ORAL DISEASES

Oral Diseases (2009) 15, 512–515. doi:10.1111/j.1601-0825.2009.01580.x

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ORIGINAL ARTICLE

Oxidant/antioxidant status in recurrent aphthous stomatitis

S Arikan, C Durusoy, N Akalin, A Haberal, D Seckin

Departments of Biochemistry and Dermatology, Faculty of Medicine, Baskent University, Ankara, Turkey

OBJECTIVE: Recurrent aphthous stomatitis (RAS) is a common oral mucosal disorder characterized by recurrent, painful oral aphthae, and oxidative stress presumably contributes to its pathogenesis. The study was performed to evaluate the involvement of oxidant toxicity in this disorder.

METHODS: Patients with RAS (*n* = 26) and age- and sex-matched healthy control subjects (*n* = 20) were included in this study. Following an overnight fast, blood specimens were obtained. Plasma malondialdehyde concentrations and erythrocytes glutathione peroxidase activities were determined. Also, plasma vitamin E and selenium levels were detected. Mann–Whitney *U*-test was performed for statistical evaluation.

RESULTS: Oxidative stress was confirmed by the significant elevation in plasma malondialdehyde levels and by the significant decrease in glutathione peroxidase activities, vitamin E and selenium levels (*P* < 0.001).

CONCLUSIONS: Our results indicated that lipid peroxidation and the inadequacy of the defense system seem to play a crucial role in the pathogenesis of recurrent aphthous stomatitis.

Oral Diseases (2009) **15**, 512–515

Keywords: recurrent aphthous stomatitis; vitamin E; selenium; glutathione peroxidase; malondialdehyde

Introduction

Recurrent aphthous stomatitis (RAS) is a common oral mucosal disorder characterized by recurrent, painful oral aphthaes. Epidemiologic studies indicate that the prevalence of RAS is between 10% and 20% in the general population (Shashy and Ridley, 2000; Ship *et al*, 2000). There have been many researches to determine the causes of RAS. Genetic, immunologic, infectious factors as well as local and systemic conditions have all been implicated as potential etiopathogenic agents (Kewkowicz *et al*, 2003; Lin *et al*, 2005; Karaca *et al*, 2008). However, to date, no principal etiology has been discovered. It has been proposed that the etiological factors lead to aphthae formation via a final common pathway based on increased oxidative stress (Gurel *et al*, 2007).

Oxidative stress can arise through the increased production of reactive oxygen species (ROS) and/or because of a deficiency of antioxidant defenses. Antioxidant deficiencies can develop as a result of decreased antioxidant intake (such as vitamins E and C), synthesis of enzymes such as superoxide dismutase and glutathione peroxidase (GPx), or increased antioxidant utilization (Bickers and Athar, 2006). Insufficient antioxidant enzyme synthesis may in turn be due to decreased micronutrient availability (such as selenium, magnese, copper, and zinc). Oxidative stress constitutes the basis for many diseases, and may account for the severity of systemic and oral disease complications (Vertuoni *et al*, 2004). Hence, diet-derived antioxidants may be particularly important in protecting against these diseases.

The aim of this study was to assess the level of lipid peroxidation and status of antioxidant vitamin E and antioxidant enzyme GPx, and trace element selenium (Se) in patients with RAS.

Materials and methods

This randomized prospective-controlled study was conducted in 26 (15 female and 11 male) patients with RAS who admitted to the Department of Dermatology at the University of Baskent in Alanya Hospital. For comparison, 20 (12 female and 8 male) healthy individuals were included as controls. There were no differences in gender or age in the study groups. The diagnosis of RAS was based on typical patients' history and clinical findings. Care was taken to the correct differential diagnosis. Taking into account the possible causes of ulcers in the oral cavity, clinical evaluation of the patients were made by the same dermatologist. All RAS patients recruited had at least three recurrences per year. None of the patients suffered from Behçet's Disease, inflammatory

Correspondence: S Arikan, Baskent University, Alanya Hospital, Yunus Emre Street No:1, 07400 Alanya/Antalya, Turkey. Tel: +902425112511, Fax: +902425135563, E-mail: serapa@baskent-aln.edu.tr
Received 4 March 2009; revised 14 April 2009; accepted 28 April 2009

bowel disease, or any systemic disease, and none received any systemic treatment that could influence the results of tests performed in this study. The study groups were also non-smokers and non-alcohol users. All the RAS patients had more than one ulcer present at the time of blood collection.

