



ORIGINAL ARTICLE

Assessment of serum malondialdehyde, uric acid, and vitamins C and E levels in patients with recurrent aphthous stomatitis



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KEYWORDS

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Abstract *Background/purpose:* Recurrent aphthous stomatitis (RAS) is a common oral mucosal disease. Recently, oxidative stress has been thought to play a major role in the etio-pathogenesis of RAS. The aim of this investigation was to compare the serum levels of an important oxidant agent [malondialdehyde (MDA)] and nonenzymatic antioxidants [uric acid (UA) and vitamins C and E] in patients with RAS versus healthy individuals.

Materials and methods: Ninety-seven patients with idiopathic minor RAS and 97 race-, age-, and sex-matched healthy individuals were included in this study. All these individuals were allocated to three groups: RAS patients in the active stage (Group A); the same RAS patients in Group A in the remission stage (Group B); and healthy individuals without RAS (Group C). The serum levels of MDA, UA, and vitamins C and E were measured by the spectrophotometric method. Independent sample *t* test and paired *t* test were performed for statistical evaluation. *Results:* Serum MDA level of Group A was significantly higher than that of Group B ($P = 0.040$) or Group C ($P = 0.011$), whereas the serum level of vitamin E was significantly decreased in Group A as compared with Group B ($P = 0.012$) or Group C ($P = 0.001$). No statistically significant differences were found between Group B and Group C in terms of MDA, UA, and vitamins C and E serum levels ($P > 0.05$).

Conclusion: With the double-faced character of oxidant/antioxidant, UA and vitamin C may not play a crucial role in the pathogenesis of RAS. However, MDA and vitamin E can be used as indicators for RAS.

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Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal disorders with prevalence of 25% in the general population,¹ characterized by recurrent and painful ulcers on nonkeratinized oral mucosa.^{2–4} Three clinical types were classified as minor, major, and herpetiform by Stanley in 1972.³ Minor RAS is the most common form, affecting about 80% of RAS patients, and can heal spontaneously within 10–14 days without scarring.⁴

To date, the exact etiology of RAS is still uncertain, but several factors such as trauma, stress, allergies, genetics, infection, and immunology have been implicated.^{2–5} All these factors mentioned are thought to have the ability to disturb the equilibrium of oxidant/antioxidant status and lead to excessive free radical formation, which can lead to mammalian cell damage through its damaging effects on peroxidation of protein, lipid, and DNA.⁶ Malondialdehyde (MDA) is the main product of lipid peroxidation and is used commonly as an oxidative stress indicator.⁷ The human body has its own innate free radical scavengers, which are classified into enzymatic antioxidants and nonenzymatic antioxidants. The latter includes glutathione, vitamins A, C, and E, and uric acid (UA).⁸

According to our knowledge, there are only several articles in the literature about the concentration of nonenzymatic antioxidants in RAS patients with active lesions,^{9,10} but no data concerning those in RAS patients in the remission stage. Therefore, the aim of the present study was to evaluate the serum levels of MDA, UA, and vitamins C and E in RAS patients in the remission stage, and compare them with the same RAS patients in the active phase and healthy individuals to find new information about RAS etiopathology.

Materials and methods

Study population

The present study included 97 patients with RAS who were admitted to the Department of Stomatology of The Third Hospital of Hebei Medical University and 97 race-, age- and sex-matched healthy individuals with no history of any episodes of RAS. The diagnosis of RAS was based on typical patients' history and clinical findings. All patients were evaluated by an expert in oral medicine during the study. The protocol of this study was approved by the Ethics Committee of Bethune International Peace Hospital (Protocol No. 2014-12-9) and informed consent was obtained from each participant.

All RAS patients recruited in the study had at least three recurrences of oral ulcer per year and all of them were idiopathic minor aphthae. The patients were otherwise healthy. The healthy controls did not have any RAS attacks before the study or during their participation in the study. The following routine laboratory tests were performed: complete blood count, erythrocyte sedimentation rate, fasting glucose, liver and kidney function tests, and measurement of thyroid stimulating hormone, electrolytes, calcium, phosphorus, globulin, albumin, serum iron, vitamin B₁₂, and folic acid. The participants with normal

biochemical analysis results mentioned above were included in the study. Individuals with any known history of systemic diseases, smoking habits, and alcohol abuse were excluded from the study. None of the participants was pregnant, nursing, or had any symptoms of active inflammation, and none was under a therapeutic regimen of immunomodulatory agents, steroids, multivitamins, or other antioxidant supplements that could influence the results of tests performed in this study for the previous 3 months.

There were three groups: Group A consisted of 97 patients with active lesions of RAS; Group B comprised the same 97 individuals in Group A in the remission stage of the disease; and Group C included 97 race-, age-, and sex-matched healthy individuals without RAS disease.

Blood samples

Venous blood samples (5 mL) were drawn into vacutainers without any anticoagulant in the early morning after at least 8 hours overnight fasting. Samples were centrifuged at 3000 g for 10 minutes at 4°C to obtain serum and stored at –80°C until being analyzed at the same time.

Measurements

The serum levels of MDA, UA, and vitamins C and E were determined with a spectrophotometer (Genesys 10 UV Scanning UV/VIS Spectrophotometer; Shimadzu, Tokyo, Japan) according to the manufacturer's recommendations of commercial colorimetric assay kits (MDA/UA/vitamin C/vitamin E Detection Kit; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The concentrations of MDA, UA, vitamin C and vitamin E were expressed as nmol/mL, mg/L, µg/mL, and µg/mL, respectively.

