Tikrit Journal for Dental Sciences 5(2)(2017) 143-149

Salivary and Serum Levels of Both Uric Acid and C-Reactive Protein (CRP) Biomarkers in Patients with Behçet's Disease

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Abstract

Behçet's disease (BD) is a systemic vasculitis identified by recurrent oral and genital ulcerations, skin inflammations and uveitis, with unknown etiology. This study had been designed to assess the level of salivary and serum uric acid (UA) and C - reactive protein (CRP) markers in BD patients as activity markers and to explore their relations in disease existence. Fifty BD patients :(32 males and 18 females) with mean age (35.3 ± 7.6) years and fifty healthy subjects (30 males and 20 females) with mean age (34.8± 9.1) years were involved in this study that extend during the period of (2014-2016). All of patients and healthy subjects were analyzed for UA by (colorimetric spectrophotometric assay that based on oxidation of UA by uricase enzyme to allantoin) and CRP by (qualitative analysis: antigen-antibody interaction Latex agglutination test) in both saliva and serum. Data was analyzed using descriptive statistics, t-test, P (ANOVA) test, Chi-square ($\chi 2$) test, and Pearson's linear correlation coefficient statistical analyses. Results of this study show that salivary uric acid was significantly elevated in BD patients than in healthy subjects. ($P<0.001$). Although serum UA was higher in patients than the healthy subjects but statistically not significant. ($P=0.57$). On the other hand, out of fifty patients there were thirteen patients (26%) significantly expressed CRP positive in their serum and saliva. The results of this study revealed that UA can act as prooxidant (pro inflammatory) marker and CRP as inflammatory marker that could be related to impaired endothelial function in BD.

Key words: Behçet's disease, Uric acid,C - reactive protein, Saliva, Serum.

Introduction:

Behçet's disease (BD) is an inflammatory systemic disorder characterized by recurrent oral and genital ulcerations, skin assaults, and uveitis. Behçet's disease is known as a multisystem vasculitis [1]. International criteria of classification for diagnosis of BD have been defined with a sensitivity of 85% and specificity of 96%. International Study Group for Behcet's Disease [2]. The etiology of this disease is uncertain, but the most involving factors were inflammatory response to an infectious agent with genetic predisposition may cause the disease [3].The precise pathogenic mechanism. Is still unknown; but it is highly considered that an underlying vasculitis leads to lining epithelial dysfunction [4]. Uric acid (UA) in human is the main final product of purine metabolism that is produced from xanthine by the enzyme xanthine oxidase. during purine metabolism, molecular oxygen as electron acceptor will generate superoxide anion and other reactive oxygen products [5]. Uric acid may be a marking of oxidative stress and may have a possible curative role as an antioxidant. Furthermore; like other reducing substances also can have a role as a pro-oxidant, especially at higher level [6]. Thus, it is indistinct whether high levels of uric acid in a disease related with

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oxidative stress are a primary cause or a preventive response [7]. C-reactive protein (CRP) is a worthy product of hepatic origin that represents the systemic inflammations [8]. Also it has been found that the concentration of CRP in serum is relevant to the endothelial tissue function [9]. However, because there are no modern laboratory markers that reflect the activity of disease in patients with Behçet's syndrome. C-reactive protein (CRP) has been utilized to assess activity of disease and medical responses to therapy [10]. Objectives of this study was to assess the level of salivary and serum UA and CRP in BD patients as activity markers and compare with healthy subjects (age and sex matched) to explore their relations in disease existence.

Materials and methods:

This study include fifty BD patients (32 males and 18 females) with a mean age of (35.3 ± 7.6 years) and fifty healthy subjects (30 males and 20 females) with mean age (34.8± 9.1) years. BD Patients were all fulfilling the International Study Group criteria for BD [2] and were clinically diagnosed by Department of Dermatology and Venereology at the BD clinic in Baghdad Teaching Hospital and Department of Dermatology and Venereology at AlYarmook Teaching Hospital during the period (2014-2016). None of the patients had any other systemic disease. Other exclusion criteria were smoking habits, alcohol consumption, regular vitamin usage,Social and medical histories from all participants were taken; systemic and rheumatological examinations were completed. Pathergy test was performed to BD patients and evaluated after 24-48 hours in terms of pustular lesions. Patients were evaluated by an ophthalmologist for the involvement of eye. The examination of oral ulcer was made regarding the size (if more than one, the mean of largest diameter of ulcer size in millimeter (mm) was taken, the number [single, multiple (more than one)], the types (minor, major, both minor and major).

Saliva collection: samples of unstimulated saliva (Five ml) were obtained with the approval of the subjects in the morning following at least 2 hours of fasting. Each individual was first asked to rinse mouth using distilled water and after 5 minutes we got salivary samples without oral stimulation. Then each individual was sited comfortably and spitting into the plastic tubes for few minutes. Then the samples were centrifuged at 3000 rpm for 10 minutes and the supernatant was aspirated then saved at (-20°C) for subsequent biochemical analyses.

