role in inflammatory diseases by controlling the immune system level of response [21]. It has been demonstrated that sCD14 is a regulatory factor capable of modulating cellular and humoral immune responses by interacting directly (without LPS) with T and B cells, decreasing antigen and mitogen-induced proliferation [22, 23]. Currently, there are no information on sCD14 levels in saliva and their associations with different periodontal conditions in Iraq. Therefore it was decided to conduct this study.

MATERIALS AND METHODS

Sample population included Eighty (80) subjects of both females and males aged from (25-50) years old. Sample recruited for this study were patients attended to the Department of Periodontics in the Teaching Hospital of College of Dentistry, University of Baghdad seeking periodontal treatment. All subjects enrolled voluntarily in the study after a well explanation about the aim and purposes of the study and gave informed consent to participate in the study in the period (November, 2012- March, 2013). From each subject, (5ml) of unstimulated whole saliva was harvested; removed particulates by cold centrifugation. The laboratory test was done in the Teaching Laboratories of Baghdad Medical City.

Exclusion criteria included the presence of less than 20 natural teeth, pregnancy and menopause ladies, smoker, Patients received periodontal treatment and /or regular used of anti-inflammatory medication, antibiotics or the use of other medications known to affect the periodontium in the past 3 months. In a cross sectional study, the subjects generally were divided into three main groups:

Group I: Composed of forty five (45) patients had chronic periodontitis, with probing pocket depth of 4 mm or more, according to WHO recommendation, with positive bleeding on probing.

Group II: Consisted of twenty (20) patients had gingivitis.

Group III: Consisted of fifteen (15) healthy volunteers with clinically healthy periodontium. The control group subjects were patients seeking treatment at other departments in the hospital. The periodontal status was evaluated by measurements of the following clinical periodontal parameters (PLI, GI, BOP, PPD, and CAL).

Measurements were performed at four sites per tooth for whole mouth excluding the 3rd molar. The readings of PPD were divided into 3 scores which are:

Score (0): Includes the examined sites with PPD range of (1-3) mm

Score (1): Includes the examined sites with PPD range of (4-5) mm.

Score (2): Includes the examined sites with PPD of (≥ 6) mm.

CAL readings were also divided into 3 scores which are:

Score (1): Includes the sites with CAL range of (1-2) mm.

Score (2): Includes the sites with CAL range of (3-4) mm.

Score (3): Includes the sites with CAL (≥ 5) mm.

Immunological analysis

Enzyme Linked Immuno-Sorbent Assay was used for quantitative determination of sCD14 Level in saliva. The work was done in the Immunology Department of Teaching Laboratories of Baghdad Medical City. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for sCD14 has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any sCD14 present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody

specific for sCD14 was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a TMB substrate solution was added to the wells and color develops in proportion to the amount of sCD14 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Statistical analysis

Data are calculated and entered into a computerized data base structure. Statistical analysis was done using SPSS software. Mean and SD, t-test, Chi square, ANOVA test, Mann-Whitney test, Kruskal-Wallis test and Spearman correlation coefficient (r) were used. Level of significance was 0.05.

RESULTS

Table (1) illustrates the mean values of PLI, GI and BOP of the three study groups, the values were expressed in mean and ±SD for PLI and GI and in percentage for non-bleeding and bleeding sites of BOP. It was clearly shown that chronic periodontitis group had the higher mean among the study groups (PLI 1.3444 ± 0.45214, GI 1.2676 ±0.37601, BOP 57.5% of sites had bleeding) followed by gingivitis group with a mean value of (PLI 1.0630 ± 0.30422, GI 1.0610± 0.38397, BOP 22.5% of sites had bleeding) and lastly the control group showed the minimum mean value of (PLI 0.1207 ± 0.08932, GI 0.0873 ± 0.08498, BOP only 1.3% of sites had bleeding).

The chronic periodontitis group was subdivided into three scores according to PPD and CAL. The distribution of the examined chronic periodontitis sites according to different scores of PPD and CAL had been illustrated in Figure (1 and 2).