The research protocol was approved by the Ethical Committee of the School of Medicine, Başkent University, and all patients or their guardians gave their signed informed consent for participation.

All blood samples were drawn into the vacutainer tubes containing Li-heparin. After the tubes had been centrifuged for 10 min at 4000 *g*, the plasma was separated, the buffy coat was discarded and packed erythrocytes were washed three times with sterile 0.9% sodium chloride (w/v). Preparation of the erythrocytes was performed immediately after the blood collection. The erythrocytes packet and plasma were stored at −80°C prior to the analysis.

Plasma malondialdehyde (MDA nmol l^{-1}) levels were measured according to the method of Buege and Aust (1978). One volume of sample was mixed with two volumes of a stock solution of 15% trichloroacetic acid (w/v), 0.375% thiobarbituric acid (w/v), and 0.25 N HCI. The combination of sample and stock solution was heated for 30 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 3200 *g* for 15 min. The absorbance of the clear supernatant was determined at 535 nm and MDA concentration was calculated using 1.56×105 M^{-1} cm^{-1} as molar absorption coefficient.

Glutathione peroxidase (U g^{-1}Hb) activity was determined by the method of Paglia and Valentine (1967). Enzyme activity was proportional to the rate of NADPH oxidation in the presence of H_2O_2 as a substrate spectrophotometrically. Erythrocyte GPx activity was determined with chemicals from Randox Chemical Company (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK).

Serum Se concentration (μg l^{-1}) was determined using the hydride generation atomic absorption spectrometry (Atomic absorption spectrometer Shimadzu, AA-6701-HV6) (Safarilazadeh *et al*, 2005).

Plasma vitamin E levels were determined according to the method of Quaife and Nevn (1949). After the proteins in plasma (1.5 ml) were precipitated by an equal volume of absolute ethanol (1.5 ml), equal volume of xylene (1.5 ml) was added to the mixture. It was centrifuged at 3000 *g* at 4°C. The α-α-dipyrimidyl (1.20 g l^{-1} in *n*-propranole) (1 ml) was added to an aliquot of the upper layer (2 ml). 1.5 ml of this mixture was transferred to another tube and absorbance was measured at 460 nm to estimate the principal interfering substance *β*-carotene. At this point, the ferric chloride reagent (1.20 g FeCI3.6H20 in 1 l of absolute ethanol) was added to the system to produce color which was measured at 520 nm. By subtracting the absorbance at 520 nm from the absorbance at 460 nm, plasma vitamin E levels were calculated; α-tocopherol was used as the standard of vitamin E (81 mg per 100 ml in absolute ethanol).

Differences in concentration between all the groups were analyzed with the Mann–Whitney *U*-test using the SPSS (SPSS Inc, Chicago, IL, USA) (11.00) program. The results are given as the mean ± standard deviation (s.d.). *P*-values < 0.05 were considered as statistically significant.

Results

Twenty-six (15 females and 11 males; mean age 38.39 years; range 17–62 years) patients with RAS and 20 healthy individuals (12 females and 8 males; mean age 34.10 years; range 25–43 years) were included in this study. The demographic data of the subjects, mean results ± s.d., and statistical comparisons are presented in Tables 1 and 2. The levels of MDA, GPx, Se, and Vitamin E in subjects are presented in Figures 1–4.

