

Assessment of serum and salivary oxidant and total antioxidant status of patients with recurrent aphthous stomatitis in a sample of Basrah city

Hussein Sh. Al-Essa, B.D.S. ⁽¹⁾

Taghreed F. Zaidan, B.D.S., M.Sc., Ph.D. ⁽²⁾

ABSTRACT

Background: Recurrent Aphthous Stomatitis (RAS) is the most common painful oral mucosal disease, affecting approximately 20% of the population. RAS presents with a wide spectrum of severity ranging from a minor nuisance to complete debility. Many of factors thought to have been involved in its etiology; that might have at the same time a direct or indirect impact upon oxidant/antioxidant system and trigger free radicals production. The aim of this study was to determine the possible association of oxidant/total antioxidant status and recurrent aphthous stomatitis (RAS).

Subjects, materials and methods: The study consisted of thirty patients with recurrent aphthous stomatitis and thirty healthy controls from which saliva and blood samples were collected. Malondialdehyde as an oxidative stress biomarker and total antioxidant status were measured in serum and saliva.

Results: Malondialdehyde in serum and saliva was significantly higher in recurrent aphthous stomatitis patients in comparison to healthy controls ($P < 0.05$). No significant differences were found in total antioxidant status between recurrent aphthous stomatitis patients and control subjects ($P > 0.05$).

Conclusions: The changes in the oxidative stress in biological systems can be induced by the consumption of antioxidants and/or by an overload of oxidant species, so the antioxidant defense system become deficient that may be important in the inflammatory reactions observed in recurrent aphthous stomatitis.

Keywords: Recurrent aphthous stomatitis, oxidative stress, Antioxidants, malondialdehyde, total antioxidant status. (J Bagh Coll Dentistry 2013; 25(Special Issue 1):83-88).

الخلاصة

المقدمة: يعتبر التهاب الفم القلاعي المتكرر (RAS) من أكثر أمراض أنسجة الفم المؤلمة شيوعاً حيث يصيب أكثر من (20%) من سكان العالم. وتمثل الألتهايات الفموية القلاعية المتكررة مديبات واسعة من الشدة تتراوح من الانزعاج البسيط الى الوهن الكامل بين المصابين.

هنالك العديد من العوامل التي يعتقد أنها من مسببات التهاب الفم القلاعي المتكرر والتي يكون لها في نفس الوقت تأثير مباشر أو غير مباشر على الشد التأكسدي (Oxidative Stress) ونظام مضادات العوامل المؤكسدة الدفاعية (Antioxidants) وبالتالي إنتاج الجذور الحرة (Free Radicals) الضارة للخلايا الحية.

الهدف من الدراسة: الدراسة صممت لتحديد العلاقة الممكنة بين الشد التأكسدي (Oxidative Stress) ومضادات الأكسدة (Antioxidants) من جهة وأصابات التهاب الفم القلاعي المتكرر من جهة أخرى.

الأشخاص والمواد وطريقة العمل: الدراسة شملت ثلاثون مريضاً بالتهاب الفم القلاعي المتكرر وثلاثون شخصاً طبيعياً للمقارنة بينهما. وكانت المجموعتان متماثلتين بالجنس والعمر الذي يتراوح بين (14-55) سنة. أخذت العينات من مصل ولعاب المجموعتين وخضعت للطرد المركزي وخنزت عند (-20°C) وبعدها تم حساب (MDA Malondialdehyde) و (TAS Total Antioxidant Status) لكلا المجموعتين.

النتائج: أظهرت النتائج أن في الألتهايات الفموية القلاعية المتكررة ارتفاع ملحوظ في (MDA) كمؤشر للشد التأكسدي مقارنة بالأصحاء ($P < 0.05$). لا توجد اختلافات هامة في مؤشر (TAS).

الاستنتاجات: أن التغيرات في الشد التأكسدي (Oxidative Stress) في الأنظمة الحيوية ناجم عن استهلاك مضادات الأكسدة الدفاعية (Antioxidants) أو حمل زائد من العوامل المؤكسدة وبالتالي عدم كفاءة النظام الدفاعي لمضادات العوامل المؤكسدة ومن هنا نستنتج الدور الفاعل للشد التأكسدي كمسبب لمرض التهاب الفم القلاعي المتكرر. كذلك يمكن استخدام لعاب كسائل حيوي تشخيصي مؤثر وملئم لقياس مؤشرات الشد التأكسدي ومضادات الأكسدة للمرضى المصابين بالالتهايات الفموية القلاعية المتكررة.