A significant difference was observed between the gingivitis & chronic periodontitis groups with both PLI and GI and a highly significant statistical difference with BOP as shown in table (2). Regarding the sCD14 level, a highly significant difference was observed among the study groups with a (p-value < 0.001). The chronic periodontitis group had the higher median with (10.359) as illustrated in table (3). Inter group comparison revealed a statistical significant difference between the control & gingivitis groups & between the gingivitis & chronic periodontitis & a highly significant difference between the control & chronic periodontitis groups (p-value <0.001) as shown in table (4). There was no correlation between salivary sCD14 and clinical periodontal parameters with gingivitis and chronic periodontitis groups while a significant positive correlation was found with the control group. Regarding the correlation between the PPD, CAL parameters and the sCD14 level we noticed a positive highly significant correlation as shown in table (5).

DISCUSSION

In the present study a significant statistical difference was observed between the gingivitis & chronic periodontitis groups with PLI, GI.and BOP. This result was in agreement with the other studies [24, 25]. These are explained by the fact that the microbial biofilm is considered the primary and the major etiological factor responsible for initiation of periodontal disease [26]. As for BOP the finding indicates the effect of plaque accumulation on blood circulation & the actual pathophysiological process that happened more in inflamed tissue. And the severity of bleeding & the ease of its provocation depend on the intensity of the inflammation [27]. For the study groups there were no pathological true pockets or clinical attachment loss for the gingivitis & control groups while for the chronic periodontitis group, there were different scores of severities. Regarding PPD, it could be due to increase in the bacterial invasion and the amount of plaque that caused destruction of the sulcular & junctional epithelium & surrounding alveolar bone. As for CAL, it could be explained by the early concepts assumed that after the initial bacterial attack, periodontal tissue destruction continued to be linked to bacterial action [27]. Regarding the sCD14 level a highly statistical significant result in sCD14 level among the study groups. This is in agreement with other studies [13, 28] who evaluated the sCD14 levels in GCF by immunoblotting, And with studies [29, 30] whom evaluated the sCD14 levels in plasma & serum (it is important to mention that there is no study evaluated sCD14 in saliva to compare with). It is thought that sCD14 either protects or enhances the host response to microbial LPS [28]. And further establish sCD14 as an acute phase protein in periodontitis, whose level increases with disease severity. Many functions have been attributed to acute-phase proteins, including tissue repair, modulation of coagulation, neuroendocrine secretion, bacterial opsonization & clearing, hemopoiesis, metal binding, and, in the case of CD14, fighting infection [21].

Regarding the Clinical-Immunological correlation, there was a weak correlation between salivary sCD14 and PLI, GI and BOP parameters. This result may be due to small number of samples and there are no data to compare the results with it. Regarding the correlation between

the PPD parameters and the sCD14 level we noticed a positive highly significant correlation and positive highly significant strong correlation between the sCD14 level and CAL. This might be interpreted as more production of sCD14 in cases with mild-to- moderate -to- severe periodontal breakdown, which is consistent with the deleterious role of sCD14 because of LPS potent stimulation of sCD14 release [30] and shedding [31] from monocytes/macrophages and activated neutrophils. As shedding implies the release of the ectodomain of a cell-surface molecule that will keep its biological activity [13], once present in the extracellular environment in a soluble and biologically active form, sCD14 can interact with cells lacking cell-surface CD14 such as endothelial and epithelial cells [32, 33]. Thus, sCD14 could mediate cell activation induced by endotoxin and whole bacteria, resulting in the production of a potent immune response and proinflammatory mediators [34], and amplifying the inflammatory process [32, 33], which further take part in the tissue destruction and bone resorption observed in periodontitis [35]. In addition, more recent evidence suggests that when bacteria propagate in the periodontal pocket, salivary sCD14 promotes their invasion and induces production of IL-8 by oral epithelial cells to recruit neutrophils and T-cells and activate neutrophils for the initiation and establishment of an innate immune response to the bacteria at the site of infection [36].