The levels of MDA in patients with RAS were found to be increased, whereas the levels of GPx, Se, and vitamin E were decreased in comparison with that of the

Table 1 Demographic data of the patients and controls

	Age (years)	*(Mean ± s.d.)*	*Female*	*Male*
Patient with RAS (*n* = 26)	17–62	38.39 ± 11.10	15	11
Controls (*n* = 20)	25–43	34.10 ± 5.07	12	8

Table 2 The statistical differences between the groups

	(Mean ± s.d.)		
	Patient with RAS (n = *26*)	*Controls* (n = *20*)	P-*value*
MDA (nmol l^{-1})	0.97 ± 0.33	0.54 ± 0.23	0.000
GPx (U g^{-1}Hb)	20.4 ± 2.68	25.9 ± 2.55	0.000
Selenium (μg l^{-1})	87.15 ± 35.25	107.25 ± 25.28	0.000
Vitamin E (mg dl^{-1})	0.74 ± 0.88	0.99 ± 0.02	0.000

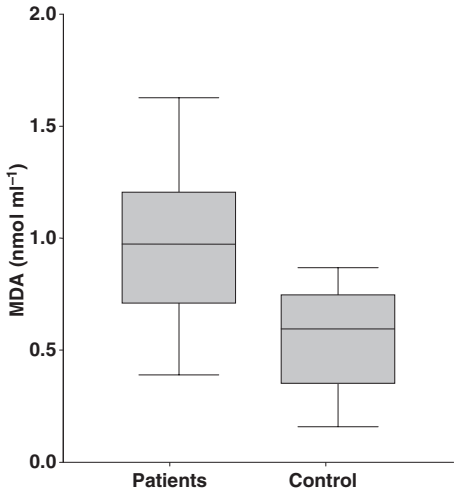

Figure 1 Serum levels of malondialdehyde (MDA) (nmol ml^{-1})

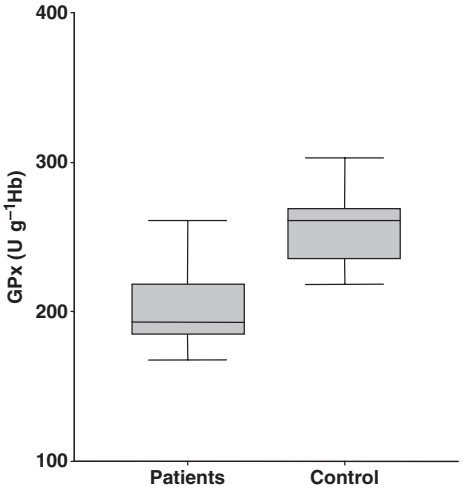

Figure 2 Serum levels of glutathione peroxidase (GPx) (U g^{-1}Hb)

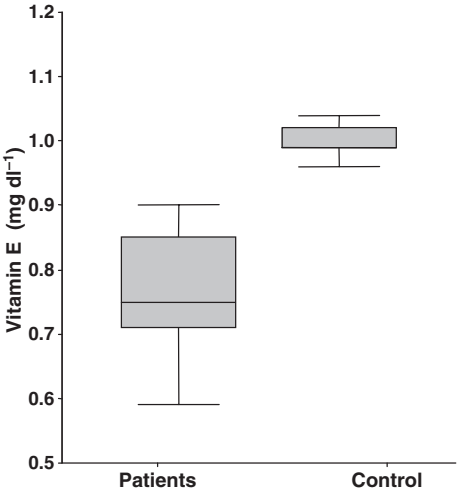

Figure 4 Serum levels of vitamin E (mg dl^{-1})

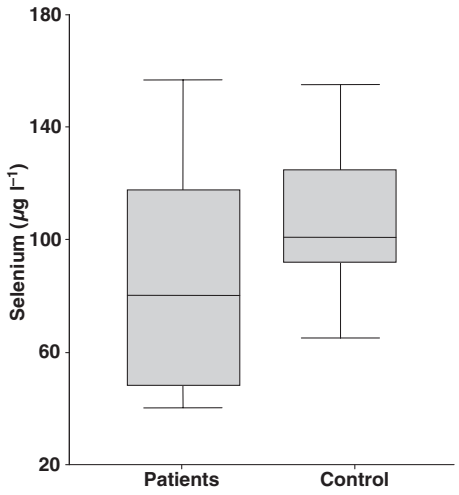

Figure 3 Serum levels of selenium (μg l^{-1})

healthy controls. The differences were statistically significant for all variables ($P = 0.000$). Also, a negative correlation between the levels of MDA and GPx activities was detected ($r = -0.418$, $P = 0.034$).