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a common condition in which recurring ovoid or round ulcers affect the oral mucosa. It is one of the most painful oral mucosal inflammatory ulcerative conditions and can cause pain on eating, swallowing and speaking ⁽¹⁾.

Aphthous ulcers are classified into three different types, minor, major and herpetic, figure-1. 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 ⁽²⁾.

(1) M.Sc. Student, Department of Oral Diagnosis, College of Dentistry, Baghdad University.

(2) Professor, Department of Oral Diagnosis, College of Dentistry, Baghdad University.

Major aphthae is typically larger and deeper in ulceration and heals slowly over weeks, or even months. It has also been shown that major aphthae are more likely to scar with healing. Herpetic aphthae are frequently more numerous and vesicular in morphology and usually heals within about 1 month ⁽²⁾.

Different subgroups of patients appear to have different causes for occurrence of aphthae. These factors suggest a disease process that is triggered by a variety of causative agents (such as trauma, stress, genetic, hypersensitivity, nutrition, immune disturbance and hormonal imbalance) ⁽³⁾. When all the subgroups are combined, the various causation clusters into three categories: primary immune dysregulation, decrease of the mucosal barrier and increase in an antigenic exposure ^(3,4).

All of the above mentioned conditions can disturb the oxidant/antioxidant balance of organism and can accelerate the formation of free radicals. Oxidative stress occurs when the intracellular concentrations of reactive oxygen species (ROS) increase over the physiological values⁽⁵⁾.

The cytotoxic effects of free radicals are detrimental for mammalian cells and lead to cell damage through its damaging effects on peroxidation of double-chain fatty acids, protein and DNA, as well as, increase oxidative stress⁽⁶⁾.

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)⁽⁷⁾.

The aim of the present study was to determine the possible association of oxidant/total antioxidant status and recurrent aphthous stomatitis (RAS) through measuring Malondialdehyde (MDA) as a biomarker for oxidative stress, and evaluate the level of serum and salivary total antioxidant status (TAS) of (RAS) patients in a sample in Basrah city.



Minor aphthae



Major aphthae



Herpetiform ulceration

Figure1: Clinical presentation of RAS.

MATERIALS AND METHODS

Patients: this study was conducted in Department of Oral and Maxillofacial surgery at AL-Basrah Teaching Hospital and Specialized Teaching Center for Dentistry in Basrah. A total of (68) subjects were in cooperated in this study. They were divided as following:-

Recurrent Aphthous Stomatitis (RAS) patients group: Thirty eight patients of both sexes with RAS were examined. Those patients had recurrent oral aphthae at least three times a year⁽⁸⁾; eight patients were excluded from this study.

Healthy Control group: Thirty gender and age-matched healthy individuals were selected from the same hospital and privates (medical staff and dental students). RAS was diagnosed clinically by an expert in oral medicine. Patients were otherwise healthy and had active aphthous lesions during the study. They were not under a therapeutic regimen for the past 3months. Patients with Behcet's disease, chronic diarrhea, trauma history, any systemic disease, smoking history, alcohol drinking, or addiction were not included in the study.

Blood samples: five ml of venous blood samples were aspirated from anticubital vein of each individual in the morning. 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, figure-2.

Saliva samples: Five ml of unstimulated salivary samples were taken with the consent of the subjects. Started to gain unstimulated saliva samples and no oral stimulus was permitted prior to collection. Then the patients were told to sitcomfortably and to spit into the plastic polyethylene tubes for five minutes. The samples then were centrifuged at 3000 rpm for 10 minutes and the supernatant was aspirated then stored at (-20°C) until biochemical analysis, figure-2.

MDA Measurement: lipid peroxidation end products, particularly malondialdehyde (MDA) react with thiobarburic acid (TBA)under acidic condition and heating to give a pink chromogen

that measured spectrophotometrically at (532) nm⁽⁹⁾.

TAS Measurement: incubation of ABTS®* with a peroxidase (*metmyoglobin*) results in production of the radical cation ABTS+®*. This species is blue-green in color and can be detected

at 600 nm⁽¹⁰⁾. Antioxidants in the added sample cause inhibition of this color production to a degree that is proportional to their concentration. This is a two-reagent assay and may be performed using either serum or saliva. TAS was estimated by the use of commercially available kits RANDOX (Randox Laboratory Ltd., UK).



Serum samples

Saliva samples

Figure 2: Serum and saliva samples.

