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EVALUATION OF TOTAL OXIDANT STATUS, ANTIOXIDANT STATUS AND OXIDATIVE STRESS INDEX IN PATIENTS WITH RECURRENT APHTHOUS STOMATITIS

REKÜRREN AFTÖZ STOMATİTLİ HASTALARDA TOTAL OKSİDAN DURUM, TOTAL ANTİOKSİDAN DURUM VE OKSİDATIF STRES İNDEKSİNİN DEĞERLENDİRİLMESİ

Hüseyin Kurku[1], Cahit Yavuz[2]

Konya City Hospital, [1]Department of Biochemistry, [2]Department of Dermatology, Konya, Turkey

ORCID iD: Hüseyin Kurku: 0000-0002-1083-4151; Cahit Yavuz: 0000-0003-4675-8127

***Sorumlu Yazar / Corresponding Author:** Cahit Yavuz **e-posta / e-mail:** yavuzcahit@yahoo.com

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Abstract

Objective: We try to evaluate total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) in patients with recurrent aphtous stomatitis (RAS) in our study.
Methods: A total of 40 patients, 23 female and 17 male, with a mean age of 29, who applied to the dermatology outpatient clinic with the complaint of oral ulcers and were diagnosed with RAS and a total of 49 healthy controls, 33 female and 16 male, with a mean age of 24, were included in the study.
Results: The TOS and OSI values of the RAS group were statistically significantly higher than the values of the control group. When the TAS values of both groups were compared; the TAS value of the RAS group was statistically indifferent from the TAS value of the control group.
Conclusion: In patients with RAS, serum TOS, serum TAS and OSI values are affected in favour of oxidative processes. In the pathogenesis of RAS, oxidant substances and antioxidant-active substances are important.

Keywords: *Recurrent aphtous stomatitis, total oxidant status, total antioxidant status, oxidative stress index.*

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Öz

Amaç: Çalışmamızda rekürren aftöz stomatitli (RAS) hastalarda total oksidan durum (TOS), total antioksidan durum (TAS) ve oksidatif stress indeksi (OSI) parametrelerini değerlendirmeye çalıştık.
Yöntem: Dermatoloji polikliniğine oral ülser şikayeti ile başvuran ve RAS tanısı alan 23 kadın ve 17 erkek olmak üzere yaş ortalaması 29 olan toplam 40 hasta ile 33 kadın ve 16 erkek olmak üzere yaş ortalaması 24 olan 49 sağlıklı gönüllü çalışmaya dahil edildi.
Bulgular: TOS ve OSI değerleri RAS grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksek bulunmuştur. İki grup arasındaki TAS değerleri karşılaştırıldığında ise RAS grubuna ait TAS değeri ile kontrol grubuna ait TAS değeri arasında fark bulunamamıştır.
Sonuç: RAS'li hastalarda TOS, TAS ve OSI değerleri oksidatif süreç lehine değişmektedirler. RAS patogenezinde oksidan maddeler ve antioksidan aktif maddeler önemlidir.

Anahtar Kelimeler: *Rekürren aftöz stomatit, total oksidan durum, total antioksidan durum, oksidatif stres indeksi.*

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common pathologies of the oral mucosa. They are recurrent, multiple, round or ovoid-shaped, painful ulcers with erythematous margins covered with a yellow-gray pseudomembrane with a necrotic center.[1,2] It can be seen in both genders, all age groups and races. Although its incidence varies according to populations, it is reported to be approximately 25%.[3] They are classified in 3 groups as minor, major and herpetiform according to clinical appearance. The minor form of RAS is the most common group, and about 80% of patients are in this group.[4] Although there are many studies showing that various factors such as local trauma, smoking, nutritional disorders, infections, genetic predisposition, hematological anomalies, drug or food allergies, and immunological status are responsible for the etiology, the etiopathogenesis of RAS is still unknown.[5,6] Factors accused in etiology also affect the balance between oxidant and antioxidant mechanisms, and when this balance is disrupted in favor of oxidant mechanisms, free radicals may form and cause some toxic reactions.[7] This phenomenon is called oxidative stress. Reactive oxygen species formed under physiological conditions are limited by antioxidants, and do not harm organs and tissues. In case of excessive formation of reactive oxygen species, they cannot be limited by antioxidants and can reach levels toxic to organs and metabolism. Antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase, as well as non-enzymatic antioxidants such as glutathione, uric acid, melatonin, A-C-E vitamins also play an active role in the fight against free oxygen metabolites.[8]

