ORIGINAL ARTICLE

Total Serum Oxidant/Antioxidant Status and Arylesterase Activity in Recurrent Aphthous Stomatitis

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Background: Recurrent aphthous stomatitis (RAS) is a chronic relapsing inflammatory disorder of the oral mucosa with unknown etiology. Oxidative stress (OS) is suggested to play a main role in the etiopathogenesis in RAS. **Objective:** In this study, we hypothesize that a systemic OS is present in patients with RAS. **Methods:** Forty-four patients with active RAS lesions and 38 healthy controls were being included in the study. Serum total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and paraoxonase 1 arylesterase (ARES) activity were being determined. **Results:** RAS patients had significantly lower TAS levels and higher TOS and OSI values than controls. The patients had a lower ARES activity when compared to healthy controls. No correlations were observed between OS parameters and age, gender, duration of disease or frequency of RAS attacks per month. **Conclusion:** A systemic OS is determined with an imbalance in oxidant/antioxidant status and lower ARES activity in RAS. Systemic OS may have an important role in the pathogenesis of RAS formation. **(Ann Dermatol 25(3) 273～277, 2013)**

-Keywords-
Arylesterase activity, Oxidative stress, Recurrent aphthous stomatitis, Total antioxidant status, Total oxidant status

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INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a chronic relapsing inflammatory disorder of the oral mucosa with unknown etiology, characterized by painful ulcerations of various sizes. RAS was reported to affect about 25% of the people in different populations[1-4]. Trauma, viral or bacterial infections, stress, nutritional deficiencies, systemic diseases, immunological status, or genetic predisposition may elicit the formation of RAS lesions[1-6]. Since these possible factors may imbalance the oxidant/antioxidant status of the organism[6-9], a relationship between systemic oxidative stress (OS) and RAS has been questioned.

OS is the reflection of an imbalance between production of reactive oxygen species (ROS) and the antioxidant mechanisms of the organism that detoxify these radicals. Under physiological circumstances, the production of ROS is not harmful to the organs and is limited by antioxidants. However, if the concentration of ROS exceeds over pathological values, the antioxidants cannot prevent excess cytotoxic effects and oxidative damages to the organisms. There are various antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT) and as well as non-enzyme antioxidant molecules including glutathione, uric acid, melatonin, and vitamins A, C, E in the organism. In OS, antioxidants are spent for the defense that detoxicifies the oxidant molecules resulting in depletions of antioxidant status[10-12]. Since the new condition is not balanced, OS affects the entire organism.

In this study, we hypothesized that there is a systemic OS in patients with RAS. Until today, many studies have investigated the amounts of oxidative reactive species, non-enzymatic molecules, and antioxidant enzymes as mentioned above in RAS[6,8,9,13-17]. However, measurements of these molecules individually do not reflect the global sta-

tus of OS. Serum (or plasma) concentrations of different oxidant species can be measured in laboratories separately, but such measurements are time-consuming, labour-intensive, costly and require complicated techniques. Since measuring different oxidant molecules separately is impractical and their oxidant effects are additive, the total oxidant status (TOS) sample is extracted instead. In this study, the method determines total oxidative status in relation to cumulative oxidative effects of various oxidants which are generated in biological systems such as hydrogen peroxide, lipid hydroperoxide, protein hydroperoxide, peroxynitrite and other reactive oxygen and nitrogen species[18,19]. Nevertheless, another recently developed method measuring the total antioxidant status (TAS) provides the evaluation efficiency of all antioxidants[20]. These measurements show the global effects of the molecules involved in OS conditions rather than individual effects for each oxidant and antioxidant molecular mechanism. To date, there have been no reports investigating the systemic OS from these methods which measures the levels of serum TOS and TAS in RAS.

