



## Assessment of relation between antioxidants level and clinical manifestation in recurrent aphthous stomatitis

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### ABSTRACT

**Introduction:** Recurrent aphthous stomatitis (RAS) is a common disease with unknown etiology and no treatment. The purpose of this study is to assess the relationship between serum antioxidants level and clinical manifestation. Change in antioxidant level can lead inflammatory reaction through oxidative stress.

**Materials & Methods:** A total 50 patients were selected for this cross sectional study. Superoxide dismutase (SOD), glutathione peroxidase (GSHPX) and total antioxidant status (TAS) was measured in plasma. Also pain intensity based on the visual analogue scale (VAS), the mean interval between lesion, number of lesions and the mean duration of complete healing were recorded. Statistical analysis was performed by using spearman, chi square and T-test.

**Results:** We had 50 patients include 28 female and 22 male. Mean and standard deviation was for TAS (1.35±0.15), GPX (178.42±39.67) and SOD (257.86±63.17). Number of lesion and duration of RAS had direct relationship with pain severity. Pain intensity and GPX and SOD were significant (respectively 0.05, 0.04). GPX and SOD level was indirectly related (p=0.04.)

**Conclusion:** We could show that RAS patients had alteration in SOD and GPX level but TAS level was stable. Against TAS level which had no change, with more lesion and severe pain GPX level is lower and SOD level was higher. These two antioxidants were related indirectly.

**Key Words:** Recurrent aphthous stomatitis, Antioxidants, Symptoms, Superoxide dismutase (SOD), glutathione peroxidase (GSHPX), total antioxidant status (TAS).

### Introduction

Recurrent aphthous stomatitis (RAS) is one of the common oral diseases all around the world with the prevalence between 5-66 % [1,2]. This is a multifactorial disease with unknown exact etiology [3]. New researches suggest that free radicals through oxidative stress have role in its pathogenesis [4].

Free radicals are neutral valance atoms or molecules with one or more unpaired electrons in outer shell which cause radicals to be highly chemically reactive [5]. Oxidative stress occurs when some conditions can change the oxidant/antioxidant balance of organism and concentration of reactive oxy-

gen radicals (ROS) increase over the physiologic values which is life threatening and lead to tissue damage [5]. Researches show that imbalance between free radicals and reactive oxygen radicals probably have main role in presence and progress of oral inflammatory reactions [6]. It seems oxidative stress is effective in presence of RAS with regard to the role of oxidative stress in inflammatory reaction [6]. It seems oxidative stress is effective in presence of RAS with regard to the role of oxidative stress in inflammatory reaction and inflammatory nature of RAS [7] so maybe antioxidants can be useful in symptoms improvement.

## Method and Material

This study is a cross sectional. The sample were selected from patients who applied to outpatient clinic of oral medicine department of Tehran University of Medical Sciences. RAS was diagnosed clinically by an expert in oral medicine. The institutional review board and Ethics Committee of Tehran University of Medical Sciences approved the study protocol and each subject signed a detailed informed consent form.

### Inclusion criteria

- 1- Patients with sufficient literacy and ability to assign the consent.
- 2- History of RAS for at least 3 times a year and being in active phase.

### Exclusion criteria

- 1- Existence of other systemic diseases such as, diabetes, hepatitis, hypertension, cardiac diseases, arthropathy, rheumatic fever, tuberculosis, neurological diseases, jaundice, renal diseases, cerebral attack, aids, dyspnea, pregnancy.
- 2- Use immunosuppressive medication within last year.
- 3- Have diet or use supplements like iron or vitamin.
- 4- Behcet' disease or other vesiculobulluse disease.

After patients were assessed for eligibility to participate in this trial, the severity of pain was recorded by VAS. Demographic information, medical history, frequency and healing period for lesions were taken. Patients were asked to recorded variables included frequency, healing time and number of the lesions. The visual analogue scale (VAS) was used for assessment of pain. Pain scores or symptom stages were used to rank the severity of the subjects' pain and discomfort which ranged from 0 (no pain) to 100mm (severe pain).

