ORIGINAL ARTICLE

Assessment of Serum Enzymatic Antioxidant Levels in Recurrent Aphthous Stomatitis Patients

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ABSTRACT

Objective: The objective of the study was to determine the serum enzymatic antioxidant levels in recurrent aphthous stomatitis patients.

Study Design: This was a case control study.

Place and Duration of Study: The study was conducted in biochemistry department of Islamic International Medical College- Trust (IIMC-T) Rawalpindi from 15 March 2016 to 14 March 2017.

Materials and Methods: The study included 160 subjects divided into two groups Aphthous group and Non-Aphthous group. Aphthous group comprised of 70 recurrent aphthous stomatitis patients with active lesion and 90 age and sex matched healthy controls were included in the non-aphthous group .From all the participants 2 ml of blood was taken. Serum glutathione peroxidase levels were measured using enzyme linked immunosorbent assay technique. This ELISA kit uses Sandwich-ELISA as the method.

Results: The comparison of mean of serum glutathione peroxidase levels between aphthous and non-aphthous group was significant (p<o.oo).This study showed that the levels of glutathione peroxidase (a biological stress marker) were lower in recurrent aphthous stomatitis patients than in control subjects.

Conclusion: Serum enzymatic antioxidant levels were lower in recurrent aphthous stomatitis patients as compared to controls, so oxidative stress plays an important role in the pathogenesis of disease.

Key words: *Glutathione peroxidase, Oxidative stress, Recurrent aphthous stomatitis*

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most commonly encountered pathology of oral mucosa, affecting nearly 20% of world population.[1] Aphthous is originated from “aphtha” a Greek word meaning ulceration.[2, 3, 4, 5] Recurrent aphthous stomatitis is a painful, recurring and distressful experience for the patients because it restricts their normal oral activities.[6,7] Ulcerations can be solitary or multiple and has a shallow necrotizing center[8] and can be classified into three classes depending on their type , size , number and their healing time i-e minor, major and herpetiform.[9] Minor recurrent aphthous stomatitis are the most common type and contributes to 80% of total cases.[10] Many studies have done to find out the causes of recurrent aphthous stomatitis but the exact etiology remains unidentified .Potential factors which have been identified in the onset of disease include hereditary predisposition, local and systemic causes, hematological and micronutrient deficiencies, emotional stress and drug allergies.[11] It has been postulated that all the predisposing factors for the formation of aphthous ulcers act through a common pathway that depends on the oxidative stress by increasing free radical synthesis. Either the enhanced production of reactive oxygen species and/or decreased defense by antioxidants result in oxidative stress that is lethal as it causes tissue damage .[12]

Studies suggest that the lack of balance between reactive oxygen species and antioxidants have a pivotal role in the development and progression of inflammatory reactions, so oxidative stress may have a crucial role in development of recurrent aphthous stomatitis as it is inflammatory in nature.[13] To prevent oxidative stress, mammalian cells have developed antioxidant defense system including both enzymatic and non-enzymatic antioxidants.

Glutathione peroxidase, a selenocysteine containing

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protein is a most potent enzyme in the defense of cells against increased reactive oxygen species. It utilizes its reduced glutathione form to convert hydrogen peroxide and lipid peroxides into alcohols.[14] Gpx-1 is found in all cells, present in mitochondria, cytosole and in few cases in peroxisomes. Studies have shown that it is more efficient in getting rid of intracellular hydrogen peroxide then catalase in a number of physiological situations. Analysis of previous studies indicates that for the activity of Gpx-1, selenium (Se) which is a trace element is required which is present in the form of amino acid Sec. Hence as Gpx-1 enzymatically detoxifies non-radical hydroperoxides, it maintains cellular oxidant/antioxidant status by two ways;

a. Via direct removal of hydroperoxides
b. Secondly through oxidation of reduced form of glutathione i-e GSH [15]Regarding the insufficient studies related to this issue in Pakistan, the present study was carried to evaluate the possible relation between levels of glutathione peroxidase as a biological marker for oxidative stress and recurrent aphthous stomatitis.

Materials and Methods

This case-control study was conducted in Biochemistry department of Islamic International Medical College Trust Rawalpindi in collaboration with dental outpatient department of Pakistan Ordanance Factories Hospital, Wah Cantt from 15 March 2016 to 14 March 2017. Convenient non-probability sampling technique was used .The study population comprised of 160 subjects, 70 patients and 90 controls .Convenient non-probability sampling technique was used. The institutional review board and ethical committee of Riphah University (Islamic International Medical College) approved the study protocol. The selected patients had active lesion of aphthous ulcer and had history of recurring oral ulcer attack at least three times a year, of any age and gender. For control, healthy individuals with no history of any episode of recurrent aphthous stomatitis and any systemic disease were selected. Patients were excluded if they had any history of systemic disease or any other concurrent oral lesions. Patients were excluded if they were on any therapeutic regimen or supplement of iron, multivitamin, steroid or other immunomodulatory agents for past two months. Pregnant and lactating mothers were excluded. Smokers and alcohol users were excluded. After the informed consent of all participants in the study 2 ml of venous blood was collected using sterile disposable syringe and transferred to serum separator tubes. Serum glutathione peroxidase levels were measured by Sandwich Enzyme linked immunosorbent assay technique (Absorbance Micro plate Reader, ELX800, BioTek Instrument, Inc.USA).

