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Serum levels of total antioxidant status, nitric oxide and nitric oxide synthase in minor recurrent aphthous stomatitis patients

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Abstract

Background: Recurrent aphthous stomatitis (RAS) is one of the most common inflammatory ulcerative conditions of oral cavity with uncertain etiology. Several studies have reported that oxidative stress may be associated with RAS. The aim of this study was to compare the serum levels of total antioxidant status (TAS), nitric oxide (NO) and nitric oxide synthase (NOS) in minor RAS (MiRAS) patients with healthy individuals and determine the possible association of MiRAS with the 3 physiological parameters mentioned above.

Methods: Ninety patients with idiopathic MiRAS and 90 race-, age- and sex-matched healthy individuals were included in this study. All these subjects were allocated to 3 groups: MiRAS patients in the active stage (Group A); the same MiRAS patients in Group A in the inactive stage (Group B); healthy individuals without MiRAS (Group C). Serum levels of TAS, NO and NOS were determined by the spectrophotometric method. Independent sample *t* test and paired *t* test were performed for statistical evaluation.

Results: Serum TAS level of Group A was significantly decreased than that of Group C, whereas the serum level of NO was significantly higher in Group A as compared to Group C ($P < .05$). The serum levels of TAS and NO in Group B were no significant differences when compared with those in Group A or Group C. No significant differences in NOS activities were also found between the 3 groups ($P > .05$).

Conclusions: MiRAS is associated with decreased TAS and increased NO levels, but NOS may not play an important role in the aetiopathogenesis.

Abbreviations: ABTS = 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate), CAT = catalase, DNA = deoxyribonucleic acid, eNOS = endothelial NOS, GSHPx = glutathione peroxidase, IL = interleukin, iNOS = inducible NOS, MiRAS = minor RAS, nNOS = neuronal NOS, NO = nitric oxide, NOS = nitric oxide synthase, RAS = recurrent aphthous stomatitis, ROS = reactive oxygen species, SOD = superoxide dismutase, TAS = total antioxidant status, TNF = tumor necrosis factor.

Keywords: nitric oxide, nitric oxide synthase, oxidative stress, recurrent aphthous stomatitis, total antioxidant status

1. Introduction

Recurrent aphthous stomatitis (RAS) is a common ulcerative disease of the oral mucosa, presenting as multiple recurrent round or ovoid ulcers with necrotic centers, yellowish-gray pseudomembranes and well-defined erythematous margins.[1,2] Both men and women of all ages, races and geographic regions can be affected. It has been demonstrated that the histology of RAS is characterized by basal cell destruction and lymphocytic infiltrates.[3] According the clinical symptoms, RAS can be classified as minor, major, and herpetiform. Minor recurrent aphthous stomatitis (MiRAS) is the most common form consisting of 80% RAS patients.[4]

Numerous studies have reported that local trauma, smoking, nutritional deficiencies, infectious agents, stress, hormonal imbalance, genetic predisposition, hematologic abnormalities, drug or food allergies and immunological status may be implicated as potential etiopathogenic agents, however, the exact etiology of RAS still remains uncertain.[5,6] These factors have a direct or indirect potential for disturbing the equilibrium between oxidant and antioxidant systems in humans, which could accelerate the formation of free radicals and the occurrence of toxic reactions.[7] Oxidative stress is defined as a process in which the dynamic redox balance between oxidants and antioxidants is intensely shifted when the concentrations of reactive oxygen species (ROS) increase over the physiological values.[8] Up to now, many studies have investigated the amounts of oxidative reactive species, antioxidant enzymes and nonenzymatic antioxidant molecules in patients with RAS.[9,10] However, measurements of oxidant and antioxidant molecules individually cannot reflect the global status of oxidative stress.

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Nitric oxide (NO) is an important endogenous vasodilator and a multifunctional intracellular and intercellular messenger molecule of immunity and inflammation.[11,12] As a free radical, it can react with superoxide and create toxic peroxynitrites, which can cause directly damage to endothelium.[13] It also can inhibit platelet aggregation and adhesion and influence the ability of immune cells to kill bacteria, viruses and tumor cells. In addition, it has been reported that NO has damaging effects against cellular proteins, DNA and lipids, eventually leading to cell death, tissue injury and organ failure.[14] It has been implicated in several pathological processes such as inflammatory processes, cancer, immunological, cardiovascular and neurological disorders.[15] Nitric oxide synthase (NOS) is helpful to synthesize NO from L-arginine in mammalian cells, which consists of 3 isoforms: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS).[16,17]

According to our knowledge, there are only several articles in the literature about the serum levels of TAS, NO, and NOS in MiRAS patients with active lesion,[11–14,17–19] but no data concerning those in Chinese MiRAS patients in the inactive stage. Therefore, the aim of this study was to compare the concentration of serum TAS, NO and NOS in Chinese MiRAS patients in the active stage and the inactive stage with healthy individuals, and to determine the possible association of MiRAS with the 3 physiological parameters mentioned above.

