Journal of Craniomaxillofacial Research

Vol. 3, No. 1 Winter 2016

Salivary antioxidant levels in pemphigus vulgaris patients compared to the healthy people

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ARTICLE INFO

Article Type:
Original Article

Received: 22 Oct 2015
Revised: 1 Nov 2015
Accepted: 3 Des 2015

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ABSTRACT

Introduction: Pemphigus comprises a group of diseases characterized by cutaneous and mucosal blistering. Pemphigus vulgaris (PV) is the most common and important variant. Recent reports have revealed the relatively high incidence of PV in Iran. The onset or aggravation of many human diseases is attributed to oxidative stress and it has been suggested as one of the several factors responsible for etiopathogenesis of pemphigus.
The present study sought to assess the association of salivary level of antioxidant enzyme glutathione peroxidase (GPx) and total antioxidant level with pemphigus vulgaris.

Materials and Methods: This case-control study evaluated patients referred to Razi Dermatology Hospital in Tehran , Iran from January to November 2011. Thirty patients with early diagnosed PV, who had never been on treatment were enrolled in this study. The diagnosis of PV was confirmed by histopathology and direct immunofluorescence. The control group consisted of 30 age- and sex-matched healthy non-smoker volunteers. Saliva was collected from subjects for evaluation of glutathione peroxidase and total antioxidant levels and comparison with controls. GPX and TAS activity was analyzed by spectrophotometry . Independent samples t-test was applied to compare the means of continuous variables. Statistical analysis was performed using SPSS software. $P \leq 0.05$ was considered significant.

Results: The mean salivary level of glutathione peroxidase was significantly lower in pemphigus patients. No statistically significant difference was observed between the groups in total antioxidant levels of saliva.

Conclusion: Our findings indicated that lower levels of glutathione peroxidase may be associated with pemphigus vulgaris. Salivary GPx level may be used for diagnosis of PV.

Key words: Antioxidant, Pemphigus vulgaris, Glutathione peroxidase.

Introduction

Pemphigus is a rare blistering auto-immune disease affecting the skin and mucosal epithelium [1]. Different types of pemphigus include pemphigus vulgaris, pemphigus vegetans, paraneoplastic pemphigus, drug-related pemphigus, and IgA pemphigus [2]. Pemphigus vulgaris (PV) as the most common type of pemphigus [3] has the annual incidence rate of 1/100,000 in Iran. The average age of onset of disease is 42 years with a female/male ratio of 1.5 to1 [4].

Blisters result from loss of contact between epithelial cells due to the production of autoantibodies against the components of cellular epithelial junctions especially Cadherin and Desmoglein [2]. These intramembranous glycoproteins are present in desmosomes and strengthen intercellular junctions. Loss of these attachments due to antigen-antibody reactions results in loosening and eventual separation of attachments between cells which is clinically manifested as blistering and vesiculation. Desmoglein 3 is mainly presented in oral epithelium and thus, mucosal involvement is the first clinical finding in 74% of patients [5,6]. In general, 80-90% of PV patients have oral involvement in their course of disease and oral lesions are the first sign of disease in 60% of patients [5].

It has recently been suggested that in pemphigus vulgaris, increased production of oxygen free radicals by activated neutrophils as well as reduced amount of vitamins and antioxidant enzymes in plasma and red blood cells lead to a condition called "oxidative stress"[7].

Several researchers have shown that the imbalance between free radicals and reactive oxygen species plays a major role in initiation and progression of oral inflammatory lesions [8]. Free radicals have harmful effects on mammalian cells due to the peroxidation of two-chain fatty acids, protein and DNA as well as increasing oxidative stress [9,10]. Since free radical damage may be life-threatening, human body has several protective pathways including superoxide dismutase, catalase, glutathione peroxidase (GPx), vitamins A, C and E, melatonin and uric acid [11-14].

In order to diagnose PV, skin or mucosal biopsy for histologic and immunologic examination is necessary [15]. Although Suprabasal acantholysis and vesicle formation are highly suggestive of PV, definite diagnosis is made through inspection of IgG deposits in intercellular epidermal spaces [16].

Topical and systemic corticosteroids, especially prednisolone, are used to manage mild to moderate forms of disease [2]. In severe forms of PV, immunosuppressive drugs such as azathioprine, cyclophosphamide and mycophenolate mofetil are also used as adjuvant drugs. Newer biologic drugs such as Rituximab and anti-CD20 antibodies are prescribed as well [17]. Chronic use of corticosteroids leads to several side effects including osteoporosis, weight gain, peptic ulcer, adrenal suppression, diabetes mellitus, increased susceptibility to infections and severe mood changes [18]. Thus, finding new approaches to treatment of pemphigus will be beneficial for patients.

Unlike other investigations in the field of PV, no study has evaluated salivary antioxidant levels in PV patients. In this study, we evaluated and compared antioxidant levels in PV patients and healthy controls.