Discussion

Recurrent aphthous stomatitis may be precipitated by local trauma, stress, food intake, drugs, hormonal changes, and vitamin and trace element deficiencies (Carrozzo *et al*, 1995; Jurge *et al*, 2006). There is strong evidence from the histopathologic and immunologic studies that T-cell-mediated immune responses have been implicated in RAS (Freysdottir *et al*, 1999; Lin *et al*, 2005).

Selenium deficiency is accompanied by a loss of immunocompetence, and both cell-mediated immunity and B-cell function can be impaired. Se supplementation has marked immunostimulant effects, including enhancement of the activated T-cell proliferation (Field *et al*, 2002). It has been demonstrated that, when taken as a supplement, Se modulates the cellular response to oxidative stress, inducing a faster restoration of the endogenous antioxidative defense system against the production of reactive oxygen species (McKenzie *et al*, 1998). In a study evaluating the efficacy of topical L-selenomethionine in reducing the effects of ultraviolet-induced acute skin damage, it was found that L-selenomethionine provided effective protection, which improved as the dose was increased (Burke *et al*, 1992). GPx also requires Se at the active site, and deficiency of GPx may occur in the presence of severe Se deficiency. It has been suggested that GPx is the main scavenger of H2O2 in cellular compartments**.** The deficiency of GPx in important components of the endogenous antioxidant defense system leads to the accumulation of ROS, inducing oxidative damage (Michiels *et al*, 1994; Devasagayam *et al*, 2004).

It is well known that Se and vitamin E show compensative effects, and either Se or vitamin E deficiency causes massive injury (Hill *et al*, 2001). Verret *et al* (2005) found that supplementation with vitamin E, Se, and the combination mildly improved skin lesions in people exposed to high levels of arsenic. Seidner *et al* (2005) suggested that oral supplementation of combination of vitamin E, vitamin C, and Se decreased the requirement for corticosteroids in patients with ulcerative colitis. This enriched oral supplement can be a useful adjuvant therapy in patients with ulcerative colitis. Ohta *et al* (2006) found that the gastric mucosa of C48/80-treated rats had decreased Se, GPx, and vitamin E activity. They suggested that vitamin E prevents acute gastric mucosal lesion progression in C48/80-treated rats possibly by suppressing the oxidative stress.

Studies investigating the antioxidant status in patients with RAS are very limited. In a recent study, Saral *et al* (2005) demonstrated that the levels of selected antioxidant vitamins in serum and saliva are lower, while the degree of lipid peroxidation, as judged by the MDA levels, is higher in patients with RAS than in the control subjects. Cimen *et al* (2003) have reported a decrease in the activity of enzymatic antioxidant capacity in patients

with RAS. Karincaoglu *et al* (2005) found decreased GPx and other enzymes' activities in the saliva and serum in patients with RAS in comparison with that in control subjects.

In this study, we found decreased GPx activities in the erythrocytes, and decreased vitamin E and Se levels and increased MDA plasma levels in patients with RAS. The reduction in plasma vitamin E and Se levels might be due to the increased consumption of these agents by the erythrocytes and/or other tissues in response to increased oxidative stress in this disease. These findings suggest that RAS patients may require additional dietary supplement of these compounds as they play an important role in the defense of the organism against the oxidative challenge.

Increased lipid peroxidation in RAS and following decreases in Se-dependent GPx activity might be the reason for such a shift of plasma Se in erythrocyte and/or other tissues. As the diet is the main source of Se, this shift might be expected to become more pronounced especially if there is a concomitant reduced Se intake.

We suggested that short-term and modest supplementation with a mixture of antioxidant nutrients may improve antioxidative capacity and reduce products of lipid peroxidation in RAS. An adequate daily intake of the individual antioxidants is therefore important to prevent the cells against oxidative damage, which may prevent the attacks in patients with RAS.

Acknowledgements

We would especially like to thank the field research assistant, Mustafa Söğüt. This work was supported by a grant of the Research Committee, Başkent University School of Medicine, Ankara, Turkey.

Author contributions

Serap Arikan, Cicek Durusoy, Nalan Akalin, Aysegul Haberal and Deniz Seckin had significant contributions in our study.

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