Salivary and Serum Oxidant/Antioxidant Level in Behçet's Disease Patients

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Abstract
Background Behçet's disease (BD) is a multi-systemic inflammatory disorder characterized by recurrent oral and genital ulceration. Free oxygen radicals may have a role in the pathogenesis of BD and can relate to the existence of the disease.
Objectives This study had been designed to assess salivary and serum lipid per-oxidation as malondialdehyde (MDA) and status of antioxidant as total glutathione (GSH) in patients with BD in comparison with healthy subjects to detect the possible involvement of oxidative stress in BD. **Materials and methods** This is a case–control study, salivary and serum samples were taken from fifty BD patients with an age range 20 to 56 years with mean age 35.3 ± 7.6 years and fifty healthy control subjects with age range 16 to 58 years and mean age 34.8± 9.1 years. All were analyzed for MDA and GSH spectrophotometrically. Data was analyzed using descriptive statistics, t-test, P (ANOVA) test, Chi-square (2X) test, Pearson correlation and Receiver Operating Characteristic statistical analyses. **Results** The study showed that salivary and serum MDA level was higher in BD patients than in healthy subjects ($P<0.05$ and $P<0.001$ respectively). The levels of saliva and serum total GSH were significantly lower in BD patients than in healthy subjects. ($P <0.001$). **Conclusion** The results of this study revealed that BD patients were subjected to oxidative stress damage and saliva could be used as pain-free alternatively to serum in determining oxidative stress in Behçet's disease.

Keywords Behçet's disease, MDA, GSH, saliva, serum

Introduction

Behçet's disease (BD) is a systemic inflammatory disorder clinically dominated by recurrent oral and genital ulcerations, uveitis, and skin lesions. It is a vasculitis, affecting vessels of different sizes, types and localizations. The Behçet's disease has a chronic course, with undetectable remissions and exacerbations with the severity and frequency that may diminish with time (Al-Otaibi et al, 2005). Behçet's disease is commonly observed among people living along the old Silk Road which extended between the Mediterranean, the Middle East and the Far East and is noted frequently between the30th and 45th degree latitudes in Asian and European populations (Keino and Okada. 2007).
The mean age of onset occurs mainly between 18 to 40 years and after 55 years the

onset of BD is rare and the diagnosis has to be made very cautiously (Saadoun and Wechsler, 2012).
Although the etiology of BD is still unknown. The main factors are genetic background and triggers of infectious agents with environmental factors as well as impairment of antioxidant defense system (Karaman et al., 2009).
It is suggested that free oxygen radicals and reactive oxygen species may have a role in the pathogenesis of BD that can lead to increase in the levels of malondialdehyde (MDA), which is the end product of lipid peroxidation and serves as an indicator of oxidative stress (Saglam et al., 2002; Isik et al., 2007; Taysi et al., 2008).
The objectives of this study were to assess the level of salivary and serum lipid peroxidation as MDA and status of antioxidant as glutathione (GSH) in Behçet›s disease patients in comparison with healthy subjects (age and sex matched) to explore the possible relation of oxidative stress in the existence of Behçet's disease and to explore the association between selected clinical features among Behçet's syndrome with oxidative stress parameter.

Materials and methods

This study was carried out in Baghdad at the multidiscipline BD clinic in Baghdad Teaching Hospital, Department of Dermatology and Venereology at Al-Yarmook Teaching Hospital and Poisoning Consultation Centre (P.C.C.) at the Specialized Surgeries Hospital for laboratory work. An ethical approval was taken from the Scientific Ethical Committee (Reference number 8 on March 29th, 2017).
Fifty patients (32 males and 18 females) with BD with an age range of 20 to 56 years with a mean age (± SD) 35.3 ± 7.6 years and fifty healthy subjects (30 males and 20 females) with an age range of 16 to 58 years and mean age of 34.8± 9.1years involved in this study.
The international diagnostic criteria for BD were used for diagnosis of patients. (International Study Group for BD, 1990). These criteria include the mandatory presence of recurrent oral ulceration, plus at least two of the followings: recurrent genital ulceration, eye inflammation, skin lesions (pseudofolliculitis and erythema nodosum), and positive pathergy test (local inflammatory reaction to scratches or intradermal saline injection). The patients did not have any other systemic diseases.
Examination of the oral ulcer was made regarding the size (if more than one, the mean of the largest diameter of ulcer size in millimeter (mm) was taken. Number (single or more) and types (minor, major or both).
Fasting blood samples and unstimulated salivary samples were obtained from patients with BD and from healthy subjects, then processed by centrifugation at 3000 rpm for 10 minutes and frozen at (-20o C) for subsequent analysis.

