

ORIGINAL ARTICLE

Oxidative stress index may play a key role in patients with pemphigus vulgaris

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Abstract

Background Increased reactive oxygen species (ROS) and lipid peroxidation are seen in many dermatologic disorders, including atopic dermatitis, psoriasis, vitiligo, acne vulgaris, pemphigus vulgaris (PV), lichen planus and alopecia areata. In PV, the increased production of ROS from activated neutrophils reduces the concentrations of antioxidant vitamins and enzymes.

Objective The present study aims to determine the oxidative stress index (OSI) by studying serum total oxidant capacity (TOC), lipid hydroperoxide (LOOH) and total antioxidant capacity (TAC) in PV patients.

Methods The study included 27 PV patients and a control group consisting of 24 healthy volunteers. Serum TOC, LOOH and TAC levels were examined and OSI was measured in the PV patients and the control group.

Results TOC ($P = 0.001$) and LOOH ($P = 0.001$) levels as well as OSI ($P = 0.001$) were found higher in the PV patients, relative to the control group. Serum TAS ($P = 0.221$) did not differ between groups. Serum TOC, LOOH and TAC levels and OSI in PV patients with mucosal involvement were not different than those in mucocutaneous PV patients.

Conclusion Serum TOC and LOOH levels and OSI were higher in the PV patients, in comparison with the control group. Serum TAC level was not different.

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Conflict of interest

None.

Introduction

Pemphigus vulgaris (PV) is an autoimmune bullous dermatosis resulting from autoantibodies that develop against the desmosomal proteins of the squamous epithelium and characterized by intra-epithelial bullae on the skin and mucosa.^{1,2} Although it is seen in all ethnic groups and races, it has a high incidence among Jews, and particularly Ashkenazi Jews. The disease does not discriminate between the genders and occurs most commonly in the 4th to 6th decades of life.³ Due to the interaction of environmental and genetic factors, PV has a complex aetiopathogenesis.¹ Although its prognosis has markedly improved after oral corticosteroids and immunosuppressant agents became available, PV still has a high morbidity and mortality.⁴

Activation of neutrophils results in the production of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), hydroxyl radicals and superoxide radicals.⁵ ROS are produced in metabolic and physiological processes and cause harmful oxidative reactions in the organism. In certain cases, increase in oxidants and decrease in antioxidants is inevitable and this leads to a shift in the

oxidant/antioxidant balance in favour of the oxidant side, resulting in oxidative stress.^{6,7}

The skin has an extensive antioxidant system consisting of an enzymatic and non-enzymatic antioxidant network. Enzymatic antioxidants include glutathione peroxidase, catalase and superoxide dismutase. Intra-cellular non-enzymatic antioxidants, on the other hand, consist of α -tocopherol, ubiquinon, β -carotene, ascorbate and glutathione.¹⁰ Cellular redox environment plays a significant role in the skin homeostasis and serves a balancing function in both pro-oxidant and antioxidant stimulations in cutaneous diseases. The skin is extensively exposed to endogenous and exogenous pro-oxidant agents.⁸ Increased ROS and lipid peroxidation are seen in many dermatologic disorders, including atopic dermatitis, psoriasis, vitiligo, acne vulgaris, PV, lichen planus, and alopecia areata. ROS and IgG-associated proteinases cause damage in the basal membrane and thus lead to the separation of the dermo-epidermal junction.⁹ Antioxidants are physiological defense mechanisms in alleviating ROS toxicity in human skin problems.^{8,10,11}

There are various studies reporting that ROS production increased, resulting in damage to the epithelial cells in PV.^{7,10,12,13} Increased production of ROS by activated neutrophils and decreased concentrations of plasma and RBC antioxidant vitamins and enzymes lead to oxidative stress.¹⁰ In the light of these data, the present study aims to determine the oxidative stress index (OSI) by studying serum total oxidant capacity (TOC), lipid hydroperoxide (LOOH) and total antioxidant capacity (TAC) levels in PV patients.

Materials and methods

Subjects

The study included 27 patients with PV and a control group consisting of 24 healthy volunteers. Whole blood samples were collected from the participants in the patient and control groups. Serum TOC, LOOH and TAC levels were studied and OSI was measured in both the patient and control groups. The study protocol followed the Declaration of Helsinki and included written consent by the parents or guardians of the participating patients. The study was approved by the Clinical Research Ethics Committee. All subjects were informed about the study protocol and written consents were obtained from all participants.

Exclusion criteria

Exclusion criteria included the presence of chronic disease, concomitant inflammatory disease, such as infections and autoimmune disorders, immuno-compromised patients, diabetes mellitus, familial hypercholesterolaemia, neoplastic diseases, liver and kidney diseases and recent major surgical procedure. Patients who were recently diagnosed with PV and whose treatment has not been started yet were sought. Patients who were on antioxidant drugs, vitamins, diuretics, hormone replacement therapy, those who used alcohol and those who smoked were also excluded.