RESULTS

The patient group comprised 16 females and 14 males, whose mean age was 34.03± 11.98 years, while the control group consisted of 16 females and 14 males, whose mean age was 29.50±10.72 years. The percentage of female patients with RAS (53.3%) was higher than the percentage of male patients (46.3%) with RAS.

Clinical examination findings

Types of Ulcer(s): Out of 30 patients; the results showed that (18) patients (60%) had minor aphthous ulceration, whereas (8) patients (26.66%) had major aphthous ulceration and (4) patients (13.33%) had both minor and major ulcers. Herpetiform type of ulcers was not found in those patients. Chi square= 7.727, p- value = 0.021, the distribution is significant (P<0.05).

The Number of Ulcer(s): On examination of thirty patients with (RAS); (11) patients (36.66%) had single ulcer, whereas (19) patients (63.33%) were with multiple ulcers (more than one ulcer at the time of examination). Chi square = 0.010, P-value = 0.919, the distribution is not significant (P>0.05).

Sites of Ulcer(s): It has been found that twenty one patients (70%) had oral ulcers located on non-keratinized mucosa: [upper and lower labial mucosa, labial part of commissures, labial sulci, Buccal mucosa (right and left), (tip of the tongue, ventral side and margins), soft palate]. While three patients (10%) had oral ulcers on keratinized mucosa [dorsal of the tongue, attached gingiva (labial & lingual)]. Whereas six patients (20%) had oral ulcers on both keratinized and non-keratinized mucosa. Chi square = 10.903, p-value = 0.004, the distribution is highly significant (P<0.01).

Biochemical findings: - table-1 & figure-3

Assessment of oxidative stress marker

Malondialdehyde (MDA):- The results of this study showed that the mean of serum and salivary MDA in RAS patients was significantly higher (P<0.01) than that of healthy controls, using t-test.

Assessment of total antioxidant status (TAS):-

The mean of serum and salivary TAS in RAS patients was also slightly lower than that of healthy controls, but both of them statistically was not significant (P>0.05), using t-test.

Table1: The mean of oxidant and total antioxidant status in serum and saliva according to the study groups

Variables	RAS patients N=30		Control groups N=30		Comparison	
	Mean	±SD	Mean	±SD	t-test	P- value
Serum MDA µmol/L	5.713	0.523	5.236	0.567	3.384	0.001
Saliva MDA µmol/L	4.426	0.762	3.886	0.639	2.971	0.001
Serum TASµmol/L	1.610	0.276	1.752	0.410	-1.571	0.122
Saliva TAS µmol/L	2.524	0.396	2.727	0.617	-1.517	0.136

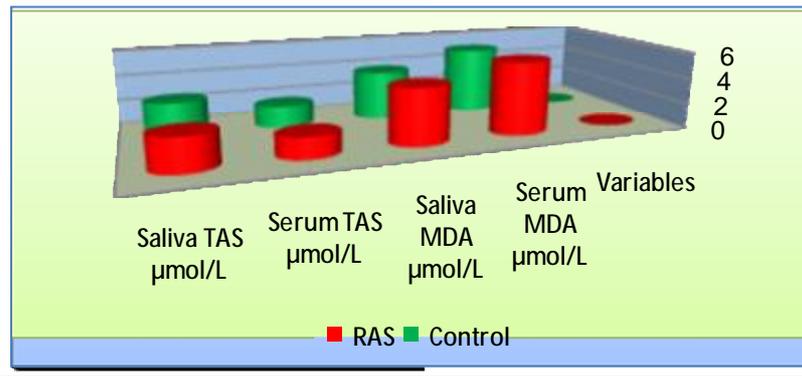


Figure 3: The mean of oxidant and total antioxidant status in serum and saliva according to the study groups

Biochemical findings according to gender

The results showed that the mean of serum and salivary MDA and TAS in female higher than

males with RAS, but statistically was not significant ($P > 0.05$) using t-test, table-2; figure-4.

Table 2: The mean of oxidant and total antioxidant status in serum and saliva of patients with RAS according to gender

Variables	Male patients N=14		Female patients N=16		Comparison	
	Mean	±SD	Mean	±SD	t-test	P- value
Serum MDA $\mu\text{mol/L}$	5.635	0.0.699	5.781	0.308	-0.720	0.481
Saliva MDA $\mu\text{mol/L}$	4.264	0.703	4.568	0.806	-1.094	0.283
Serum TAS $\mu\text{mol/L}$	1.571	0.307	1.643	0.250	-0.710	0.468
Saliva TAS $\mu\text{mol/L}$	2.555	0.474	2.496	0.325	0.400	0.692