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Table 1: The mean values of PLI, GI & the percentages of sites with BOP among the study groups

		Control No. = 15	Gingivitis No. = 20	Chronic Periodontitis No. = 45
PLI	Mean	0.1207	1.0630	1.3444
	± SD	0.08932	0.30422	0.45214
GI	Mean	0.0873	1.0610	1.2676
	± SD	0.08498	0.38397	0.37601
BOP	Score 0	98.7%	77.5%	42.5%
	Score 1	1.3%	22.5%	57.5%

Table 2: Groups comparison among the study groups of PLI, GI & the percentages of sites with BOP

	Group Comparison	t-test	p-value	Sig.
PLI	Gingivitis x Chronic Periodontitis	2.535	0.014	S
GI	Gingivitis x Chronic Periodontitis	2.031	0.046	S
BOP	Gingivitis x Chronic Periodontitis	Chi 710.913	0.000	HS

Table 3: The median of sCD14 level among the study groups

	Groups	No.	Median	Chi	df	p-value	Sig.
sCD14 Level	Control	15	5.428	21.772	2	0.000	HS
	Gingivitis	20	7.069				
	Periodontitis	45	10.359				

Table 4: Inters groups comparison of sCD14 level among the study groups

Inter Groups Comparison	Z-value	p-value	Sig.
Control x Gingivitis	2.471	0.013	S
Control x Chronic Periodontitis	4.587	0.000	HS
Gingivitis x Chronic Periodontitis	1.991	0.046	S

Table 5: Correlation between the periodontal parameters & the sCD14 level among the study groups

sCD14 Level	PLI		GI		BOP		PPD		CAL	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Control	0.570	0.027	0.269	0.333	0.380	0.163	-	-	-	-
Gingivitis	0.095	0.692	0.241	0.306	0.383	0.096	-	-	-	-
Ch. PD	0.217	0.153	0.058	0.706	0.287	0.056	0.489	0.001	0.504	0.000

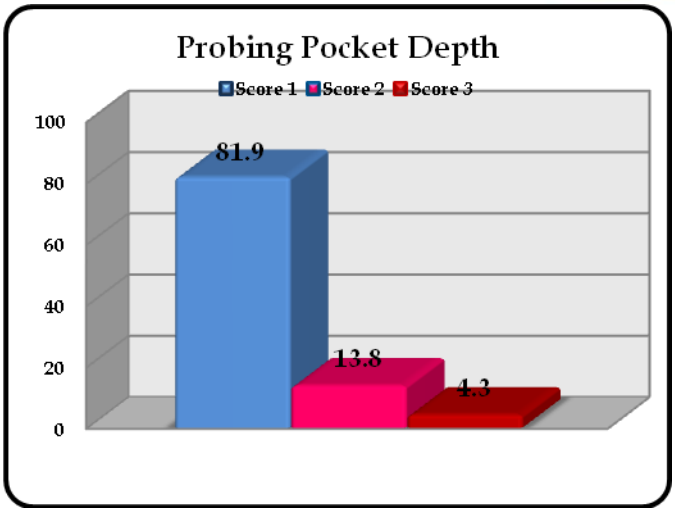

Figure 1: Bar chart of percentage distribution of PPD scores among the chronic periodontitis group

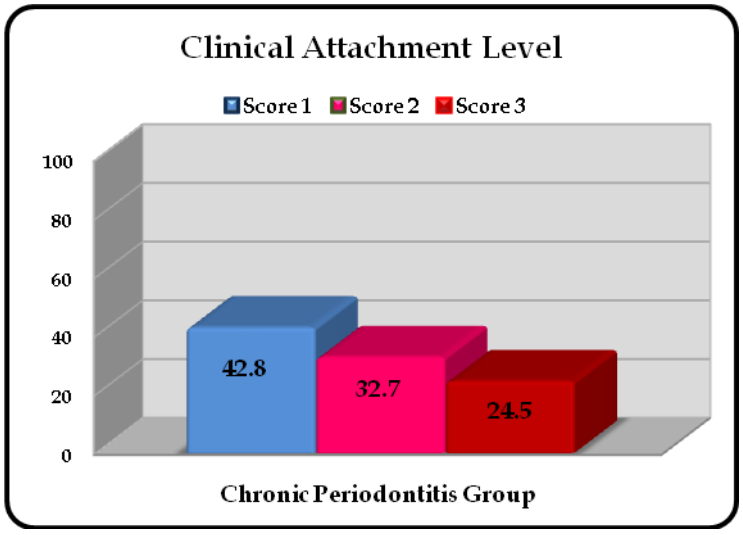

Figure 2: Bar chart of percentage distribution of CAL scores among the chronic periodontitis group