The paraoxonase 1 (PON1) enzyme is an antioxidant and anti-inflammatory enzyme. The PON1 enzyme has both paraoxonase and arylesterase (ARES) activities. The PON1 ARES activity of the organism protects low-density lipoprotein (LDL) and lipoproteins from oxidation, which are important biomolecules produced during oxidative damages in atherosclerosis[21,22]. The PON1 enzyme activities have been demonstrated to reduce in metabolic syndrome, atherosclerosis, and diabetes mellitus, in which the OS plays a major role[23,24]. However, alterations in ARES activities of the patients with RAS have not yet been investigated.

In this study, we determined the status of OS in RAS patients by measuring the levels of TOS, TAS, and oxidative stress index (OSI), and ARES activity.

MATERIALS AND METHODS

Subjects

This study included 44 patients with RAS presented in dermatology out-patient clinic and 38 age and sex matched healthy control subjects. Patients had at least 3 minor aphthous lesions. They had RAS attacks recurring at least 3 times a year. These patients were not on any treatment for RAS, non-RAS related conditions, or any systemic diseases for the last 3 months. The healthy controls did not have any RAS attacks before the study and during participation. They did not have any systemic or dermatological diseases. The participants were not pregnant, nursing, smokers or alcohol users. They had not taken any systemic treatment and vitamins. None of them had Behçet disease, inflammatory bowel disease, chronic diarrhea, or any systemic diseases. The study was approved by the hospital's ethics committee. All participants signed written informed consent.

Blood sampling

Venous blood samples were drawn into 5 ml vacutainer tubes after at least 8-hour of overnight fasting state in the early morning. Then, the samples were centrifuged at 3,600 rpm for 6 minutes and sera were stored at $-80^{o}C$ until all samples were assayed at the same time.

Measurement of serum total oxidant status

The TOS of the serum was measured using an automated colorimetric measurement method for TOS[19]. In this method, oxidants presented in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules, which are abundantly presented in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules presented in the sample. The assay was calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/L).

Measurement of serum total antioxidant status

The TAS of the serum was measured using a novel automated colorimetric measurement method for TAS[20]. This method is based on the bleaching of color characteristics of a more stable ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L.

Determination of oxidative stress index

The ratio of TOS to TAS represents the OSI, an indicator of the degree of OS. The OSI value is calculated according to the formula: OSI (arbitrary unit) = TOS (mmol H_2O_2 Eq/L)/ TAS (mmol Trolox Eq/L) × 100[25].

Measurement of serum PON1 arylesterase activities

Serum PON1 ARES activity was measured by using commercially available kits (Rel Assay Diagnostics, Mega Tıp, Gaziantep, Turkey). Serum ARES activity was determined by the presence of phenol following the reactions of phenylacetate. The molar extinction coefficient of phenol was 4,000 $M^{-1}cm^{-1}$; the results were expressed as kU/L.

Statistical analysis

Evaluation of normality was performed with the Shapiro-Wilk test. Normally distributed continuous variables were expressed as mean and standard deviation (mean±standard deviation), and were compared with Student's t-test for independent groups. Non-normal distributed continuous variables were expressed as median and interquartile ranges (IQR) of 25 and 75 (median [IQR 25～IQR 75]), and were compared with Mann-Whitney U test for independent groups. Categorical variables are reported as numbers and percentages and compared with Fischer's exact test. The association between continuous variables was explored by Pearson (normally distributed) or Spearman (non-normally scattered) correlation analysis. A $p<0.05$ was accepted to be statistically significant. All statistical analyses were performed using SPSS version 15.0 statistical software package (SPSS Inc., Chicago, IL, USA).

RESULTS

The study enrolled a total of 44 patients (21 females, 23 males; mean age: 30.5 years; range: 19～55 years) with RAS lesions (Table 1). The patients and healthy controls matched according to age and gender (*p*-values are 0.825 and 0.389, respectively).

