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ORIGINAL ARTICLE

Oxidative stress and myeloperoxidase levels in saliva of patients with recurrent aphthous stomatitis

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OBJECTIVE: Recurrent aphthous stomatitis (RAS) is the most common oral ulcerative condition affecting 5–25% of the general population. The aim of this study was to evaluate the oxidative stress parameters in saliva of patients with RAS and to investigate the relationship among these parameters in either group.

MATERIALS AND METHODS: The study involved 50 patients with RAS of whom 24 were male and 26 were female, and 25 healthy controls of whom 13 were male and 12 were female.

RESULTS: There was no statistically significant difference in the salivary total antioxidant capacity, total oxidant status, oxidative stress index levels, and myeloperoxidase activity between patients with RAS and those in the control group.

CONCLUSIONS: The results show that reactive oxygen species may not play a role in the etiology of RAS.

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Keywords: recurrent aphthous stomatitis; saliva; total antioxidant capacity; total oxidant status; oxidative stress; myeloperoxidase

Introduction

Recurrent aphthous stomatitis (RAS) is an inflammatory condition of unknown etiology characterized by painful recurrent, single or multiple ulcerations of the oral mucosa. RAS is the most common oral ulcerative condition affecting 5–25% of the general population (Shashy and Ridley, 2000; Scully *et al*, 2002; Porter and Leao, 2005; Jurge *et al*, 2006) and in selected groups, it reaches a prevalence of more than 50% (Shashy and Ridley, 2000; Scully *et al*, 2002). The peak age of RAS onset is during childhood, with a tendency to decrease in severity and frequency with age (Field and Allan, 2003). Localized burning or pain can be seen as a prodromal symptom 24–28 h before classical clinical appearance. Typical RAS lesions involve self-limited, painful, round or oval 1–3 mm ulcers, each with a shallow necrotic center. These ulcers are covered with a grayish yellow pseudomembrane and are surrounded by minimally raised margins and an erythematous halo representing superficial vasculitis and extravasated erythrocytes located in the superficial layers of the lamina propria (Scully *et al*, 2002).

Stanley classified RAS as minor, major, and herpetiform in 1972 (Natah *et al*, 2004). Minor RAS (MiRAS) is the common variety, affecting about 75–85% of RAS patients (Scully *et al*, 2002; Field and Allan, 2003; Natah *et al*, 2004). It is a round or oval ulcer and <10 mm in diameter. It heals within 10–14 days without scarring. Major RAS (MaRAS) affects approximately 10% of all RAS patients (Scully *et al*, 2002; Field and Allan, 2003; Natah *et al*, 2004). The lesions are similar to those of MiRAS, but they are more than 10 mm in diameter. The ulcers of MaRAS persist up to 6 weeks or longer and often heal with scarring. Herpetiform RAS (HuRAS) is the least common variety of RAS and affects 5–10% of all RAS patients (Scully *et al*, 2002; Field and Allan, 2003; Natah *et al*, 2004).

Despite extensive investigations, the exact etiology of RAS is still unknown. However, most patients who suffer from RAS are usually healthy individuals. Factors such as trauma (Scully *et al*, 2002; Natah *et al*, 2004), stress (Andrews and Hall, 1990; Scully *et al*, 2002), family tendency (Scully *et al*, 2002; Field and Allan, 2003), atopy (Veller-Fornasa *et al*, 2003), nutrition (Eversole *et al*, 1982; Shashy and Ridley, 2000), drug reactions (Healy and Tornhill, 1995; Boulinguez *et al*, 2000), immune disturbance (Scully *et al*, 2002), hormonal imbalance (Field and Allan, 2003; Natah *et al*, 2004), and microbial factors (Scully *et al*, 2002; Field and Allan, 2003; Jurge *et al*, 2006), which are thought to be associated with the etiology of RAS, can disturb the equilibrium of oxidant/antioxidant status of the organism and can accelerate the formation of free radicals. Reactive oxygen species (ROS) are the most common

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free radicals in biologic systems. Oxidative stress occurs when the intracellular concentrations of ROS increase over the physiologic values. Several conditions, including viral and bacterial infections, hyperthermia, ionizing and UV irradiation, and environmental pollutants can cause oxidative stress. The cytotoxic effects of free radicals are harmful for mammalian cells, and they are associated with atherosclerosis, diabetes, inflammatory conditions, neurologic diseases, cancer, aging, etc. (Sultan Beevi *et al*, 2004; Hoelzl *et al*, 2005; Meneses *et al*, 2005; Ziyatdinova *et al*, 2006). However, mammalian cells have developed elaborate antioxidant defense systems to prevent oxidative damage and to allow survival in an aerobic environment.

Myeloperoxidase (MPO) is a heme-containing enzyme found in azurophilic granules of human neutrophils (Floris and Wever, 1992; Wei *et al*, 2004; Kaner *et al*, 2006). As the enzyme has a unique ability to catalyze the H_2O_2-dependent peroxidation of Cl^- to HOCl, which is an anti-microbial agent, MPO is thought to play an important role in the killing of micro-organisms and it shows non-specific peroxidase activity (Floris and Wever, 1992; Liskmann *et al*, 2004; Wei *et al*, 2004). MPO is considered to reflect the strength of oxidative stress. There are a lot of articles in the literature mentioning that microbial agents play a role in the etiology of RAS (Scully *et al*, 2002; Natah *et al*, 2004; Jurge *et al*, 2006). As MPO has an anti-microbial property, it may be important in RAS patients.