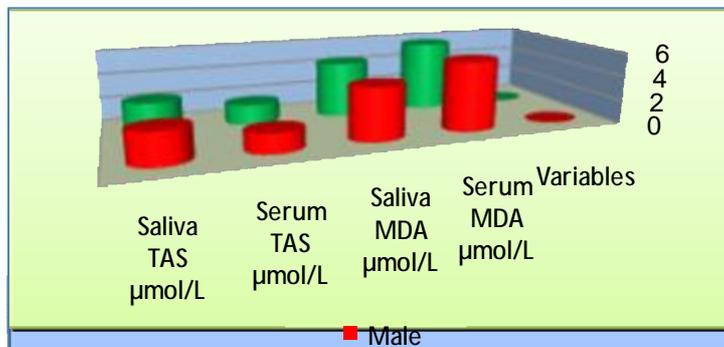


Figure 4: The mean of oxidant and total antioxidant status in serum and saliva of patients with RAS according to gender.

DISCUSSION

Considerable activity of reactive oxygen radicals may lead to destroyed normal cell functions and integrity of cell structures. 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. It appears that imbalance between free radicals and antioxidants causes many inflammatory oral soft tissue disease

varying from infections and immunological diseases to lethal cancers (6,11).

Clinical examination findings:

Gender: In the present study the results showed that females with RAS formed (53.3%), while the males was (46.6%), this was reported in most other studies which suggested a female predilection (12,13), but other studies showed a males predominance (14,15).

Age: The study showed that the mean age of the patients with RAS was (34.03±11.93) years, which means that RAS was found to be around the 3rd decade. While, other studies have noted that the average age group to be around 4th decade (16, 17).

Type of ulcer(s): The results showed that patients with minor aphthous ulceration were significantly higher. This result agreed with other studies (2,4), but disagreed with (15).

Number of ulcer(s): This study showed that (36.66%) of aphthae cases were with single ulcer on examination; whereas (63.33%) were with multiple ulcers; this is in a positive agreement with clinical study that had been done by Hashem (15). In other epidemiological study on RAS by Safadi (18) demonstrated that approximately half of participants reported that ulcerations were single while the other half reported them as multiple ulcerations.

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, which means that the percentage of patients with RAS on non-keratinized mucosa was significantly higher. This could be explained on the basis that the non-keratinized mucosa areas were movable structure 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 (6, 15).

Biochemical findings

Malondialdehyde (MDA):- Oxidation of lipids initiates a process that result in impairment of structural/ functional properties of the cell membrane, lysis of the cell and tissue damage occurs as a final result. In the current study, the serum and salivary MDA which is used as a marker of lipid peroxidation was found to be significantly higher in patients with RAS if compared with the control groups. These were agreed with results of Hashem (15). Likewise, elevation of MDA was reported in previous studies in saliva and serum (19) and in erythrocytes (20) and in serum (21) of RAS patients.

Total antioxidant status (TAS):- In the present study, it has been shown that there was a slightly decreased in serum and salivary TAS in RAS patients than healthy controls and the results

showed that the mean of serum and salivary TAS in males were lower than females, but not reach the significant level. These were agreed with the study of Aylin Sepici-Dinçel et al. (22); who demonstrated that serum TAS was statistically decreased in RAS patients, and oxidative stress levels increased in Behcet's disease and RAS patients than those in healthy controls.

Cimen et al. (23) concluded finally that enzymatic and non-enzymatic antioxidant defenses in RAS patients are defective. It can be concluded that the RAS samples were under reactive oxygen species stress due to significant increase production of serum and salivary (MDA) as an oxidative stress biomarker. 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 (24).

