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SERUM AND SALIVARY ANTIOXIDANT BIOMARKERS IN PATIENTS WITH RECURRENT APHTHOUS STOMATITIS

HUSSEIN SH. AL-ESSA[1] & ADIL G. FADIL[2]

[1]Assistant Lecturer, Department of Oral Diagnosis, College of Dentistry, Basrah University, Iraq

[2]Assistant Lecturer, College of Dentistry, Basrah University, Iraq

ABSTRACT

Background: Recurrent aphthous stomatitis (RAS) constitutes the most common oral mucosal lesion affecting approximately 20% of the population. In which painful, recurrent, oval or round ulcers of various size and location of oral mucosa Many factors thought have been involved in its etiology; these factors might have at the same time a direct or an indirect impact upon the oxidant/antioxidant system of the body that trigger free radicals which cause traumatic effect on mucosal tissue. The aim of this study was to assess the antioxidant biomarkers in patients with recurrent aphthous stomatitis (RAS).

Materials and Methods: The study included 30 patients with (RAS) as cases who attended to outpatient clinic of Oral Medicine (oral medicine clinic at the College of Dentistry, Basrah University) and 30 healthy individuals as control. Both groups were matched for age and sex, from whom saliva and blood samples were collected. The RAS patients had oral ulcer attack recurring at least three times a year and had active lesions at time of the study. Catalase (CAT) enzyme and Uric acid (UA) as antioxidant biomarkers were measured in serum and saliva of both groups.

Results: The mean of serum and salivary CAT and UA in patients with recurrent aphthous stomatitis was slightly lower than that of healthy controls, but both of them statistically was not significant ($P>0.05$). UA showed a highly statistically significant correlation ($P<0.01$) between serum and salivary of patients with RAS which had a direct (positive) linear correlation ($r= 0.516$).

Conclusions: The antioxidant defense system (enzymatic or non-enzymatic) become deficient due to consumption of antioxidants and/or by an overload of oxidant species lead to changes in the oxidative stress in biological systems which important in the inflammatory reactions observed in recurrent aphthous stomatitis.

KEYWORDS: Recurrent Aphthous Stomatitis, Antioxidant Biomarkers, Catalase Enzyme, Uric Acid

INTRDUCTION

Recurrent aphthous stomatitis (RAS) or recurrent aphthous ulceration (RAU) is an extremely common disorder of the oral cavity, estimated to affect 20% of population. There is some evidence of a familial tendency to RAS[(1)]. The RAS can occur in any age group, but it is more commonly found in young adults[(2)]. Three clinical patterns are recognized:- Minor aphthae are generally located on labial or buccal mucosa, the soft palate and the floor of the mouth (***non- keratinized mucosa***), they can be singular or multiple and tend to be small (less than 1 cm in diameter) and shallow, this type of RAS is the most common (80% of cases), and usually heals within 7-14 days[(3)]. Major aphthae are typically large and deep ulceration and heals slowly over weeks, or even months. It has also been shown that major aphthae are more

likely to heal with scar. Herpetiform aphthae are frequently more numerous and vesicular in morphology and usually heals within about 1 month [(3)].

The exact etiology of RAS remains elusive, recently oxidant-antioxidant imbalance of the body has been implicated in the pathogenesis of recurrent aphthous stomatitis[(4)].

Oxygen is a double edged sword; it is a vital component for living. Oxygen meditates chemical reactions that metabolize fats, proteins and carbohydrates to produce energy. While, on other hand Oxygen is a highly reactive atom that is capable of becoming

Part of potentially damaging molecules commonly called “free radicals”. Accordingly, mammalian cells have developed complicated antioxidant defense system to prevent oxidative damage and allow survival in an aerobic environment. This system includes: - ***enzymatic activities*** such as superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). Or ***non-enzymatic antioxidants*** such as vitamins (A, C & E) and Uric Acid (UA)[(5), (6)].

Antioxidants are a class of molecules that are capable of inhibiting the oxidation of another molecule. Antioxidants play a significant role in your health, as they can control how fast you age by fighting free radicals[(7)].