The median duration of RAS attacks of patients was 3.5 years (range: 1.0～30.0 years). The range of frequency of RAS attacks per month was 1 to 6. Twenty-three patients (52.3%) had two attacks per month. The first degree relatives of 12 patients (27.3%) also had medical histories of RAS.

RAS patients had significantly lower TAS levels than control subjects (2.95±0.41 vs. 3.49±0.49; $p<0.001$). The TOS levels were higher in patients as compared to controls (15.32 [10.83～23.71] vs. 10.58 [7.46～15.08]; $p=0.008$). The OSI, as calculated by the formula, was significantly higher in RAS patients than the control subjects (0.53 [0.40～0.79] vs. 0.30 [0.23～0.47]; $p\leq 0.001$) (Table 2).

The TG amounts were similar between groups ($p=0.115$); however, HDL levels were lower in RAS patients than control subjects ($p<0.001$). The ARES activity was lower in patients with RAS than healthy controls (176.43±21.61 vs. 210.47±27.64; $p<0.001$) (Table 3).

In the patient group, no correlations were observed between OS parameters (TAS, TOS, OSI, ARES activities) and age, gender, duration of disease or frequency of RAS attacks (all $p>0.005$).

DISCUSSION

In this study, we determined that there was a systemic OS reflected by an imbalance between the TOS and antioxidant status and lower ARES activities in patients with RAS. The results of serum TOS, TAS, and OSI values demonstrated the global effect of the molecules involving in OS rather than individual effects of each oxidant and antioxidant molecules. To date, many oxidant and/or antioxidant products in patients with RAS have been investigated in recent studies[6,8,9,13-17]. Several laboratory kits and different methods were used to determine the oxidant reactive species, antioxidant enzymes, and non-enzymatic molecules. In general, studies defined alterations of

Table 1. Demographic characteristics of patients with RAS and healthy controls*

Charicteristic	RAS patient (n=44)	Control subject (n=38)	*p*-value
Age (year)	30.5 (25.25～37.75)	33.0 (26.0～45.0)	0.389
Males	23 (52.3)	18 (47.4)	0.825

RAS: recurrent aphthous stomatitis. *Continuous variables are reported as median (interquartile ranges 25～75) for asymmetrically distributed data and compared with Mann-Whitney U test. Categorical variables are reported as number (%) and compared with Fischer's exact test.

Table 2. Total antioxidant status, total oxidant status, and the oxidative stress index of RAS patients and control subjects*

Variable	RAS patient (n=44)	Control subject (n=38)	*p*-value
TAS (mmol Trolox Eq/L)	2.95±0.41	3.49±0.49	<0.001
TOS (μmol H_2O_2 Eq/L)	15.32 (10.83～23.71)	10.58 (7.46～15.08)	0.008
OSI (arbitrary unit)	0.53 (0.40～0.79)	0.30 (0.23～0.47)	<0.001

RAS: recurrent aphthous stomatitis, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index. *Normally distributed data are reported as mean±standard deviation and compared with t-test; asymmetrically distributed data are reported as median (interquartile ranges 25～75) and compared with Mann-Whitney U test.

Table 3. Serum total cholesterol, TG, HDL-C levels, and ARES activities in RAS patients and healthy controls*

Variable	RAS patient (n=44)	Control subject (n=38)	*p*-value
Total cholesterol (mg/dl)	179.38±34.96	179.16±46.46	0.982
TG (mg/dl)	116.00 (90.50～160.75)	94.00 (77.75～142.50)	0.115
HDL-C (mg/dl)	36.50 (33.85～43.00)	46.2 (37.80～53.25)	<0.001
ARES activity (kU/L)	176.43±21.61	210.47±27.64	<0.001

TG: triglyceride, HDL-C: high density lipoprotein-cholesterol, ARES: arylesterase, RAS: recurrent aphthous stomatitis. *Normally distributed data are reported as mean±standard deviation and compared with t-test; asymmetrically distributed data are reported as median (interquartile ranges 25～75) and compared with Mann-Whitney U test.