Blood collection: venous blood samples (five ml) were pulling up from antecubital vein of each person in the morning. The Entire blood was gathered in a sterilized disposable tube. The blood was left to clot thereafter the supernatant serum that was gained by centrifugation at 3000 rpm for 10 minutes was drawn and transmitted directly into other tube and frozen at (-20°C) for later analyses.

Biochemical analysis:

Determination of UA (colorimetric kit Bio Maghreb, Tunis): UA is oxidized by uricase to allantoin forming hydrogen peroxide, and according to the action of peroxide, react with 4-aminoantypirine (PAP) and 3, 5 dichloro-2-hydroxybezenesulfonic acid (DCHBS) forming **a red-violet quinoneimine dye** as indicator; the intensity of the measured coloration (by spectrophotometry) is proportional to the concentration of uric acid in the sample. It was read at a wavelength of (510 nm).

Determination of an inflammatory marker (CRP)(colorimetric kit, Spin react, Spain).: was measured by qualitative method (Latex slide agglutination) assay that based on principle: CRP reagent is a suspension of polystyrene latex particles plated with the gamma globulin part of anti-human CRP specific serum. When CRP exists in the sample, the appearance of **agglutination** indicates a CRP content of greater than or equal to 6 mg/l, without preceding sample dilution. Both tests had been done at

Poisoning Consultation Centre (P.C.C.), Specialized Surgeries Hospital in Baghdad.

Statistical analyses:

The statistical analyses were performed by using IBMSPSS version 23 computer software (IBM Statistical Package for Social Sciences). Data analysis was performed for independent samples. T-test was used to test the difference in statistical significance in the mean between 2 groups. ANOVA test was used to test the difference in statistical significance in the mean among more than 2 groups. A cross-tabulation was used to explore the association between 2 categorical variables; Chi-square ($\chi 2$) test of homogeneity was used to assess the statistical significance of such associations. Pearson's correlation coefficient was used to measure the strength and direction of linear correlation and the statistical differences between 2 quantitative normally distributed variables. P value less than the 0.05 level of significance was considered statistically significant.

Results:

Table (1) shows the mean salivary and serum uric acid in BD patients and in controls. Salivary UA was higher significantly in BD patients compared with healthy subjects. (P<0.001). Serum UA level was higher in patients than in healthy subjects but statistically not significant. (P=0.57). Out of fifty BD patients, thirteen patients (26%) expressed positive salivary and serum CRP, while all healthy subjects were negative for salivary and serum CRP, this is a highly significant result. (P<0.001). In this study, there was a total agreement between salivary and serum CRP positive expression, Table (2) as all the 13 BD patients who expressed positive serum CRP, they also expressed positive salivary CRP. Concerning the type of oral ulceration, the frequency of expression of positive salivary and serum CRP were significantly high (33.3%) in BD patients who had minor type oral ulcer Fig.(1,2) then followed by major ulcer (27.8%) then both minor and major ulceration (20%) (Table 2, P<0.001). Regarding correlation between UA and CRP, The mean salivary uric acid was slightly high in patients with positive salivary CRP (149.2±43.94) µmol/L. (P=0.46), (Table 3) and the mean serum uric acid was high in patients with positive serum CRP (259.8±76.97) µmol/L, but statistically not significant. (P=0.78). Linear correlation for Behçet's disease showed no correlations among salivary or serum UA, the age of patients in (years) and the total size of oral ulcer(s) in millimeter (mm) (P>0.05, Table (4)).

Discussion:

Saliva is an aquatic mixture collected easily that contains organic complex such as (uric acid, urea, glucose, fatty acids, cholesterol glycerides, lipids, amino acids) and inorganic ingredients such as electrolytes, in addition to high molecular weight compounds like the proteins [(11)].Up to our knowledge, this study was the first in Iraq to detect salivary UA (prooxidant agent) and CRP (inflammatory marker) in Behçet's disease. Due to paucity of data on CRP and uric acid levels in patient with BD. Uric acid (2, 6, 8-trihydroxypurine, UA) is the essential final product of purine metabolism. It is the main urinary nitrogenous compound and it is also found in other biological fluids: blood, serum and saliva [(12)]. Some studies reported that salivary UA is the most important non-enzymatic antioxidant and accounts for about 70% of the total antioxidant capacity in saliva [(13,14)]. UA share in a large extent of antioxidant capability in both saliva and blood. Also, its production will cause generation of free radicals and many studies have shown that UA can work as a pro-oxidant and pro-inflammatory factor [(15,16)]. In the current study, salivary uric acid was significantly higher in BD patients than in healthy subjects, as salivary UA may act as antioxidant so its level increased as a compensatory mechanism trying to counteract oxidative stress in a response to the damaging effect of free radicals release associated with Behçet's disease. Antioxidants have