Statistical analyses

The statistical analyses were performed by IBMSPSS version 23. For independent samples T-test was used in the mean and standard deviation (SD) in two groups. ANOVA test was used to test the statistical significance of differences in mean between more than two groups. Chi-square (X2) test of homogeneity was used for tables with frequencies. Pearson's coefficient was used for linear correlation analysis between two quantitative normally distributed variables. The results were considered statistically significant when P value ≤ 0.05 level of significance. ROC analysis was used to calculate the validity parameter of a quantitative test at successive cut-off

values to predict Behçet's disease differentiating it from healthy subjects.

Biochemical analysis

Malondialdehyde (MDA) assay: The MDA level was measured according to the method of Shah and Walker, (1989) which is based on the reaction of MDA (the last product of fatty acid peroxidation) with thiobarbituric acid (TBA), under heat and acidic condition to give a pink chromogen, which was measured by the spectrophotometer at 532 (nm). The results were expressed in µmol/L.

Glutathione (GSH) assay: GSH level (µmol/L) was measured according to the method of Burtis & Ashwood, (1999). The concentration of GSH is directly proportional to the absorbance of the reduced chromogen that is measured at 405 (nm) by the spectrophotometer.

Results

The mean ± SD age of BD patients and healthy subjects were (35.3 ± 7.6) and (34.8± 9.1) years respectively (P >0.05). This study was conducted on fifty BD patients (32 males and 18 females) and fifty healthy subjects (30 males and 20 females) (P>0.05). There was no statistically significant difference in age and gender between patients and healthy subjects.

Table (1) showed that salivary and serum MDA was significantly elevated in patients than in healthy subjects. The P value is P <0.05 and P <0.001), respectively. Salivary and serum GSH was significantly diminished in patients compared with healthy subjects (P <0.001).

There were no statistically significant correlations between the number and type of oral ulcer(s) in BD patients with salivary and serum MDA and GSH level as shown in tables 2 and 3.

Figure (1) shows that salivary MDA considered highly sensitive and less specific in the detection of the existence of Behçet's disease differentiating it from healthy subjects (ROC area did not differ significantly from the 0.5 area) while serum MDA was highly specific and less sensitive in this context. (ROC area >0.9), (P <0.001).

Salivary and serum GSH were very strong (highly specific and less sensitive) tests as shown in Figure 2, (ROC area >0.8), (P <0.001) to predict the existence of Behçet's disease and differentiating it from healthy subjects

There was a strong negative linear correlation between salivary GSH and MDA (r= (-0.603), P <0.001) as shown in Table 4 and Figure 3 and there was no correlation between oxidant/antioxidant markers with oral ulcer size in mm and age of patients.

Table (1): Mean ± SD Salivary and Serum oxidant/antioxidant in Behçet's disease patients compared with healthy subjects.

	B.D. patients (Mean±SD) N=50	Healthy subjects (Mean±SD) N=50	t-test (P value)
SalivaryMDA(µmol/L)	4.3±2.59	3.5 ±1.09	P<0.05
Salivary GSH (µmol/L)	1.7±1.06	3 ±1.15	P<0.001
Serum MDA (µmol/L)	9.2±2.11	6±1.04	P<0.001
Serum GSH (µmol/L)	2.9±1.1	5.8±1.72	P<0.001

Table (2): Mean ± SD Salivary and Serum oxidant/antioxidant levels according to the number of oral ulcer(s) among Behçet's disease patients.