oxidant and antioxidant status in RAS although there are some conflicting results. Cimen et al.[13] demonstrated decreased CAT, GSHPx, and antioxidant potential; and increased malondialdehyde levels in RAS patients. In contrast, Gunduz et al.[14] did not find a significant difference between RAS patients and controls with regards to SOD, CAT, nitrite, and nitrate levels. In the study of Karincaoglu et al.[15], plasma SOD and CAT were lower and GSHPx was higher in RAS patients than healthy controls; however their salivary levels were opposite to the plasma concentrations. The salivary antioxidant defense seemed to be stimulated against ulcerated lesions. Momen-Beitollahi et al.[9] detected similar concentrations of erythrocyte GSHPx and CAT concentrations, salivary and plasma TAS in patients and controls. Only the erythrocyte SOD activity was lower in patients. They concluded that antioxidant system in saliva and plasma might not reflect the TAS of the whole body.

Serum TAS and TOS levels and OSI were evaluated in RAS patients in only one recent study by different methods used in the present study[16]. The investigators demonstrated that RAS patients had higher TOS and lower antioxidant levels than healthy controls. The results of our study were compatible with these findings. However, the values of these parameters were higher in our study. These differences may be related to the different methods used when measuring the TAS and TOS levels. Since the Erel method[19,20] was developed more recently and has been shown to be more specific and sensitive to detect the TAS and TOS levels; higher levels of oxidant and antioxidant molecules might have been measured in the present study. The OSI detected in our study was also higher in RAS patients than in healthy controls, which indicated the exact degrees of imbalance of OS towards the oxidant status. The OSI shows the ratio of TOS and TAS, which is more objective to evaluate the conditions of OS rather than evaluating the TAS and TOS separately[25]. All results suggested that the release of excessive ROS represents an increase in serum TOS levels in patients with RAS, which affects the entire organism in general. In a recent study, TAS, TOS, and OSI were measured by Erel methods in the saliva of RAS patients and no significant differences were found between patients and healthy controls[8]. The measurements of same markers in the serum of RAS patients in our study suggest that global serum oxidant and antioxidant molecules and the imbalance between them may be more important than local oxidant and antioxidant markers detected in saliva of RAS patients. Since the patients had minor aphthous lesions, it is interesting that the organism showed an obvious imbalance of OS. Therefore, the micro-environmental tissue damages in small ulcerations may be related to factors causing OS and thus, resulting in RAS formation. Although the peripheral neutrophils of the immune system cells were shown to produce reactive oxygen intermediates[6], the origin of OS in RAS still needs further investigations. The total serum oxidants and antioxidants were measured simultaneously, thus, the cause and effect relationship is not certain in the present study. To demonstrate such a relationship between these OS markers, a longitudinal assessment of post-treatment status may be considered after an optimal therapeutic attempt, such as supplementations with antioxidants or treatment with various agents, in order to reduce the level of OS levels. In addition, studies which concurrently compare the circulating levels of OS markers (TAS, TOS, OSI, ARES activity) with tissue levels may help to analyze the details of OS in the pathogenesis of RAS.

In our study, the ARES activity of PON1 enzyme of the RAS patients was found to be lower than healthy individuals. The ARES activity represents one of the antioxidant enzymatic activities of PON1 enzyme, which has an important role in modulating OS and in protection from cardiovascular diseases. This enzyme is located on high density lipoprotein cholesterols (HDL-C) and protects LDL cholesterol from OS by hydrolyzing lipid peroxides[26,27]. The lower TAS, ARES, HDL-C levels and higher TOS and OSI values provide support that a systemic OS is present in RAS patients. All observations suggest that a systemic OS is responsible for RAS formation as well as

affected the entire organism.

In conclusion, systemic OS may have an important role in the etiopathogenesis of RAS. Future studies investigating the origin of OS and the systemic OS effects of the whole organism in RAS patients are needed.

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