Fasting venous blood samples were taken and were centrifuged at 3000 g for 5 min at 4 °C. Plasma was separated and buffy coat was discarded by aspiration. Erythrocytes were washed two times with physiological saline and recentrifuged with the same conditions. After washing 15 ml packed cells were separated from the sediment and transferred into indurf tube. The status of Superoxide dismutase (SOD) was measured by the use of commercially available kit RANSEL. SOD is an accelerator to convert toxic radical into hydrogen peroxide and molecular oxygen. In this method xan-

thin and xanthin oxidase produce superoxide radicals.

The SOD and GSHPx activities of erythrocytes were estimated for the hemoglyzsates by the use of commercially available kit RANSEL (Randox Laboratory Ltd., Ardmore, UK). The erythrocytes were hemolysed by the addition of ice-cold deionized water and vigorously vortexed. SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazoliu-chloride (INT) to form a red formazan dye. The SOD activity was measured by the degree of inhibition of this reaction.

Erythrocyte GSHPx determination was based on the following principle: GSHPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and reduced nicotinamide adenine dinucleotide, phosphate (NADPH) the oxidized glutathione (GSSG) is immediately converted to the reduced form with a contaminant oxidation of NADPH to NADP<sup>+</sup>. The decrease in absorbance at 340 nm was measured by a spectrophotometer [11,12].

Activity of GSHPx was measured by RANSEL kit through measuring the decrease of light absorbance by NADPH consumption. This antibody has been shown to cross-react with human xanthine oxidase. Total blood antioxidant status was measured by spectrophotometry. (Mmol/l). All values were expressed as mean  $\pm$  SD. Statistical analyses were done by Spearman's, t test and chi square.

## Result

There were 50 patients include 28 females (26-55y M=35) and 22 males (19-51y M=35), whose mean age was  $34.5 \pm 8.5$  years.

32 patients (64%) always had ulcer, 5 patients (10%) had ulcer every 2 weeks, 6 patients (12%) had ulcer every months and 7 patients (14%) had ulcer every 2 months.

13 patients (26%) had more than 3 lesions and 74% had 3 or fewer lesion in each lesion. The average healing time was  $11.03 \pm 0.3$  days.

No patients had mild pain and all the patients had moderate to severe pain and 31 patients (62%) had very severe pain.

Mean and standard deviation was for TAS ( $1.35 \pm 0.15$ ), GPX ( $178.42 \pm 39.67$ ) and SOD ( $257.86 \pm 63.17$ ).

SOD and GPX, had inverse relationship. Sex and age had no significant relationship with antioxidants level and clinical manifestation. TAS level was not signifi-

cant.

Qualitative variables and quantitative variables were reported as absolute frequency and average and standard deviation respectively. We used t test for measuring the pain, healing time, frequency and serum antioxidant rate.  $P < 0.05$  considered significant. Number of lesion and duration of RAS had direct relationship with pain severity.

The number of lesions and pain severity had direct relationship with antioxidants. Mean duration between lesions and number of lesions was significantly related ( $p=0.002$ ). The correlation between the number of lesions and pain, GPX, SOD level intensity was significant (respectively  $p=0.005, 0.002, 0.05$ ) and also between duration of lesions and pain intensity is significant ( $p=0.01$ ). Pain intensity and GPX, SOD level was significant (respectively  $0.05, 0.04$ ). GPX and SOD level was indirectly related ( $p=0.04$ ).

### Discussion

Recurrent aphthous stomatitis (RAS) is a common clinical condition producing painful ulcerations in oral cavity without any other component [1]. RAS is more common in 2nd decade of life and in 20s-30s [3]. Some studies showed a little predilection in female [8]. In this study mean age is 35 years old and females are more than males.