2. Material and methods

2.1. Subjects

The Ethics Committee of Bethune International Peace Hospital approved the study protocol (Protocol No. 2014-12-9) and each subject signed a detail informed consent form. Minor RAS patients were chosen from those who presented in the Department of Stomatology of Bethune International Peace Hospital and control subjects were chosen from those who took routine medical examination in our hospital. The diagnosis of MiRAS was based on detailed patients' history and clinical examination. All patients were evaluated by the same oral medicine specialist during the study.

Patients with RAS had oral ulcer attack at least 3 times a year and all of them were idiopathic minor aphthae. These patients were otherwise healthy. The healthy controls did not have any RAS attacks before the study or during their participation in the study. All participants were not under a therapeutic regimen of immunomodulatory agents, steroids, multivitamins, or other antioxidant supplements for the past 3 months. None of them suffered from diabetes mellitus, rheumatoid arthritis, coronary heart disease, Behçet's disease, inflammatory bowel disease, celiac disease, chronic diarrhea, or any systemic diseases. In addition, they were not pregnant or nursing, nor did any of them smoke tobacco or use alcohol. Finally, there were 90 patients with idiopathic MiRAS (52 females and 38 males; range 20–42 years) and 90 race-, age-, and sex-matched healthy individuals (50 females and 40 males; range 22–45 years) with no history of any episodes of RAS meeting these above-mentioned requirements.

There were 3 groups: Group A consisted of 90 patients with active lesions of MiRAS; Group B comprised the same 90 individuals in Group A in the inactive stage of the disease, without any prodromal symptoms or active lesions; and Group C included 90 race-, age-, and sex-matched healthy individuals without RAS disease.

2.2. Blood samples

Following an overnight fast, 5 ml of venous blood was drawn from the antecubital vein using sterile disposable syringe in the morning hours (8:00–10:00) and transferred into a vacutainer without any anticoagulant. All the blood samples were centrifuged at 3000 g for 10 min at 4 °C to obtain serum and stored at −80 °C until analysis.

2.3. Measurement of TAS, NO, and NOS

The serum concentrations of TAS, NO and NOS were measured with a spectrophotometer (Genesys10 UV Scanning UV/VIS Spectrophotometer, Shimadzu Co., Tokyo, Japan) according to the manufacturer's recommendations of commercial colorimetric assay kits (TAS/NO/NOS Detection Kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The estimation for serum TAS was based on the bleaching of color characteristics of a more stable ABTS radical cation by antioxidants.[20] The variation of assay data was lower than 3% and the results were expressed as millimolar Trolox equivalent per liter (mmol Trolox Eq/L). As a very labile molecule, NO can react with molecular oxygen and generate nitrite (NO_2^-) and nitrate (NO_3^-) in aqueous solution.[11–13] Therefore, only the sum of nitrite and nitrate could accurately represent the serum levels of NO, which was expressed as μmol/L in this study. The NOS activity was calculated according to the serum levels of NO and was expressed as U/ml.

2.4. Statistical analysis

The results were expressed as mean ± standard deviation. Independent sample *t* test and paired *t* test were used to compare those quantitative data of unpaired samples and paired samples, respectively. A $P < .05$ was accepted to be statistically significant. All statistical analyses were performed using SPSS version 20.0 statistical software package (SPSS Inc., Chicago, IL).

3. Results

Table 1 shows the demographic characteristics of the subjects with MiRAS and controls. There was no significant difference between MiRAS patients and healthy subjects with respect to age and gender ($P > .05$). The serum levels of TAS, NO and NOS in MiRAS patients and controls are presented in Figures 1–3. The serum TAS level of Group A (2.61 ± 0.78 mmol Trolox Eq/L) was significantly decreased than that of Group C (3.08 ± 0.49 mmol Trolox Eq/L, $P = .03$), whereas the serum level of NO was significantly higher in Group A (60.08 ± 23.75 μmol/L) as compared to Group C (46.45 ± 13.01 μmol/L, $P = .03$). The serum levels of TAS and NO in Group B (2.82 ± 0.77 mmol Trolox Eq/L and 50.28 ± 21.20 μmol/L) were no significant differences when compared with those in Group A ($P = .56$ and .38, respectively) or Group C ($P = .24$ and .51, respectively). No

Table 1

Demographic data of recurrent aphthous stomatitis patients and healthy individuals (n = 90).