REFERENCES

1. Jurge S, Kuffer R, Scully C, Porter SR. Mucosal disease series. Number VI. Recurrent aphthous stomatitis. *Oral Dis* 2006; 12 (1): 1–21.
2. Greenberg MS, Glick M, Ship JA. *Burkett's oral medicine*. 11th ed. Hamilton: BC Decker Inc; 2008. p. 57-60.
3. Cawson RA, Odell EW. *Cawson's essential of oral pathology and oral medicine*. 8th ed. London: Elsevier Science Limited; 2008. p. 220-24.
4. Neville BW, Damm DD, Allen CM, Bouguot JE. *Oral and maxillofacial pathology*. 3rd ed. Philadelphia: W.B. Saunders Company; 2008. p. 333-336.
5. Momen-Beitoallahi J, Mansourian A, Momen-Heravi F, Amanlou M, Obradov S, Sahebjamie M. Assessment of salivary and serum antioxidant status in patients with recurrent aphthous stomatitis. *Med Oral Patol Oral Cir Bucal* 2010; 15(4): e557-61.
6. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis: a consensus approach. *J Am Dent Assoc* 2003; 134:200-7.
7. Karıncaoglu Y, Batcioglu K, Erdem T, Esrefoglu M, Genc M. The levels of plasma and salivary antioxidants in the patient with recurrent aphthous stomatitis. *J Oral Pathol Med* 2005; 34:7-12.
8. Albanidou-Farmaki E, Deligiannidis A, Markopoulos AK, Katsares V, Farmakis K, Parapanissiou E. HLA haplotypes in recurrent aphthous stomatitis" a mode of inheritance. *Int J Immunogenet* 2008; 35: 427-32.
9. Shah SV, Walker PD. Evidence suggesting a role for hydroxyl radical in glycerol induced acute renal failure. *Am J Physiol* 1988; 255(3 pt 2): F438-443.
10. Erel O. A new automated colorimetric method for measuring total antioxidant status. *Clin Biochem* 2005; 38: 1103-11.
11. Beevi SS, Rasheed AM, Geetha A. Evaluation of oxidative stress and nitric oxide levels in patients with oral cavity cancer. *Jpn J Clin Oncol* 2004; 34: 379-85.

12. Porter SR, Scully C, Pedersen A. Recurrent Aphthous Stomatitis. *Crit Rev Oral Biol Med* 1998; 9(3): 306-321.
13. Eris S, Ghaemi EO, Moradi A, Mansourian AR, Rabiei MR, Nosrat SB, Amirchaghmaghi A, Ahmadi AR. Aphthous ulcer and the effective factors on its incidence among the students of Golestan Medical Sciences. University in the North of Iran. *J Biol Sci* 2007; 7(5): 830-832.
14. Fernades R, Tuckey T, Lamp, Allidina S, Shaarifi S, Nia D. The best treatment for recurrent aphthous Ulcer. An evidence based study of the literature 2000: 30-34.
15. Hashem MN. Assessment of salivary and serum lipid peroxidation/ antioxidant status and C-reactive protein marker in patients with recurrent aphthous stomatitis (RAS) in selected sample in Baghdad city. A master thesis. Department of Oral Diagnosis, College of dentistry, Baghdad University 2012. p. 78-86.
16. Ship JA. Recurrent aphthous stomatitis. An Update. *Oral Surg. Oral Med Oral Path Oral Radiol Endod* 1996; 81: 141-7.
17. Oh SH, Han EC, Lee JH, Bang D. Comparisons of the clinical features of recurrent aphthous stomatitis and Behcet's disease. *British J Assoc Dermatol. Clinical and Experimental Dermatol* 2009; 34: e208-e212.
18. Safadi RA. Prevalence of recurrent aphthous ulceration in Jordanian dental patients. *BMC Oral Health* 2009; 9: 31.
19. Saral Y, Basak K, Perihan O, Fikret K, Ahmet A. Assessment of salivary and serum antioxidant vitamins and lipid peroxidation in patients with Recurrent Aphthous Stomatitis. *Tohoku J Exp Med* 2005; 206: 305- 312.
20. Altinyazar HC, Ahmet G, Rafet K, Ferah A, Murat U. The status of oxidants and antioxidants in the neutrophils of patients with Recurrent Aphthous Stomatitis. *Turk J Med Sci* 2006; 36: 87-91.
21. Gurel A, Altinyazar HC, Unalacak M, Armutcu F, Koca R. Purine catabolic enzymes and nitric oxide in patients with Recurrent Aphthous Ulceration. *Oral Dis* 2007; 13: 570-74.
22. Aylin Sepici-Dinçel, Yeşim Özkan, Sevgi Yardim-Akaydin, Gülçin Kaymak-Karataş, Meltem Önder, Bolkan Şimşek. The association between total antioxidant status and oxidative stress in Behcet's disease. *Scand J Rheumatol* 2006; 26(11):1005-1009.
23. Cimen MY, Kaya TI, Eskandari G, Tursen U, Ikizoglu G, Atik U. Oxidant/antioxidant status in patients with recurrent aphthous stomatitis. *Clin Exp Dermatol* 2003; 28:647-50.
24. Babae N, Mansourian A, Momen-Heravi F, Moghadamnia A, Momen-Beitollahi J. The efficacy of a paste containing *Myrtus communis* (Myrtle) in the management of recurrent aphthous stomatitis. A randomized control trial. *Clin Oral Investig* 2010; 14: 65-70.