The etiology of RAS is not entirely clear [3]. there is no definitive treatment for RAS and supportive treatment is not enough [9]. Recently it was suggested that free radicals through creating oxidative stress take part in the pathogenesis of RAS [4]. besides unbalanced free radicals and reactive oxygen species have main role in onset and progress of oral lesion [6]. oxidative stress has role in inflammatory process and with concern to RAS nature, it seems that oxidative stress can be effective in its pathogenesis [7]. free radicals produce intrinsically through oxidative system, metabolism, inflammatory system and extrinsically through stress, sun, air pollution, professional exercise, x ray exposure, smoking and alcohol. Antioxidants work as network.

Although Gunduz and Azizi [10,11] did not find significant relation between antioxidant status and RAS but Momen-beitollahi, Saral, Karıncaoglu and Cimen showed impaired antioxidant system [12-15] so maybe inhibitors can ameliorate symptoms.

Until this publication, there is no other study in which the relation between antioxidant level and pain was assessed. In this study we investigated whether there is any alteration in SOD, GPX, TAS level in RAS

cases. We found indirect relation between GPX level and severity of pain, but SOD level was related directly. There was not any significant alteration in TAS level. We could show that RAS patients had alteration in SOD and GPX level but TAS level was stable. Against TAS level which had no change, with more lesion and severe pain GPX level is lower and SOD level was higher. These two antioxidants were related indirectly.

### Conclusion

The results of this study showed that changes in antioxidants level can affect in occurrence and severity of sign and symptoms. So we recommend patients to use regiments rich in antioxidants.

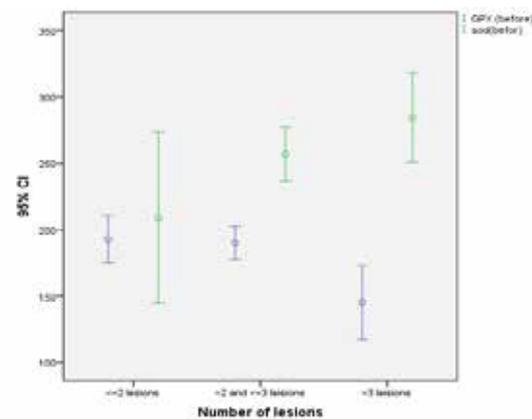


Fig 1: Number of lesion and duration of RAS had direct relationship with pain severity.

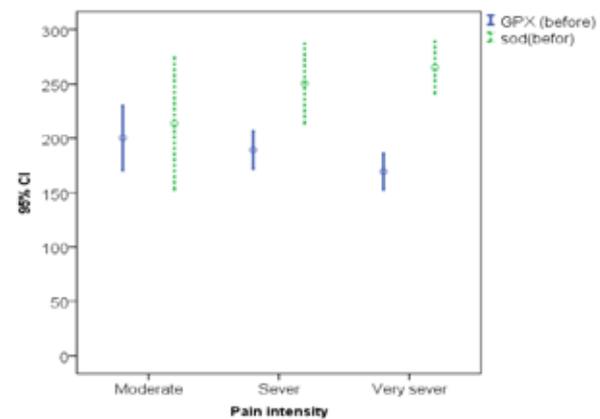


Fig 2: The number of lesions and pain severity had direct relationship with antioxidants.

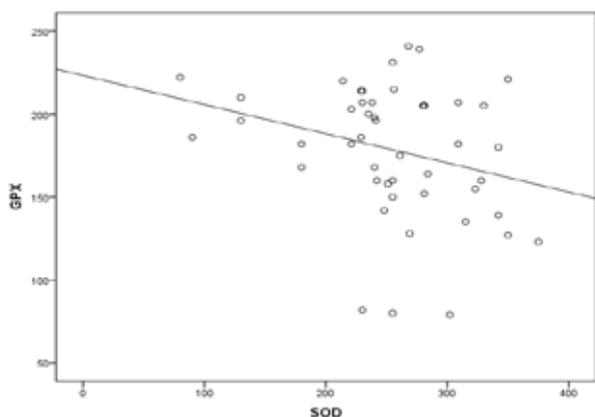


Fig 3.

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