Catalase enzyme (CAT) is an antioxidant enzyme, like superoxide dismutase (SOD) and glutathione peroxidase, Catalase works closely with superoxide dismutase to prevent free radical damage to the body. Superoxide dismutase (SOD) converts the dangerous superoxide radical to hydrogen peroxide, while catalase converts to harmless water and oxygen. Catalases are some of the most efficient enzymes found in cells; each catalase molecule can convert millions of hydrogen peroxide molecules every second [(8)].

Uric Acid (UA) is one of the important antioxidants and contributes approximately (70%) of the total salivary antioxidant capacity [(9)]. It is the final product of purine metabolism in human.

During purine metabolism, molecular oxygen was used as electron acceptor and generation of superoxide anion and other reactive oxygen products occur. Uric acid may be a marker of oxidative stress and may have a potential therapeutic role as an antioxidant. On other hand; like other strong reducing substances can also act as a pro-oxidant, particularly at higher level [(10)]. Thus, it is unclear whether elevated levels of uric acid in disease associated with oxidative stress are a protective response or a primary cause [(11)].

MATERIALS AND MATHODS

The study included 30 patients with RAS as cases who attended the outpatient clinic (Oral medicine clinic at the College of Dentistry, Basrah University), and 30 healthy individuals as control who were selected from the patients attending the same clinic with other dental problems. Age and sex were matched for both groups. The RAS patients had oral ulcer attack recurring at least three times a year and had active lesions during the study without therapeutic regimen for the past 3months. The informed consent had been taken from all subjects under study. Venous blood samples (5ml) were aspirated from anticubital vein of each individual in the morning from the two groups. The whole blood was collected in sterile disposable plain tube. The blood was left to clot then the supernatant serum which was obtained by centrifugation at 3000 rpm for 10 minutes was aspirated and transferred immediately into another tube and frozen at (-20°C) for subsequent analysis. Haemolyzed samples were discarded. Non stimulated salivary samples (5ml) were taken from subjects of two groups. The patients were told to sit comfortably and to spit into the plastic polyethylene tubes for five

minutes. The samples were centrifuged at 3000 rpm for 10 minutes and the supernatant was aspirated then stored at (-20°C) until biochemical analysis.

Assay of Antioxidant Biomarkers

Determination of Catalase Enzyme (Human Catalase (CAT) ELISA Kit, Mybiosource, USA): CAT enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay The coated well immunoenzymatic assay for the quantitative measurement of CAT utilizes a polyclonal anti-CAT antibody and a CAT-HRP conjugated. The assay sample and buffer were incubated together with CAT-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells decanted and washed five times. The wells were then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms *a blue colored complex*. Finally, a stop solution is added to stop the reaction, which will then turn the solution *yellow*. The intensity of color is measured spectrophotometrically at 450 nm in a micro plate reader. The intensity of the color is inversely proportional to the CAT concentration since CAT from samples and CAT-HRP conjugate compete for the anti-CAT antibody binding site.

In order to measure the concentration of CAT in the sample, this CAT ELISA kit includes a set of calibration standards. The calibration standards are assay at the same time as the sample and allow the operator to produce a standard curve of Optical Density (OD) versus CAT concentration.

The concentration of the sample is then determined by comparing the OD of the samples to the standard curve.

Determination of Uric Acid (Colorimetric Kit, Bio Merieux, France): Uric acid is oxidized by uricase to allantion forming hydrogen peroxide. And according to action of peroxide, react with 4-aminoantypirine (PAP) and 3, 5dichloro-2-hydroxybenzenesulfonic 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.

Statistical Analysis

The data collected were analyzed by using program SPSS version 20. All values were expressed as mean ± SD, t-test, ANOVA test and chi square were used to analyze the significance between parameters, the Pearson correlation coefficient (r) was used to test the statistical significance, direction and strength of linear correlation between two quantitative variables. The P value of less than 0.05 was considered to be statistically significant.

RESULTS

Demographics

The mean age of subjects in study and control group was (34.03±11.98) years with age range from (14-55) years and (29.50±10.72) years with age range from (12-46) years respectively. Both the study and control groups comprised 16 (53.3%) males and 14 (46.6%) females.