To our knowledge, there is simultaneously no study on the total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI) levels, and MPO activity in the saliva of patients with RAS. Therefore, in the present study, we investigated these parameters of oxidative stress in the saliva of patients with RAS.

Materials and methods

Patients

The Health Science Institute Ethics Committee of Ataturk University approved the study. The study involved 50 patients with RAS of whom 24 were male and 26 were female, and 25 healthy controls of whom 13 were male and 12 were female. The RAS patients had oral ulcer attack at least three times a year and all of them were minor aphthae. Patients were otherwise healthy. They were not under a therapeutic regimen for the past 3 months. Patients with Behçet's disease, trauma history, and any systemic diseases were not included in the study. Patients who were smokers and had periodontal problems were also not included in the study. The control group included 25 healthy volunteers who denied having RAS, using medications and smoking.

Saliva samples

Saliva samples were taken with the consent of patients. Samples were obtained in the morning following an overnight fast. The patients were first asked to rinse their mouth using distilled water. After 5 min, we started to gain unstimulated saliva samples. Then the patients were told to sit comfortably and to spit into the plastic tubes five times per min for 5 min. The samples were then stored at −80°C until biochemical analyses.

Measurement of TAC

Saliva TAC levels were determined using a novel automated colorimetric measurement method developed by Erel (2004a). In this method, the hydroxyl radical, the most potent biologic radical, is produced by the Fenton reaction and reacts with the colorless substrate *O*-dianisidine to produce the dianisyl radical, which is bright yellowish brown in color. Upon the addition of a sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the sample preventing the color change and thereby providing an effective measure of the total antioxidant capacity of the sample. The assay has excellent precision values, which are lower than 3%. The results are expressed as micromolar Trolox equivalent per liter (mmol Trolox equivalent l^{-1}).

Measurement of TOS

Measurement of TOS was determined by using a novel automated measurement method, developed by Erel (2005). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of the oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (mmol H_2O_2 equivalent l^{-1}).

OSI

The percent ratio of TOS to TAC yields the OSI, an indicator of the degree of oxidative stress (Kosecik *et al*, 2005). OSI (arbitrary unit) = TOS (mmol H_2O_2 equivalent l^{-1})/TAC (mmol Trolox equivalent l^{-1}).

Measurement of MPO concentration

The method described by Wei and Frenkel (1993) was used for the tissue MPO activity assay, and data are expressed as U g^{-1} protein.

Statistical analysis

The results were expressed as mean ± s.e.m. Statistical analysis was undertaken using the Student's *t*-test (two-tailed) and Pearson's correlation test. A P-value < 0.05 was accepted to be statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 11.0, SPSS Inc., Chicago, IL, USA).

Results

The demographic data of the subjects are summarized in Table 1. There were no significant differences in age and

Table 1 Demographic data of recurrent aphthous stomatitis patients and controls

	Patients (n = *50*)	*Controls* (n = *25*)	P
Age (years)	27.50 ± 8.54	24.13 ± 5.68	>0.05
Gender (female/male)	26/24	12/13	>0.05

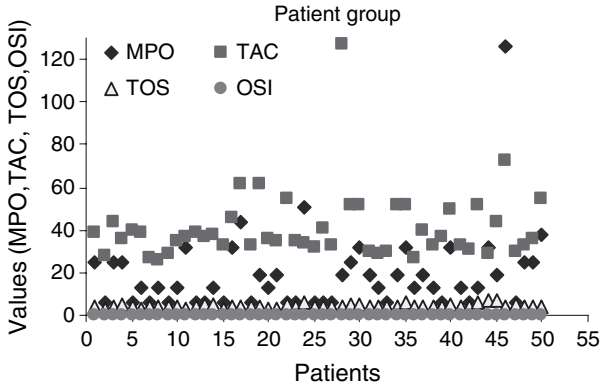

Figure 1 Myeloperoxidase, total antioxidant capacity, total oxidant status, oxidative stress index values of RAS patients

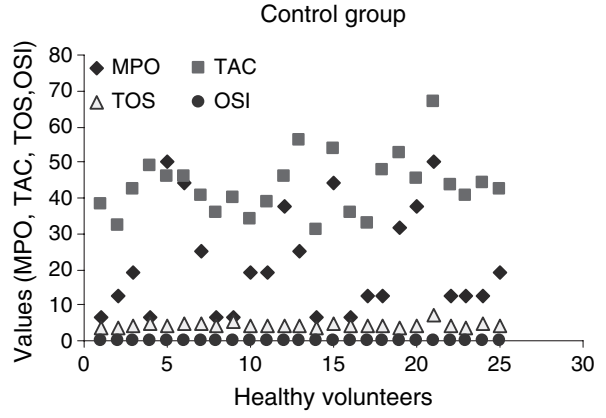

Figure 2 Myeloperoxidase, total antioxidant capacity, total oxidant status, oxidative stress index values of healthy controls