	The Number of oral ulcer(s)		
	Single (Mean±SD) N=15	Multiple (Mean±SD) N=35	t-test (P value)
Salivary MDA (µmol/L)	5± 2.61	4±2.55	P=0.2
Salivary GSH (µmol/L)	1.7±1.25	1.7±0.99	P=0.94
Serum MDA (µmol/L)	9.9±2.28	8.9±1.99	P= 0.12
Serum GSH (µmol/L)	3±1.01	2.8±1.14	P=0.44

Table (3): The Mean ± SD of Salivary and Serum oxidant/antioxidant by the type of oral ulcer among Behçet's disease.

	Types of oral ulcer(s)			
	Minor (Mean±SD) N=12	Major (Mean±SD) N=18	Both types (Mean±SD) N=20	P(ANOVA)
SalivaryMDA(µmol/L)	5.5±2.63	3.7±2.16	4.2±2.78	P=0.15
Salivary GSH (µmol/L)	1.5±1.15	1.9±1.12	1.6±0.96	P=0.45
Serum MDA (µmol/L)	10.3±2.4	8.9±1.9	8.7±1.96	P=0.1
Serum GSH (µmol/L)	3.2±1.04	2.8±1.16	2.7±1.09	P=0.45

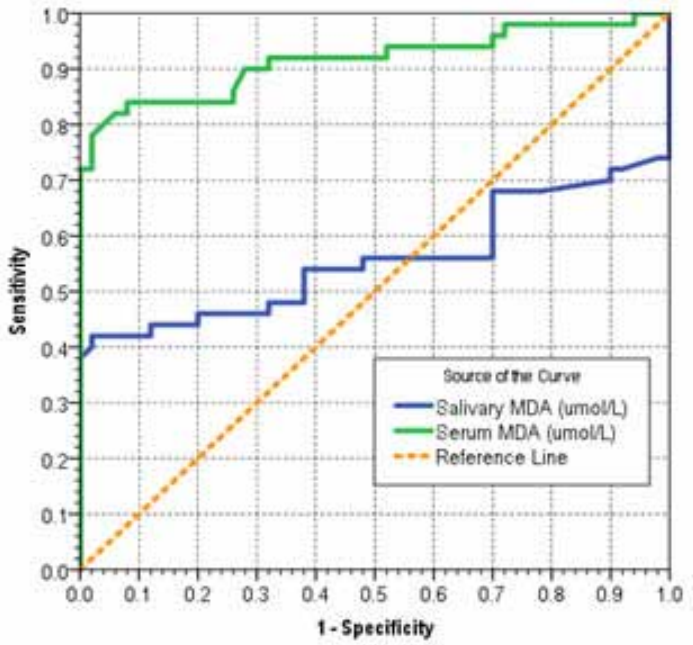

Figure 1: Receiver operating characteristic (ROC) curve showing the trade-off value between sensitivity and specificity for the salivary and serum MDA to predict a positive diagnosis of Behçet's disease, differentiating it from healthy subjects.

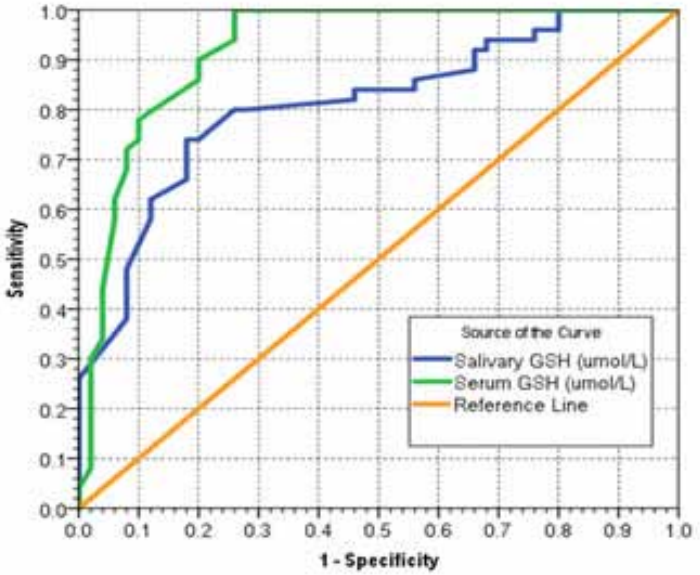

Figure 2: ROC curve showing the trade-off between sensitivity and specificity for the serum and salivary GSH when used as test to predict a positive diagnosis of Behçet's disease, differentiating it from healthy subjects.