Determining either reactive oxygen species or antioxidant molecules as either singular or multiple molecule is insufficient to evaluate oxidative status or antioxidant status collectively. Monitoring these molecules individually is time consuming, costly and tiring. Therefore, a total oxidant status (TOS) assessment was made. Measurements were made based on the principle of cumulative determination of oxidant substances and antioxidant substances produced by the human body. Among the oxidants are hydrogen peroxide, lipid hydroperoxide, protein hydroperoxide, peroxynitrite and other oxygen metabolites.[9,10] TOS is determined by cumulative measurements of oxidant metabolites formed in oxidative stress conditions, and total antioxidant status (TAS) is determined by the cumulative measurements of antioxidant substances against them.[11] In our study, we aimed to investigate the oxidative stress level in patients with RAS through the oxidant/antioxidant balance. For this purpose, we planned to compare a group of patients with RAS with a healthy control group. We hypotize that the oxidant/antioxidant balance will increase in favor of oxidative stress in patients with RAS.

Methods

The study was planned in accordance with the principles of the Declaration of Helsinki and the approval of the ethics committee was obtained from KTU Karatay University, School of Medicine with approval number 2021-19097. A total of 40 patients (23 female and 17 male) with a mean age of 29, who applied to the dermatology outpatient clinic with the complaint of oral ulcers and were diagnosed with RAS, and a total of 49 healthy controls, 33 female and 16 male, with a mean age of 24, were included in the study. The diagnosis of RAS was made by anamnesis, clinical examination, and exclusion of systemic causes that could cause oral ulcers. Behcet's Disease was evaluated with anamnesis and examination, so those patients were excluded. Patients with oral ulcers that recur at least every 3 months were diagnosed with RAS according to the criteria defined by Scully et al.[12] All participants were informed verbally before the study and their consent was obtained. Those under the age of 18, those with additional systemic disease and oral mucosal ulcers caused by systemic causes were excluded from the study. After approximately 12 hours of fasting, 5 mL venous blood samples were taken from the patient and control groups between 08:00 and 12:00 a.m. into routine biochemistry tubes (with gel and vacuum). The venous blood samples taken were centrifuged at 3000g for 5 minutes, the sera were separated then stored at -80 °C until the study day. On the working day, the sera were thawed and all samples were run on the same day.

Study of TOS and TAS tests and calculation of oxidative stress index (OSI): TOS and TAS commercial kits (Rel Assay Diagnostics, Megatip Sanayi ve Ticaret Ltd. Sti. Gaziantep, TURKEY) were applied to the autoanalyzer Olympus AU 400 (Olympus life & Material Science Europe, Hamburg, Germany), and the samples were studied. Calculation of oxidative stress index (OSI); The percent OSI values were calculated by proportioning the TOS values of the samples to the TAS values (OSI (arbitrary unit, AU) = ((TOS, μmolH_2O_2 eq/L)/(TAS, μmolTroloxeq/L)*100).

Statistical Analysis

A statistical package program SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was used to evaluate the data. Before the analysis, the preconditions of normality of continuous variables and homogeneity of variances were checked (Shapiro Wilk and Levene Test). During data analysis, the differences between the two dependent groups were evaluated using the Independent Samples T Test (Student t-test) when the parametric test prerequisites were met, and using the Mann-Whitney U Test when they were not. "Chi-square Test" was used in the analysis of categorical data. The mean (±standard deviation), median [Interquartile Range(IQR): 25th percentile-75th percentile], percentage and frequency values of the variables were used. A value of $p<0.05$ was accepted for the significance level of the tests.