Table 2 Oxidant/antioxidant parameters and myeloperoxidase levels of recurrent aphthous stomatitis patients and controls

Parameters	*RAS* (n = *50*)	*Controls* (n = *25*)	P
TAC (mmol Trolox equivalent l^{-1})	41.47 ± 16.24	43.62 ± 8.23	>0.05
TOS (mmol H_2O_2 equivalent l^{-1})	4.11 ± 0.86	4.31 ± 0.78	>0.05
OSI (arbitrary unit)	0.11 ± 3.23	0.10 ± 1.55	>0.05
MPO (U g^{-1})	19.22 ± 18.97	21.36 ± 14.73	>0.05

TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index; MPO, myeloperoxidase.

gender between patients with RAS and controls. The MPO, TAC, TOS, and OSI values of RAS patients and controls are shown in Figures 1 and 2. As seen in Table 2, no statistically significant difference was found in the salivary TAC, TOS, OSI, and MPO levels of RAS patients and controls. Furthermore, there was a positive correlation between OSI and TOS and a negative correlation between OSI and TAC.

Discussion

In this study, we investigated whether there is any alteration in TAC, TOS and OSI values, and MPO activity in patients with RAS. We found unchanged TAC, TOS and OSI values, and MPO activity in RAS patient when compared with those of the control group.

Normal cell functions and integrity of cell structures may be broken via considerable activity of ROS. The organism has enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (e.g. vitamin C, vitamin E) antioxidant mechanisms that work as scavenger for this harmful ROS. Radical-scavenging antioxidants are consumed by the increased free-radical activity associated with several conditions, and the total antioxidant response has been used to indirectly assess free-radical activity. The effects of various antioxidants in plasma and other biologic samples are additive and the cooperation of antioxidants in human serum provides protection against attacks by free radicals. Therefore, the measurement of TAS may reflect accurately the antioxidant status of the organism (Erel, 2004a,b; Bolukbas *et al*, 2005).

Moreover, it is widely accepted that imbalance between free radicals and antioxidants causes many inflammatory oral pathologies (Battino *et al*, 2002; Nagler *et al*, 2002; Yang *et al*, 2002; Brock *et al*, 2004; Sultan Beevi *et al*, 2004; Karıncaoğlu *et al*, 2005). Saliva is the first defense against the oxidative stress that is caused by free radicals, because chewing function may cause lipid peroxidation. Lately, in the literature, it is emphasized that attacks of free radicals on the oral mucosa may lead to many situations, from infection to cancer (Nagler *et al*, 2002; Yang *et al*, 2002; Sultan Beevi *et al*, 2004). Dayan *et al* (1997) showed the anticancer property of saliva by using animal models. We used whole and unstimulated saliva to detect the oxidant/antioxidant parameters in this study. Whole saliva is the most relevant, as it contains gingival crevicular fluid, immune cells, and tissue metabolites and reflects more closely the predominant intra-oral condition. Stimulation may increase the flow of gingival crevicular fluid and this may result in false increases in the concentration of antioxidants in the saliva (Buduneli *et al*, 2006).

As the involvement of inflammatory mechanisms has been suggested in the pathogenesis of RAS, oxidant/antioxidant systems were investigated in patients with RAS. This methodology has previously been used in various inflammatory disorders such as inflammatory bowel diseases, ulcerative colitis, and psoriasis (Cimen *et al*, 2003). Cimen *et al* (2003) found that enzymatic and non-enzymatic antioxidant defense systems were impaired in erythrocytes and plasma samples of RAS patients. Ohashi *et al* (1999) found elevated salivary nitric oxide (NO·) levels in RAS

patients in comparison with controls. Gunduz *et al* (2004), however, could not find a significant difference on plasma NO· levels between RAS patients and controls. Karıncaoğlu *et al* (2005) found oxidant and antioxidant parameters to be diverse in the saliva and plasma of RAS patients. They found no differences in the salivary uric acid (UA) levels of RAS patients and controls. UA is one of the most important antioxidant molecules in the saliva and compose 70–85% of TAC (Battino *et al*, 2002; Nagler *et al*, 2002; Karıncaoğlu *et al*, 2005). Moreover, we could not find any significant difference between salivary TAC of RAS patients and controls. Furthermore, there were no significant differences in salivary TOS levels of RAS patients and controls. So, there was no difference in OSI values in the saliva of RAS patients and control group.

The exact etiology of RAS is unknown. Probably, its etiology is multifactorial with many predisposing factors and immunologic bases. We found no significant difference in the salivary oxidant/antioxidant parameters of RAS patients and controls. Oxidant/antioxidant systems do not play a role in the pathogenesis of recurrent aphthous stomatitis whole saliva. These findings also show the need for further studies on this subject.

Author contributions

Fatma Çağlayan, Özkan Miloglu and Oğuzhan Altun contributed in the study design, Özcan Erel contributed in the biochemical analyses, and A. Berhan Yılmaz contributed in drafted papers.

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