Clinical Examination Findings

Types of Ulcer(S): The minor aphthous ulceration is significantly higher than major aphthous ulceration in case study group which comprised (60 %, 26.6 %) respectively, while remaining was the mixed type (minor and major aphthous ulceration). As shown in figure (1a).

Sites of Ulcer(S): The aphthous ulceration on non-keratinized mucosa (70%) was higher than that of aphthous

ulceration on keratinized mucosa (10%), and 20 % on both keratinized and non-keratinized mucosa. With highly statistical significant (p value < 0.01). As shown in figure (1b)

Assessment of Catalase Enzyme (CAT) and Uric Acid (UA): The mean of serum and salivary CAT & UA in RAS patients was slightly lower than that of healthy controls, but both of them statistically was not significant. As shown in (table 1and figure 2).

The Correlation Coefficient (R) Between Serum and Saliva Parameters in Patients with (RAS): Uric acid showed a highly statistical significant correlation (P<0.01) between serum and salivary of patients with RAS which had a direct (positive) linear correlation. As shown in Table 2.

DISCUSSIONS

Considerable activity of reactive oxygen radicals may lead to destroy normal cell functions and integrity of cell structures by the effect of oxidative stress (oxidative stress occurs when the reactive oxygen radicals increase over the physiological value), this oxidative stress in biological systems can be induced by the consumption of antioxidants and/or by an overload of oxidants species, so that antioxidant levels become deficient. [5, 12]

Clinical Examination Findings

Type of Ulcer(S): The results showed that patients with minor aphthous ulceration were significantly high. This result agreed with other studies ***(Neville et al., 2008; Greenberget al., 2008)*** [2, 3], but disagreed with ***(Mohammad, 2012)*** [13].

Site of Ulcer(S): In the present study the results showed that ***(70%)*** of patients with RAS have aphthous lesions on *non-keratinized* mucosa, while ***(10%)*** located on areas of *keratinized* mucosa and ***(20%)*** located on both areas of keratinized and non-keratinized mucosa, and this changes was statistically significant. This could be explained on the fact that the non-keratinized mucosa areas were movable tissue and least resistant and mostly affected by trauma which was the most precipitating factor in developing the aphthous ulcer. These were agreed with the results of ***Scully et al., 2003; Mohammad, 2012)*** [5, 13]**.**

BIOCHEMICAL FINDINGS

Catalase Enzyme (CAT)

The current study showed that there was no statistical significant difference in the mean of serum and salivary CAT in RAS patients and healthy controls. In spite that the mean of both of them was slightly lower than that of healthy controls and the mean of serum CAT was significantly higher in males with RAS than that in females; this may be due to hormonal influence. These results agree with the study of (Karincaoglu et al., 2005)[6]; they reported that SOD and CAT activity in plasma of RAS patients was lower than that of control group. Cimen et al., 2003[8] measured SOD, GPx, and CAT activity in plasma of RAS patients and control group; they observed a relative reduction in CAT and GPx activity. While other studies conducted by (Gunduz et al., 2004)[14] obtained exactly the opposite results. From the best of our knowledge no previous studies were found on saliva CAT in RAS patients.

Uric Acid (UA)

In this study, the serum and salivary UA concentration was lower in RAS patients than healthy controls, but this difference was not statistically significant. Serum and salivary UA were higher in males than that in females, Also the

results showed a highly statistically significant correlation between serum and saliva of patients with RAS which had a direct positive linear correlation (r=0.515).

These disagree with the results of **(Mohammad, 2012)** [13] who found that salivary UA was significantly higher in RAS patients than healthy control and no significant difference in serum UA was found.