Statistical analysis

All values 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. Statistical analysis was performed with SPSS version 19.0 statistical software package (SPSS Inc., Chicago, IL, USA). A *P* value < 0.05 was accepted to be statistically significant.

Results

Ninety-seven RAS patients (55 female and 42 male) with a mean age of 31.4 ± 6.2 years (range 17–42 years) and 97 healthy controls (55 female and 42 male) with a mean age of 30.1 ± 5.8 years (range 19–40 years) were included in the present study. No significant difference was found between patients with RAS and healthy individuals in terms of age (*P* = 0.136).

The serum levels of MDA, UA, and vitamins C and E are presented in Table 1. The serum MDA level of Group A was significantly higher than that of Group B (*P* = 0.040) or Group C (*P* = 0.011), whereas the serum level of vitamin E was significantly decreased in Group A as compared with

Table 1 Serum levels of MDA, UA, and vitamins C and E in RAS patients and control individuals.

Parameters	Group A (n = 97) (Active RAS)	Group B (n = 97) (Remission RAS)	Group C (n = 97) (Control)
MDA (nmol/mL)	5.7 ± 1.8*	4.5 ± 1.2	4.5 ± 0.8
UA (mg/L)	61.4 ± 19.7	66.4 ± 20.8	60.8 ± 10.1
vitamin C (µg/mL)	14.9 ± 2.6	15.6 ± 2.8	15.9 ± 2.4
vitamin E (µg/mL)	6.7 ± 2.5*	8.9 ± 1.6	9.0 ± 1.2

* Significant difference when compared with control group.

MDA = malondialdehyde; RAS = recurrent aphthous stomatitis; UA = uric acid.

Group B ($P = 0.012$) or Group C ($P = 0.001$). No significant differences were found between Group B and Group C with respect to serum MDA, UA, and vitamins C and E levels ($P = 0.966, 0.282, 0.733,$ and 0.864 , respectively). There were no significant differences in serum vitamin C and UA levels between Group A and Group B ($P = 0.651$ and 0.489 , respectively) or between Group A and Group C ($P = 0.313$ and 0.894 , respectively).

Discussion

In the present study, the serum levels of MDA and some nonenzymatic antioxidants (UA and vitamins C and E) were investigated in RAS patients with active lesions, the same RAS patients in the remission stage, and healthy individuals. To the best of our knowledge, there are no previous reports concerning the above indicators in RAS patients in the remission stage.

MDA is an important reactive carbon compound, which is generated during the disintegration of lipid peroxides, and is one of the most frequently used indicators of lipid peroxidation.⁹ As an end-product of peroxidation of membrane lipids, excessive MDA has a toxic effect that leads to changes in proteins, modification of amino-acid chains, and impairment of structure and function of cell membranes.¹¹ In our study, a significantly increased serum MDA level was found in Group A when compared with Group B or Group C, which was in accordance with previous studies.^{7,9,12} However, there was no significant difference between Group B and Group C, which indicated that the antioxidative ability of the organism was elevated for the reason of the self-regulation mechanism, and the balance of oxidant/antioxidant systems was recovered in RAS patients in the remission stage.

As a final oxidation product of purine nucleotides, UA is a powerful scavenger of free radicals in plasma.¹³ However, the antioxidant effects of UA are manifested only in the hydrophilic environment of biological fluids. With the hydrophobic environment created by lipids, UA may become a prooxidant by forming free radicals in reactions with other oxidants, and even could be converted into an oxidant by oxidized lipids.¹⁴ In this study, there was no significant difference in serum UA level between any two groups, which may be due to the oxidant/antioxidant paradox of UA, which indicates that UA may not play a role in the etiology of RAS.

Vitamin E (α -tocopherol) is a major lipid antioxidant in the membranes of cells and subcellular organelles. It can react directly with free radicals and interrupt lipid peroxidation by scavenging the thiobarbituric acid reactive substances.¹⁵ Vitamin C (ascorbic acid) is an important water-soluble vitamin and has a synergistic effect with vitamin E, which can reduce the tocopheroxyl radical, an oxidized form of α -tocopherol, back to α -tocopherol.¹⁶ It is not only a potent antioxidant against free radicals, but also has prooxidant activity as a result of the Fenton reaction; a process in which free radicals are formed.¹⁷ The results reported about the serum levels of vitamins in RAS patients with active lesions were inconsistent. Decreased serum levels of vitamins A, C, and E in RAS patients with active lesion were demonstrated by Saral et al,⁹ whereas a converse conclusion was observed by Khademi et al,¹⁰ who detected no significant differences in vitamins between RAS patients with active lesions and healthy controls. In the current study, the minimum serum vitamin E level was in Group A, which indicated that there was a deficiency of vitamin E during the active phase of RAS. However, there was no significant difference in serum vitamin C level between any two groups, which may be explained by the double-faced character of vitamin C.

There were two limitations to the current study. First, the age of the participants was low, which may have been due to the frequent occurrence of minor RAS in young adults. Second, disease severity was not considered. As the patients with major and herpetiform RAS were excluded, it is uncertain whether the current results obtained would be the same with different types of RAS patients. Future studies with a larger number of patients should be performed to clarify this question.

In conclusion, the elevated serum MDA level and decreased vitamin E level in RAS patients with active lesions indicate that there is increased oxidative stress. Moreover, there were no significant differences between RAS patients in the remission stage and healthy individuals with regards to the parameters investigated in this study, indicating that the balance of oxidant/antioxidant systems is recovered in the remission stage of RAS.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

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