Table (4): Linear correlation between Behçet's disease patients between clinical and laboratory parameters.

	Salivary MDA (µmol/L)	Salivary GSH (µmol/L)	Serum MDA (µmol/L)	Serum GSH (µmol/L)	The mean of size of oral ulcer(s) in millimeter (mm)
Salivary GSH (µmol/L)	r=(-0.603) P<0.001				
Serum MDA (µmol/L)	r=0.148 P=0.31	r=0.033 P=0.82			
Serum GSH (µmol/L)	r=0.217 P=0.13	r=0.162 P=0.26	r=0.035 P=0.81		
The mean of size of oral ulcer(s) in (mm)	r=(-0.251) P=0.08	r=0.14 P=0.33	r=(-0.288) P=0.043	r=(-0.122) P=0.4	
Age (years)	r=0.019 P=0.9	r=(-0.136) P=0.35	r=0.138 P=0.34	r=0.076 P=0.6	r = (-0.154) P=0.29

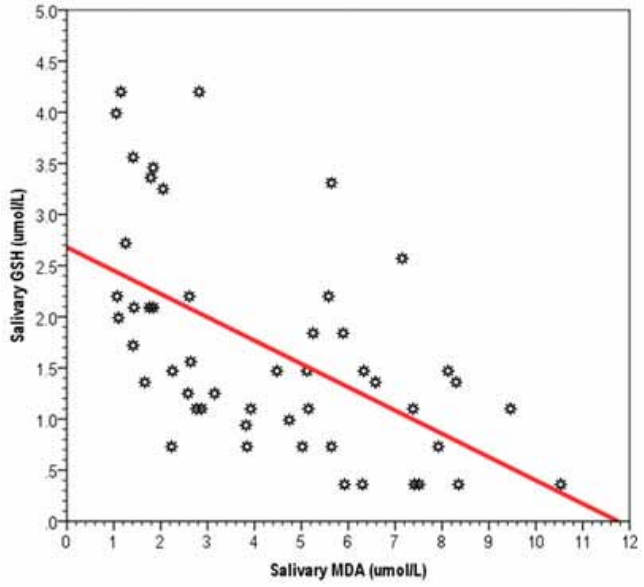

Figure 3: Scatter graph showing a strong negative (inverse) linear correlation between salivary GSH and MDA (r=-0.603, P <0.001) among BD patients.

Discussion

BD is a chronic inflammatory multi-systemic syndrome with uncertain etiology and pathogenesis and there were many studies in the literature that detect markers of oxidant/antioxidant level in blood. In this study the use of saliva as a diagnostic, substitutive tool for oxidant/ antioxidant markers and correlation with serum level was made, since saliva can be used in many studies in diagnosis and implications of oral and systemic disorders.

Saliva has become a widespread diagnostic fluid for medical research and clinics in the last years. Since it's available, easy to collect and repeated non-invasively. However, sensitivity as well as specificity of saliva as a diagnostic aid has to be validated before the routine clinical use (Lee and Wong, 2009).

MDA is the most prominent product of lipid peroxidation that can be used as a mark for the evaluation of oxidative stress (Marnett, 1999). Oxidative stress can also be estimated by measuring the antioxidant capacity in most types of biological fluid (Suresh et al., 2009).

Elevated MDA levels reflect increased oxidative stress, which is one of the suspected factors in the existence of BD (Onur et al., 2011).