Result

A total of 40 patients, 23 female and 17 male, were included in the study. The mean age of the patients was 29.0 (23.5-38.0). The control group consisted of 49 individuals, 33 females and 16 males, with a mean age of 24.0 (21.0-34.5) (p=0.090, Mann-Whitney U Test). There was no statistical difference between the patient and control groups in terms of age and gender. Demographic data of the patient and control groups are presented in Table 1.

The TOS (6.876 [3.332-9.963]) and OSI (0.531 [0.265-0.888]) values of the RAS group were statistically significantly higher than the TOS (3.354 [1.523-6.483]) and OSI (0.261 [0.121-0.484]) values of the control group (respectively; p= 0.040, p=0.002, Mann-Whitney U Test). When the TAS values of both groups were compared; The TAS of the RAS group (1.26 [1.142-1.375]) was statistically indifferent from the TAS of the control group (1.255 [1.179-

1.435]) (p= 0.380, Mann-Whitney U Test). TAS, TOS and OSI values of the study groups are shown in Table 2.

Discussion

Our study was a single-center, prospective, controlled study. In our study, TOS, TAS and OSI in patients diagnosed with RAS were compared with a control group that was similar in terms of age and gender. In conclusion, there was a statistically significant difference between the two groups in terms of TOS and OSI, but no significant difference was found in terms of TAS.

Oxidative stress is one of the factors that is frequently emphasized in the pathogenesis of RAS. Oxidative stress; It occurs when the balance between oxidant processes and protective antioxidant mechanisms is disrupted in favor of oxidant processes.[13] Antioxidant mechanisms can be of endogenous or exogenous origin and mainly work to reduce cellular damage by preventing free radical formation.[14] There are many studies evaluating oxidant and antioxidant processes in patients with RAS. Cimen et al. studied superoxide dismutase, glutathione peroxidase, catalase and malondialdehyde levels in patients with RAS, and reported that malondialdehyde levels were high in the patient group.[15] Another study, which reported a decrease in superoxide dismutase and catalase levels and an increase in glutathione peroxidase levels in patients with RAS was published by Karincaoglu et al.[16] Similar results were obtained in different studies. In a study by Arikan et al, an increase in plasma malondialdehyde levels and a decrease in glutathione peroxidase, vitamin E and selenium levels were reported in RAS.[17]

Table 1. Demographic features of patients and control group

	Patient group (n:40)	**Control group** (n:49)	***p***
Sex			*0,339* ¥
Female, n (%)	23 (57,5)	33 (67,3)	
Male, n (%)	17 (42,5)	16 (32,7)	
Age#	29.0 (23,5-38,0)	29.0 (23,5-38,0)	*0,090**

RAS: Recurrent aphthous stomatitis, #:values are given as Median[IQR: 25th percentile-75th percentile], IQR: Interquartile Range, *:*p* value for Mann-Whitney U Test, ¥ :*p* value for Chi-square Test

Table 2. Total Oxidant Status (TOS), Total Antioxidant Status (TAS) and Oxidative Stress Index (OSI) in patients and control group

Variables	**Patient group** (n: 40)	**Control group** (n: 49)	***p***
TAS (mmol Trolox Equiv./L) #	1.26 [1.142-1.375]	1.255 [1.179-1.435]	*0.380**
TOS (μmol H_2O_2 Equiv./L) #	6.876 [3.332-9.963]	3.354 [1.523-6.483]	***0.004****
OSI (AU) #	0.531 [0.265-0.888]	0.261 [0.121-0.484]	***0.002****