Methods and Materials

This was an analytical case-control study. The study subjects were selected among patients presenting to Razi Hospital from February 2011 to April 2011. Thirty patients with newly diagnosed pemphigus vulgaris and 30 age- and sex-matched healthy controls enrolled in this study. Patients with autoimmune diseases other than PV, those using immunosuppressive drugs or nutritional supplements, pregnant women, and tobacco users were excluded from the study.

The Ethics Committee of Tehran University of Medical Sciences approved this study and all participants provided written informed consent. The patients were asked not to eat, drink or brush their teeth for at least 90 minutes before obtaining salivary samples. "Spitting" saliva collection method was taught to patients. The patients were asked to sit in a comfortable position for 5 minutes and spit saliva in a plastic container. Saliva samples with a volume of 3 ml were collected and prepared for laboratory tests. All samplings were carried out between 10am and 12am.

We centrifuged the samples at 3000 g for 10 minutes and then stored them at -70°C before laboratory testing. Activity of glutathione peroxidase (GPx) and total antioxidant status (TAS) of saliva were measured by spectrophotometric method using Ransel and TAS kits recipe (Randox Laboratories).

Statistical analysis

We used SPSS version 11.5 software for statistical analysis. Quantitative data were reported as mean and standard deviation. Paired t-test served to compare the means between the two groups (level of significance<0.05).

Results

The mean age of PV patients (18 women, 12 men) was 49 yrs. (SD ±12) and no significant difference existed in age between the case and control groups.

The mean GPx level was 114.30 (SD±8.23) in the case and 121.87 (SD± 8.58) in the control groups, respectively. The difference between the two groups in this regard was statistically significant (p= 0. 001) (Fig.1). No significant difference was found in mean TAS between the case (p= 0.33, SD±0.16) and the control (p= 0.32, SD±0. 17) groups (Fig. 2).

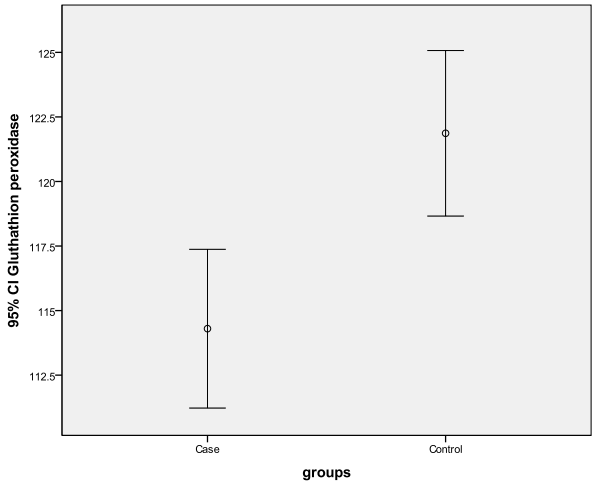

Figure 1. Salivary levels of antioxidant enzyme glutathione peroxidase in pemphigus vulgaris patients compared to healthy people.

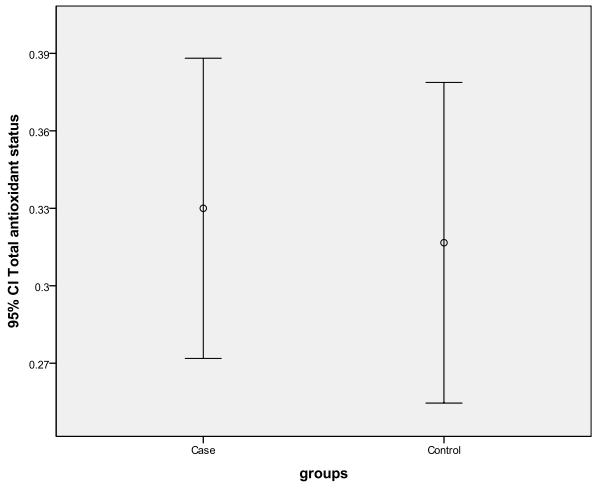

Figure 2. Total antioxidant levels of saliva in pemphigus vulgaris patients compared to healthy people.

Discussion

Pemphigus comprises a group of potentially life-threatening diseases that are characterized by cutaneous and mucosal blistering .PV is the most common and important variant .Recent reports have revealed the relatively high incidence of PV in Iran.The interaction between oxidative stress and inflammatory responses has received special attention in the past decade and it is now considered as one of the several factors responsible for the etiopathogenesis of pemphigus.Antioxidant enzymes like GPx are the first line of cellular defense against toxic free radicals. These enzymes react directly with free oxygen radicals to yield non-radical products. There are increasing evidences that support the role of oxidative stress in several human pathological conditions. It seems that only a few studies have been interested in the association of salivary levels of antioxidant enzymes with pemphigus vulgaris. To the best of our knowledge, there is no publication on the salivary level of GPx in PV patients.