	MiRAS patients	Healthy individuals	*P*
Age (years)	29.8 ± 5.6	31.2 ± 6.7	.13
Gender (females/males)	52/38	50/40	.76

MiRAS = minor recurrent aphthous stomatitis.

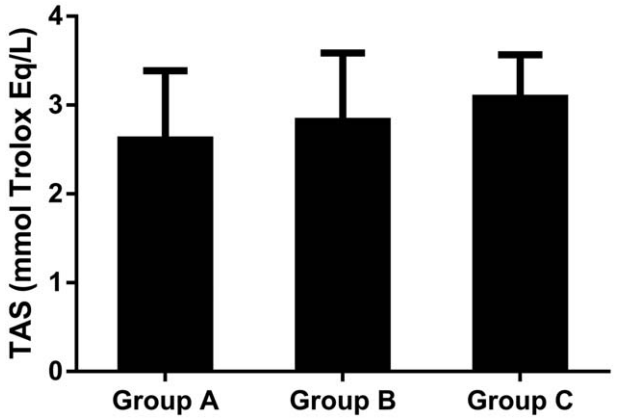

Figure 1. Serum levels of total antioxidant status (TAS) (mmol Trolox Eq/L).

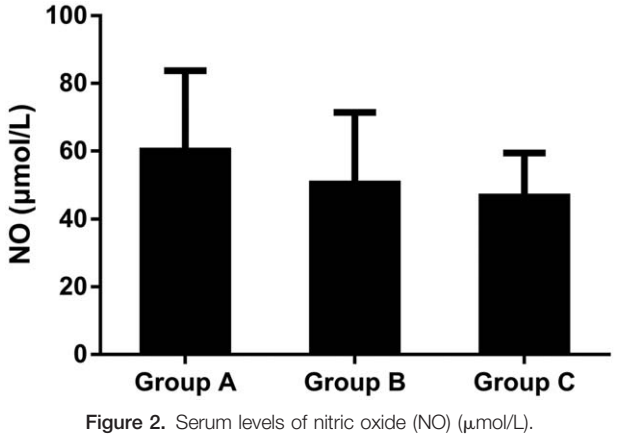

Figure 2. Serum levels of nitric oxide (NO) (μmol/L).

significant differences in NOS activities were also found between the 3 groups (35.90 ± 8.47 U/ml, 35.17 ± 5.49 U/ml, and 32.87 ± 4.21 U/ml; $P = .15$, .82 and .19, respectively).

4. Discussion

In the current study, we investigated the serum levels of TAS, NO, and NOS in MiRAS patients with active lesion, the same MiRAS patients in the inactive stage and healthy subjects. We found that there were decreased TAS and increased NO in MiRAS patients in the active stage when compared with those of the control group.

Oxidative stress occurs when intracellular concentrations of free radicals (O_2^-, H_2O_2, OH^-, $ONOO^-$) exceed over the physiological values, which can result in cell damage by lipid peroxidation, DNA and protein damage, enzyme oxidation, and stimulation of proinflammatory cytokine release.[9] In the oral cavity, it has also been demonstrated that increased oxidative stress maybe correlated with dentinogenesis, periodontitis, Behçet's disease, systemic sclerosis and Sjøgren's syndrome.[21] To prevent oxidative damage from excessive free radicals, there are antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) and as well as nonenzymatic antioxidants including glutathione, melatonin, vitamins A, C, E, and uric acid in the organism.[10] The concentrations of different antioxidants in the serum can be measured in laboratories separately, but these measurements are time-consuming, costly and require complicated techniques. It has been reported that the effects of various antioxidants in the plasma and other biologic samples are additive and the measurement of TAS may reflect accurately the overall antioxidant status of the organism.[18] Most of those results reported in the literature have demonstrated that MiRAS patients with active lesion have lower TAS level than healthy subjects.[18,19] The same result was also obtained in our study, which indicated that the dynamic redox balance was changed in favor of the oxidant side. The results of no significant differences between Group B and Group C or Group A may be due to the fact that the excessive ROS was being detoxified and the equilibrium between oxidant and antioxidant systems was recovered gradually in MiRAS patients in inactive stage.