Measurement of total antioxidant capacity (TAC)

Serum TAC was determined using a novel automated measurement method, developed by Erel.¹⁴ In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution which is present in Reagent 1 is mixed with H₂O₂ which is present in Reagent 2. The sequentially produced radicals, such as brown coloured dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, the antioxidative effect of the sample serum against the potent-free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has excellent precision values of lower than 3%. The results are expressed as mmol Trolox eq/L.¹⁵

Measurement of total oxidant capacity TOC

The TOC of serum was determined using a novel automated measurement method, developed by Erel.¹⁶ Oxidants present in the

sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with H₂O₂ and the results are expressed in terms of $\mu\text{mol H}_2\text{O}_2 \text{ eq/L}$.¹⁵

Measurement of total LOOH

Serum LOOH levels were measured with the ferrous ion oxidation-xylenol orange (FOX-2) assay. The principle of the assay depends on the oxidation of ferrous ion to ferric ion *via* various oxidants and the ferric ion produced is measured with xylenol orange. Serum are reduced by triphenyl phosphine (TPP), which is a specific reductant for lipids. The difference with and without TPP pretreatment gives LOOH levels.¹⁵

Measurement of oxidative stress index

The per cent ratio of the TOC to the TAC gave the OSI, an indicator of the degree of oxidative stress. To perform the calculation, the result unit of serum TOC, mmol Trolox eq/L, was converted to $\mu\text{mol eq/L}$ and the OSI value was calculated by the formula: $\text{OSI (arbitrary unit)} = \text{TOC } (\mu\text{mol H}_2\text{O}_2 \text{ eq/L}) / 10 \times \text{TAC (mmol Trolox eq/L)}$.⁶

Statistical analysis

All analyses were conducted using SPSS 11.5 statistical program (SPSS for Windows 11.5, Chicago, IL, USA). The normality of distributions was evaluated with the Kolmogorov–Smirnov test. Results were expressed as mean \pm SD. Differences were considered significant at a probability level of $P < 0.05$. Student's *t*-test was used to compare patient and control groups. Mann–Whitney *U*-test was employed in the comparison between the clinical forms in the patient group.

Results

The patient group consisted of 10 male and 17 female PV patients. Mean age of the patients was 32.7 ± 16.9 years. The mean age in the control group, which consisted of 9 male and 15 female healthy volunteers, was 27.2 ± 17.0 years. Of the PV patients

Table 1 Mean (SD) serum TOC, TAC and LOOH levels and OSI in PV patients and control group

	PV patients (n = 27)	Controls (n = 24)	P value
TAC	1.13 \pm 0.27	1.09 \pm 0.33	0.221
LOOH	75.63 \pm 36.19	7.95 \pm 67.59	0.001
TOC	99.68 \pm 44.95	13.48 \pm 96.18	0.001
OSI	9.61 \pm 3.82	1.15 \pm 9.08	0.001

TAC total antioxidant capacity TOC total oxidant capacity LOOH, lipid hydroperoxide; OSI, oxidative stress index; PV, pemphigus vulgaris. Bold values ($P < 0.05$) were considered statistically significant.

Table 2 Mean (SD) serum TOC, TAC and LOOH levels and OSI in patients with mucosal and mucocutaneous involvement

	Mucosal (n = 8)	Mucocutaneous (n = 18)	P value
TAC	1.01 + 0.30	1.14 + 0.26	0.559
LOOH	93.3 + 46.6	69.9 + 27.87	0.672
TOC	121.9 + 49.9	93.7 + 33.2	0.447
OSI	11.1 + 4.76	88.7 + 3.6	0.197

TAC total antioxidant capacity TOC, total oxidant capacity LOOH, lipid hydroperoxide; OSI, oxidative stress index; PV, pemphigus vulgaris.

33.34% ($n = 9$) had only mucosal involvement, whereas 66.66% ($n = 18$) had mucocutaneous involvement. There was no case with only cutaneous involvement among the PV patients. As shown in Table 1, serum TOC ($P = 0.001$) and LOOH ($P = 0.001$) levels in the study group were found higher than those in the control group. Serum TAC ($P = 0.221$) level of the study group was not different than that of the control group. OSI, which is determined on the basis of serum TOC/TAC ratio, was established to be higher in the study group, relative to the control group ($P = 0.001$). Serum TOC ($P = 0.559$), LOOH ($P = 0.672$) and TAC ($P = 0.447$) levels, as well as OSI ($P = 0.197$) in PV patients with mucosal involvement were not different than those in the mucocutaneous PV patients (Table 2).

Discussion

The ROS has an important role in the inflammation process. In PV, increased production of ROS leads to a decline of antioxidants in plasma and red blood cells which results in oxidative stress.¹³ Inflammatory pathways lead to neutrophil activation and release of ROS, which mostly implicate their adverse effect through lipid peroxidation, inflammatory cytokines and the disruption of dermal-epidermal junction in PV.^{8,10,11,13} Although the pathophysiology of PV is nearly understood and the interaction between desmoglein as autoantigens and autoantibodies is in the prior line of the hypothesis, the role of the decline in antioxidants and redox state sequencing further oxidative damage in the formation of PV lesions remains unknown.¹³

Naziroğlu *et al.* found an increase in plasma oxidant (MDA) levels together with a decrease in plasma antioxidant (GSHPx, GSH and catalase) levels in PV patients.¹⁰ Similarly, Yousefi and colleagues reported that glutathione peroxidase and various antioxidant enzymes and vitamins were low in patients with PV.¹³ In our study, serum TOC and LOOH, as well as OSI were higher in the PV patients relative to the control group and TAS level was not different. In this respect, our study is consistent with the two foregoing studies.

Yousefi *et al.* did not find any difference between PV patients with mucosal and mucocutaneous involvement in terms of antiox-

idants.¹³ Likewise, in our study there was no difference between serum TOC, LOOH and TAC levels and OSI of PV patients with mucosal involvement and those of the patients with mucocutaneous involvement.

In conclusion, high serum TOC and LOOH levels and low serum TAC in PV patients cause the pro-oxidant/antioxidant balance to shift towards the oxidant side and this shift results in oxidative stress. Elevated serum oxidant levels and OSI may contribute to the explanation of the peroxidation of structural proteins and lipids that may arise in PV patients and the inflammation in the pathogenesis of the disease. Further and more comprehensive studies on oxidants and antioxidants with more patients may provide new information about the aetiopathogenesis of PV disease and present new alternatives in its treatment.

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