This study was conducted on 30 patients with early diagnosed PV, registered at the Department of Dermatology, Razi Hospital in Tehran, Iran. The control group consisted of 30 age- and sex-matched healthy non-smoker volunteers. The present study was conducted to analyze the association of salivary levels of antioxidant enzyme glutathione peroxidase (GPx) and total antioxidant level with pemphigus vulgaris.

In the current study, the mean salivary level of glutathione peroxidase was significantly lower in pemphigus patients.

In a recent study by Yazdanpanah et al, in Iran, zinc and copper levels were measured in patients with pemphigus vulgaris. Also, oxidative stress was measured using the new method PAB. In this study, 25 PV patients and 25 healthy sex- and age-matched controls were evaluated and PAB was measured in the two groups. Results showed that PAB values were significantly higher in patients compared to controls. Authors concluded that increased oxidative stress and decreased serum concentrations of zinc and copper may be associated with pemphigus vulgaris [19].

In a recent study by Yousefi et al, in Iran, antioxidant levels were measured in patients with pemphigus vulgaris. Thirty PV patients and 30 healthy controls participated in their study. Venous blood samples were collected to determine plasma levels of glutathione peroxidase, vitamin C, selenium, bilirubin, and uric acid. In their study, the mean uric acid levels in patients were significantly lower than in controls. Concentrations of other antioxidants were not significantly different between the two groups. Uric acid concentration in patients with mucosal involvement was significantly lower than in patients with mucocutaneous involvement. They introduced uric acid as an antioxidant involved in pemphigus disease. Also, they were the first to assess the role of selenium in patients with pemphigus vulgaris [20]. Naziroglu et al, in 2003 measured the levels of lipid

oxidation (which represents the amount of produced reactive oxygen species), antioxidant vitamins, reduced glutathione, glutathione peroxidase (GPx) and catalase enzyme activity in blood samples. Levels of lipid peroxidation (malondialdehyde) in plasma and red blood cells of patients with pemphigus vulgaris were significantly higher compared to the control group. Plasma concentrations of antioxidant vitamins (vitamin E and beta carotene) and vitamin A, antioxidant enzymes (catalase in erythrocytes and plasma, glutathione peroxidase in red blood cells) and reduced glutathione activity in red blood cells and plasma were significantly lower in the patients group. The concentration of glutathione peroxidase in plasma showed no significant difference between the two groups. These findings provide evidence for the potential role of increased amounts of lipid peroxidation and decreased levels of antioxidants in the inflammatory nature of pemphigus vulgaris.

It is not clear whether the increased oxidative stress is secondary to inflammatory reactions in pemphigus or it is one of the causes of disease [7]. Although the pathophysiology of pemphigus is mainly known and the interaction between genes and autoantibody against Desmoglein is the first line hypothesis in pathophysiology of disease, role of antioxidants and oxidative stress status in pemphigus lesions have been questioned. Naziroglu et al. proposed that decreased activity of glutathione peroxidase and catalase in erythrocytes of patients may result from enhanced enzyme degradation due to increased levels of free radicals such as hydrogen peroxide. Reduced activity of glutathione peroxidase and catalase in erythrocytes may be an outcome of decreased synthesis or inhibition of these enzymes by some inhibitory substances in patients' blood [7].
In this study, Glutathione peroxidase was measured in salivary samples of PV patients for the first time and its mean values in the patients group were significantly lower than in the control group. This difference may indicate an imbalance and increase in levels of antioxidants and free radicals in PV patients. It is likely that increased production of free radicals during inflammatory process leads to a reduction in amounts of antioxidants, or, conversely, factors that reduce the levels of antioxidants and produce oxidative stress may accelerate the process of inflammation. Total antioxidant status of saliva in our study showed no significant difference between the two groups and therefore, we recommend future studies with a larger sample size to further examine this variable. We found 3 studies that have evaluated the antioxidant status in patients with pemphigus vulgaris, supporting the hypothesis that antioxidant defenses are altered in patients with PV. We think there are reasonable grounds to suggest that antioxidant defense Possible mechanisms behind lower serum levels of GPx in this disease and whether these changes should be considered as risk factors for pemphigus vulgaris render further investigations.

Also, the results of this study may lead to an increase in use of dietary antioxidants or antioxidant drugs in order to reduce noticeable side effects of long-term corticosteroid use.

Acknowledgments

This research project was financially supported by the Research Council of Pardis International Dentistry Faculty of Tehran University of Medical Sciences. The authors would like to thank Mrs. Faeze Amiri for her assistance in editing the manuscript.

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Please cite this paper as:
Najafi Sh, Bahrami N, Esmaili N, Mohammadzadeh M, Gholizadeh N, Mahdavi S, Mohamadnia A; Salivary antioxidant levels in pemphigus vulgaris patients compared to the healthy people. J Craniomaxillofac Res 2016; 3(1): 161-165