#:values are given as Median [IQR: 25th percentile-75th percentile], IQR: Interquartile Range, *:*p* value for Mann-Whitney U Test, TAS:Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index , AU: Arbitrary unit

A similar study to our study was conducted by Caglayan et al. In that study, TAS, TOS, and OSI levels were evaluated in saliva samples of patients with RAS, and it was reported that there was no significant difference between the patient and control groups.[18] Unlike in this study, the relevant values in plasma were not examined. Studies show the relationship between oxidative stress and DNA damage. In a study conducted by Tugrul et al., it was reported that DNA damage was also seen more frequently in patients with RAS.[14] In the same study, a significant increase in TOS and OSI values was also found in patients with RAS, and the positive correlation between oxidative stress and DNA damage was emphasized.

Dincel et al. reported an increase in TOS and OSI levels and a decrease in TAS levels in patients with Behcet's disease that goes with genital ulcers and other findings at different levels accompanying RAS lesions.[19] In our findings, an increase was observed in TOS and OSI levels, but no significant difference was found in TAS levels. We think that the reason for this may be that the age range of 20 patients in the study by Dincel et al. varied between 22 and 62. The age range of 40 patients examined in our study ranged from 23 to 38 years. It can be emphasized that there may be a regression in antioxidant mechanisms with advanced age. In addition, in the light of this information, it would not be wrong to emphasize the possibility that antioxidant nutritional and support supplements will reduce the course and severity of the disease in patients with decreased TAS levels.

Another oxidative stress-increasing factor is neutrophil infiltration into the region of tissue damage induced by the microenvironment of aphthous lesions. It was reported by Lewkovicz et al. that neutrophils in the peripheral circulation form reactive oxygen species.[20] In our study, total serum oxidant and antioxidant status in RAS patients were evaluated collectively. It is obvious that evaluating oxidant and antioxidant enzymes and substances separately and observing the differences before and after treatment will help to understand the role of oxidative stress in the pathogenesis of RAS.

There are many studies examining serum TOS and TAS values, and it was reported that serum TOS values of patients with RAS increased and serum TAS values decreased compared to the control group, as a common finding in most of these studies.[8,19] In our study, we found that serum TOS values increased compared to the control group, but serum TAS values did not change statistically. In different studies, increased sensitivity of peripheral blood lymphocytes to hydrogen peroxide-like oxidative agents and an increase in reactive oxygen species produced by peripheral blood neutrophils are shown as possible reasons for the different serum TAS values.[16,20]

Oxidative stress values studied in minor RAS patients by Zhang et al. They reported that serum TAS values were decreased in minor RAS patients with active oral ulcers than minor RAS patients without active oral ulcer and healthy control group.[21] Ekinci et al. reported as similar with our study that, TOS and OSI levels are significantly higher in RAS patients and TAS levels were statistically indifferent in RAS patients as paraoxonase/arylesterase 1 and prolidase values.[22]

Some of the limitations of our study can be declared as the limited number of patients, the lack of as certaining the relation between the subgroups of RAS lesions and serum TAS, TOS and OSI values, and not determining the post-teratment clinical response, TAS, TOS and OSI values of the patients. Patients were not been evaluated for daily food or drug intake that act like antioxidants supplements. Controlled and large-scale studies with larger patient series, in which the subgroups of RAS lesions are handled separately and the values before and after treatment are compared will allow a better interpretation of the total oxidant status, total antioxidant status and oxidative stress index parameters in the pathogenesis of recurrent aphthous stomatitis.

Conclusion

In patients with recurrent aphthous stomatitis, serum total oxidant status, serum total antioxidant status and oxidative stress index values are affected in favor of oxidative processes. In the pathogenesis of RAS, oxidant substances and antioxidant-active substances are important.

Conflict of interest

Authors declare no conflict of interest.

Funding information

None.

Ethical statement

Study approved by KTO Karatay University School of Medicine Ethical Committee with approval number 2021/19097.

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