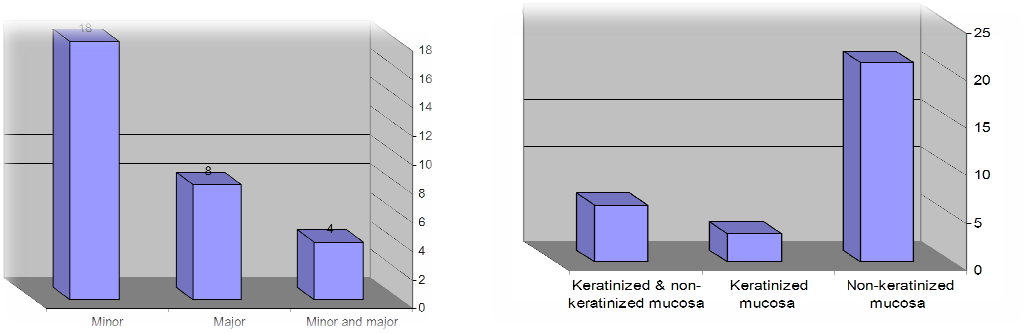
Saxena, 2011[15] found that salivary UA level was significantly higher in RAS patients.

Karincaoglu et al., 2005[6] contradict these studies by their results that showed that no significant changes in salivary UA level between RAS patients and their controls.

Moreover, in the study of **Yardim-Akaydin et al., 2006**[16] they found that the difference between serum UA of RAS patients and controls was not statistically significant. While **Sachin, 2009**[17] showed an elevation in the activity of serum UA of RAS patients that were a highly significant, but the level in the saliva was not statistically significant compared with healthy controls.

Cimen et al., 2003[8] concluded finally that enzymatic and non-enzymatic antioxidant defenses in RAS patients are defective.

In recent years, there are increasing reports on literature regarding application of natural antioxidant products on management of RAS. These herbal preparations including extracts and/or essential oils of medical plants exhibits promising effects on shortening healing time and severity of pain in RAS patients [18].



(a) **(b)**

Figure 1: Distribution of Patients According to the Clinical Presentation (Type and Site of Ulcers)

Table 1: The Mean of Antioxidant Biomarkers in Serum and Saliva According to the Study Groups

	RAS Patients N=30		Control Group N=30	
Variables	**Mean**	**±SD**	**Mean**	**±SD**
Serum CAT µmol/L	0.642	0.571	0.973	0.947
Saliva CAT µmol/L	0.807	0.662	0.827	0.613
Serum UA µmol/L	5.980	0.707	6.246	0.809
Saliva UA µmol/L	2.657	0.931	2.927	0.984

P value > 0.05 *t*- test

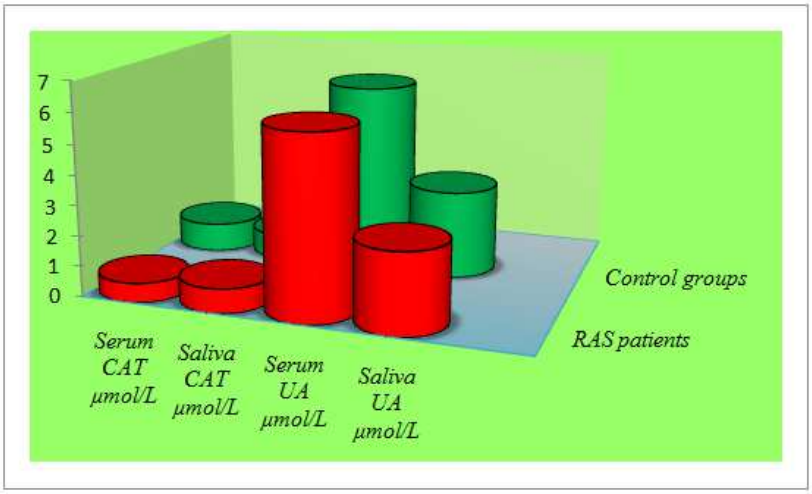


Figure 2: The Mean of Antioxidant Biomarkers in Serum and Saliva According to the Study Groups

Table 2: Pearson Correlation Coefficient(R) Between Serum and Salivary Antioxidant Biomarkers in RAS Patients

Parameters	Correlation Coefficient (R)	P- Values
Serum and saliva UA	**0.516**	**0.003**
Serum and saliva CAT	**0.187**	**0.322**

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