shown to be elevated as a result of increased oxidative stress in BD in various studies [17, 18]. In the present study, serum UA level was higher in patients than in healthy subjects but statistically not significant, because concentrations of uric acid were affected by nutrition, age, intense practice, kidney failure and considerable metabolic ailments [19] .Thus, the level of serum UA may not correctly represent total antioxidant capacity. In agreement with our study, others reported that there was no significant variation between BD patients and the healthy subjects regarding the level of serum UA [20, 21]. CRP has been discovered in human saliva providing pathway to assess danger of disease and observing the reaction to therapy [22]. Although examining inflammatory biomarkers in blood is a standard practice, its identification in saliva is more convenient and non-invasive. These studies were in agreement with findings of the present study, salivary and serum levels were significantly express positive CRP in 13 BD patients. There were conflicting results in the preceding studies regarding the accuracy of CRP as a marker to explore BD activities [25]. Some stated that there were no statistically significant differences in the level of CRP in serum between BD patients and healthy subjects [26,27]. This might be due to the differences in sample size, disease durations or treatment modalities for BD patients. In a study by Pelin et al, [28], reveal that CRP had a considerable relation with particular clinical features of BD like The association with vascular lesion, genital ulcer ocular lesion.

Conclusions:

1-Uric acid level was significantly increased in saliva but insignificantly increased in serum of patients with BD as compared with healthy control subjects.

2-Both salivary and serum CRP level serve as markers of systemic inflammatory status that could be used in BD.

In the current study, regarding type of oral ulcer, Minor type had high significant percentage of the frequency of expression of positive salivary and serum CRP than the major type, this might be due to minor oral ulcers (<10mm in diameter) are the most common type (85%) in BD than the major type [29] . In the present study, there was a strong relation between salivary and serum CRP in term of positive patients and this is because concentrations of CRP measured in the saliva reflect its systemic level, as confirmed by Ouellet-Morin et al. [30].

3-Saliva could be used as an alternative for the serum in assessing CRP level in patients with BD.

4-Minor type oral ulcer in BD patients had significantly higher percentage of inflammatory marker (CRP) in both saliva and serum.

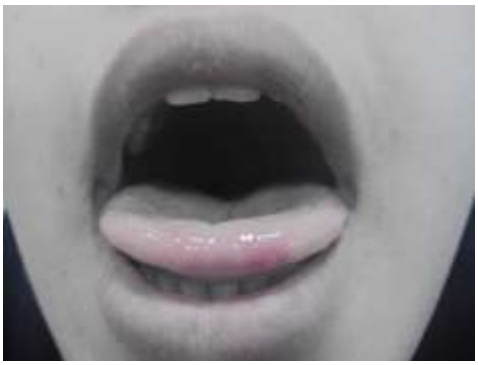

Fig. (1): Minor oral ulcer in BD patient.

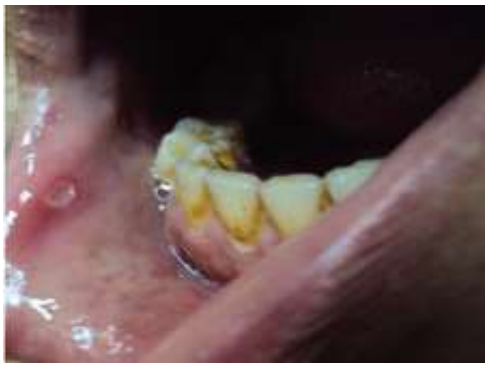

Fig. (2): Minor oral ulcer in BD patient.

Table (1): The mean±SD of salivary and serum uric acid levels (µmol/L) in Behçet's disease patients and control subjects.

Uric acid (µmol/L)		Healthy controls	Behçet's disease	P
Salivary	**Range**	60.96 -150.73	75.56 -290.95	
	Mean±SD	99.7±26.06	141.6±42.93	<0.001
Serum	**Range**	60.96 -396.66	120.78 -471.83	
	Mean±SD	246.6±73.42	254.9±72.34	0.57

Table (2)1: The frequency distribution of positive salivary and serum CRP of total Behçet's disease patients and in relation to the type of oral ulcerations.

CRP expression		Total patients		Type of oral ulcer						P
				Minor		Major		Both types		
		N	%	N	%	N	%	N	%	
Salivary	**Negative**	37	74	8	66.7	13	72.2	16	80.0	<0.001
	Positive	13	26	4	33.3	5	27.8	4	20.0	
Serum	**Negative**	37	74	8	66.7	13	72.2	16	80.0	<0.001
	Positive	13	26	4	33.3	5	27.8	4	20.0	

Table (3): The range, mean±SD of serum and salivary uric acid levels in relevance to serum and salivary CRP expression.

Uric acid (µmol/L)		Salivary and serum CRP Expression		P
		Negative 37	Positive 13	
Salivary	**Range**	75.56 -290.95	95.2 -263.66	
	Mean	138.9±42.85	149.2 ±43.94	0.46
Serum	**Range**	120.78 -471.83	126.73 -389.72	
	Mean	253.2±71.66	259.8 ±76.97	0.78

Table (4): Linear correlation among patients with Behçet's disease.

	Salivary Uric acid (µmol/L)	Serum uric acid (µmol/L)	Age (years)
Serum uric acid (µmol/L)	r=0.169 P=0.24		
Age (years)	r=0.074 P=0.61	r=0.17 P=0.24	
Total size of oral ulcers in millimetre (mm)	r=-0.196 P=0.17	r=0.098 P=0.5	r=-0.139 P=0.34

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