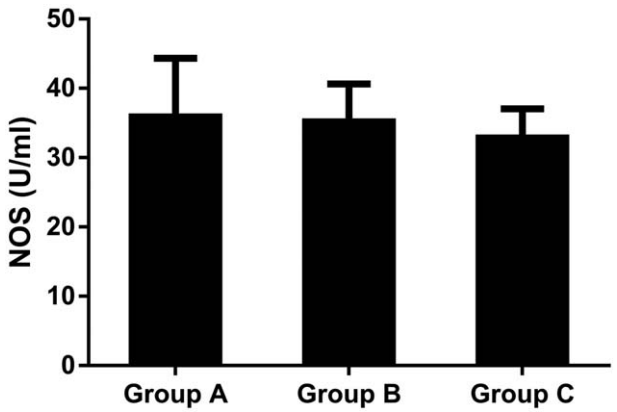

Figure 3. Serum levels of nitric oxide synthase (NOS) (U/ml).

NO is one of the most abundant free radicals in the body and can react with superoxide anion (O_2^-) to yield peroxynitrite ($ONOO^-$), which is a concentration dependent oxidizing agent and can result in decreased ATP production and cell death.[22] Under physiological conditions, NO can be rapidly cleared by reaction with oxyhemoglobin and does not cause tissue damage. However, in pathological conditions such as disorders of cardiovascular, nervous, and immunological systems, increased NO can give rise to cell damage, tissue injury and organ failure.[13,22] Studies investigating the concentration of serum NO in patients with RAS are very limited. In this study, the serum NO level of Group A was significantly increased than that of Group C, which is not completely consistent with previous research results reported. In the literature, there are some reports that support[13,14] and contradict[11,12] our results, which may be related to the use of various methods for measurement and subjects recruited with different racial backgrounds, dietary and life habits. Avci et al[11] in their study demonstrated that NO levels were decreased in serum of MiRAS patients compared with controls, but the change was not statistically significant. In the study of Gunduz et al[12], no significant differences between MiRAS patients and healthy subjects with regards to plasma NO levels was obtained. However, in another study conducted by Yildirim et al[13], the serum NO metabolite levels were found to be significantly higher in MiRAS patients than healthy controls.

As is known to all, NO is synthesized from L-arginine by NO synthase (NOS). There are 3 NOS enzymes. NOS-1 is the first to be purified and cloned from neural tissue and also called nNOS.

NOS-2 is an inducible isoform and also called iNOS. NOS-3 is the isoform found in vascular endothelium cells and also called eNOS. Both of nNOS and eNOS are dependent on the Ca^{2+} concentration in the corresponding tissue and can produce low and transient concentration of NO. Conversely, iNOS is Ca^{2+} independent isoform and can synthesize NO continuously throughout the life of the enzyme.[13,14] It has been reported that psychological stress, which may be a potential etiology of RAS, causes NO release in correlation with increase of nNOS activity.[14] Another study reported that eNOS was no significant association with RAS.[17] It has been proved that many proinflammatory cytokines, such as interleukin (IL)-IB, IL-2, IL-6, and tumor necrosis factor (TNF), are found in the ulcerative tissue of RAS patients, which can induce the expression of iNOS and increase NO production.[17,22] In our study, the serum levels of total NOS in Group A were higher when compared with Group B or Group C, but the differences were no statistical significance, which imply that NOS may not play a primary role in the occurrence of RAS.

This study has 2 limitations. First, most of these subjects came from satellite cities, so the results only indicate the serum TAS, NO and NOS levels of the province's adults. Second, several underlying factors were not considered in the study, such as oral habits, sleeping duration, conditions of oral hygiene or periodontal health, occupation and emotional stress index, which will be included in our future study.

In conclusion, the higher NO levels and lower TAS levels in serum of MiRAS patients with active lesion indicate that there may be an increased oxidative stress in RAS. In addition, no significant differences between MiRAS patients in the inactive stage and healthy subjects with regards to all these parameters investigated in this study indicate that the balance of oxidant/antioxidant systems maybe recovered gradually in the inactive stage of MiRAS. To offer an optimal treatment for patients with MiRAS, additional clinical and experimental studies are needed to determine how to shift the oxidative balance to the antioxidant side.

Author contributions

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Supervision: Yi Xue, Guang Chen.
Validation: Zhongyin Wu, Huiqing Fang.
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Writing – review & editing: Qian Zhang, Zhongyin Wu, Huiqing Fang.

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