In the current study there is a statistically significant elevation in serum MDA level in BD patients compared to healthy subjects. ($P < 0.001$). Serum MDA levels may reflect the disease activity and this is consistent with previous studies of Kose et al., 2002, Aydin et al., 2004, Buldanlioglu et al., 2005, Nassar et al., 2006, Taysi et al., 2007, Bilen et al., 2016. These results revealed that the lipid oxidation represented by high level of MDA could be responsible for oxidative damage (particularly the endothelial tissue damage) presenting in BD patients. Saliva being non-invasive, easy to collect and bearable to the patient with convenient approach can be used to assess MDA levels. Regarding salivary MDA level, there is a statistically significant elevation in BD patients than in healthy subjects ($P. < 0.05$). On the other hand, the specificity and sensitivity of salivary and serum MDA in the detection of BD by ROC curve, Figure 1 showed that the serum MDA was an accurate test (highly specific and less sensitive) in the detection of the existence of Behçet's disease. (ROC area > 0.9), ($P < 0.001$); while salivary MDA was considered highly sensitive and less specific (ROC area did not differ significantly from the 0.5 areas). This might be because salivary markers of oxidative stress seem to be locally produced, example, in chronic periodontitis, the local production of types of reactive oxygen by oral microorganisms or activated neutrophils appears to be of interest. In systemic diseases subjected to oxidative stress, the transmission of molecules from plasma to saliva by active transport or intracellular transfusion within salivary glands could be the main source of salivary oxidative stress (Tóthová et al., 2015). Saliva contains local and serum-derived markers that have been shown to be of benefit in the screening of a wide range of systemic disorders; however, the levels of specific markers in saliva are not always a correct reflection of their levels in serum. The transmission of serum ingredients which are not part of the normal salivary composition into saliva is related to the chemical and physical properties of these molecules. A salivary composition determination could be affected by the salivary flow rate and its method of collection. Furthermore, certain salivary enzymes could influence the constancy of definite diagnostic markers. Some particles were also decomposed during intracellular transport into saliva (Kaufman and Lamster, 2002). Numerous antioxidant molecules present in blood that suppress the deleterious effects of free oxygen radicals (Young and Woodside, 2001). There are studies with a

variety of results on enzymatic and non-enzymatic system and trace elements that are a part of antioxidant defense system in Behçet's disease. The total antioxidant defense capacity in BD patients has been significantly diminished than healthy subjects in numerous studies of Orem et al., 2002, Najim et al., 2007, Harzallah et al., 2008, Korkmaz et al., 2011, Gul et al., 2014. The current study showed that the levels of salivary and serum total glutathione were significantly lower in BD patients than in healthy controls. (P <0.001), this confirms the presence of oxidative stress state in BD. Regarding (ROC) curve method, Salivary and serum GSH were very strong (highly specific and less sensitive) tests as shown in figure 2, (ROC area >0.8), (P <0.001) to predict the existence of Behçet›s disease and differentiating it from healthy subjects. In contradiction to the present study of Sandikcis et al., (2003) and Bekpinar et al., (2005), reported no significant difference in the total antioxidant capacity among BD patients and healthy subjects. The probable explanation for this difference in the capacity of the antioxidant system in BD is that the activity of the disease has a noticeable role in the efficiency of antioxidant system; there is a various activity index for Behçet›s disease.

In consistence with the above findings, Pearson correlation coefficient was applied in this study between parameters revealed a strong negative (inverse) linear correlation between salivary GSH and MDA (r= (-0.603), P <0.001), as shown in Table 4 and Figure 3 and this could support the oxidative stress hypothesis in BD.

There was no correlation between the studied salivary and serum oxidant/antioxidant markers and the mean of the largest diameter of oral ulcer size in mm. This may be because of the imbalance between oxidant/antioxidant levels is related to systemic involvement of BD regardless the size of the oral ulcer. The study conducted by Najim et al., (2007) found that serum MDA levels were found to be positively correlated with the oral ulcer size and there is an inverse correlation between serum total glutathione and oral ulcer size.

Conclusion

BD patients are subjected to oxidative stress damage reflected by an elevation in saliva and serum MDA level and diminution in antioxidant levels (GSH). Saliva could be used as a simple, noninvasive laboratory tool. The study reveals that there is no correlation between oxidant/antioxidant markers and clinical characteristics of the oral ulcer in BD. Supplementation of the antioxidant in addition to medical therapy may be helpful in the management of BD patients.

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