The collected data was entered in statistical package for social sciences (SPSS) version 21 for analysis. Gender was expressed as percentages and frequency. Numerical variables like age, glutathione peroxidase were expressed as mean and standard deviation (±SD). Independent t –test was used to determine the significant difference of means between controls and patients.*p* values less than 0.05 were considered as significant.

Results

The Aphthous group had 45 females (64%) and 25 males (36%) while Non-Aphthous group had 54 females (60%) and 36 males (40%).The gender distribution of the both group I and group II is shown in the figure no 1.

Mean age of the Aphthous group was 28.76±9.144 and of the Non-Aphthous group was 30.53±6.404. The comparison of mean ages between the aphthous and non-aphthous group was not significant (p>0.05).

Biochemical findings:

Assessment of oxidative stress marker, Glutathione peroxidase:

With regard to oxidant/antioxidant status, mean of serum glutathione peroxidase in RAS patients was significantly lower (p <o.oo) than that of healthy controls, using t-test.

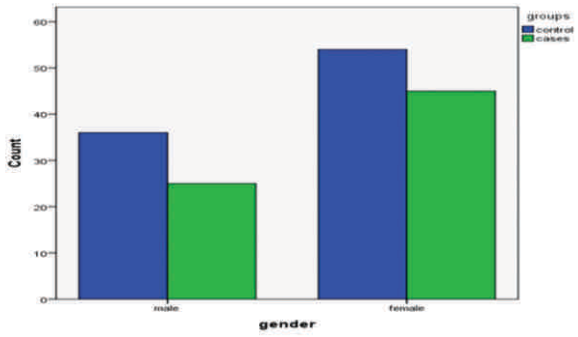

Fig 1: Gender distribution of study population

Table I: Enzymatic antioxidant levels of recurrent aphthous stomatitis patients and controls

Antioxidant parameter	RAS patients Mean±SD	Controls Mean±SD
Glutathione peroxidase -1 (Gpx-1)	733.160 ±220.500	1997.100±75.10

Discussion

Recurrent aphthous stomatitis or canker sores is an inflammatory ulcerative lesion and is one of the most common pathological disease of oral cavity.[1]

Even though extensive research regarding the predisposition of recurrent aphthous stomatitis have been done, the definitive cause of RAS remains unclear. Moreover directly or indirectly different factors involved in the pathogenesis of disease results in the loss of balance between oxidant and antioxidant system of the body. The present study was undertaken to determine serum enzymatic enzymatic antioxidant levels in patients with recurrent aphthous stomatitis and healthy controls.

Loss of balance among oxidant species and antioxidants causes variety of inflammatory oral diseases that varies from simple infections to lethal cancers. Recurrent aphthous stomatitis is also a sign of this imbalance. In several studies a possible relationship between free radical metabolism and inflammatory reaction have been demonstrated.[16] Lih-Brody et al.[17] has found that in mucosa of inflammatory bowel disease patients there is an imbalance between oxidant/antioxidant statuses. Also a study conducted by Kokcam and Naziroglu.[15] on psoriasis patients demonstrated a possible role of increased lipid peroxidation

Oxidative stress increases by the cellular damaging cytotoxic effects of reactive oxygen species. Nowadays in the maintenance of health and prevention from diseases, functional role of antioxidants has received huge attention.

Glutathione peroxidase -1 is the chief antioxidant in getting rid of hydrogen peroxide by utilizing reduced form of glutathione. Elimination of excessive hydrogen peroxide by Gpx-1 is directly related to utilization of reduced form of glutathione resulting in its decreased levels. So in recurrent aphthous stomatitis patients there is a decrease in serum levels of Gpx-1.

In the present study, serum glutathione peroxidase-1, an antioxidant marker showed significantly lower levels in recurrent aphthous stomatitis patients than the controls with a p value of zero.

This result was in agreement with the findings of Arikan et al and Cimen et al.[12] In a study conducted by Arikan, glutathione peroxidase levels were significantly lower in recurrent aphthous stomatitis patients as compared to controls .Results of our study were in contrast to the findings by Saxena who in his study found increased levels of glutathione peroxidase-1 in recurrent aphthous stomatitis patients.[19]

Selenium deficiency can occur with the decreased cellular immunity. By activating T-cell proliferation selenium supplement has an ability to markedly stimulate the immune response. Immunostimulatory role of selenium is supported by the study conducted by McKenzie.et.al who demonstrated that in the presence of oxidative stress selenium supplementation regulates the cellular response by rapidly promoting and activating the cellular antioxidant defense system.[14]

Devasagayam et al postulated that Gpx-1 deficiency causes free radical accumulation.[20]

Regarding the association of antioxidant and recurrent aphthous stomatitis, the disagreement amongst different studies may be due to different factors including different sample size, genetic variation of different population

There is a need to carry out experimental studies to find out the causative relationship between recurrent aphthous stomatitis and impaired oxidant/antioxidant status.

Further prospective studies with larger sample size are also required to identify new preventive and treatment options in recurrent aphthous stomatitis patients.

Conclusion

Glutathione peroxidase levels were significantly lower in recurrent aphthous stomatitis patients as compared to controls, which suggests there is an alteration of oxidant/antioxidant status in RAS patients.

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