

INVESTIGATIVE REPORT

Lipid Peroxidation and Antioxidant Defence System in Patients with Active or Inactive Behçet's Disease

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To evaluate plasma lipid peroxidation and enzymatic and non-enzymatic antioxidant systems in patients with Behçet's disease, plasma malondialdehyde levels and total antioxidant status, erythrocyte superoxide dismutase and whole blood glutathione peroxidase activities were studied in 15 patients with active disease and in 30 with inactive disease, and compared with 20 age-matched healthy control subjects. Plasma malondialdehyde levels were significantly higher in patients with active Behçet's disease than in patients with inactive disease, who had significantly higher levels than control subjects. The plasma total antioxidant status of both groups of patients was significantly lower than that of controls. Furthermore, whole blood glutathione peroxidase activity was significantly lower in patients with active versus inactive Behçet's disease. There were no significant differences in erythrocyte superoxide dismutase levels between the groups. In conclusion, there is an increase in oxidative stress in Behçet's disease. Despite this stress, the antioxidant system is deficient and inadequate, especially in patients who are in an active phase of the disease. **Key words:** glutathione peroxidase; malondialdehyde; superoxide dismutase.

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Behçet's disease (BD) is defined as a triad of aphthous lesions in the oral mucosa, uveitis and genital ulcerations. Recent studies have shown that the condition is a multi-systemic disorder characterized by recurrent systemic inflammation involving the vascular bed in particular. Although it has been suggested that bacterial and viral infections, as well as genetic, environmental and immunological factors, may play a role in the aetiology of the disease, its actual cause is still unknown. Histopathology of BD is characterized by increased neutrophil infiltration into the perivascular area (1). A large number of abnormalities, such as an

increase in one or more of the following, have been observed: neutrophil functions, chemotaxis (2, 3), *in vivo* leucocyte migration (4), phagocytic activity (2, 4), lysosomal enzyme production (5), and superoxide radical anion (O_2^-) production. Moreover, superoxide radical-retaining activity has been shown to be decreased in both neutrophils (6) and plasma (7).

In addition to studies showing that neutrophil-related tissue damage is associated with lysosomal degranulation (8), there are others showing that tissue damage, particularly damage involving endothelial tissue, is caused mainly by neutrophil-related oxygen derivatives, oxidase lipids and lipoproteins that form peroxides, which in turn damage the cell membrane and its components.

A limited number of studies conducted in recent years have shown that patients with BD have decreased levels of polymorphonuclear leucocyte (PMNL) (9), erythrocyte (10) and plasma (11, 12) antioxidant enzymes. As a result, free oxygen radicals may increase and contribute to tissue damage.

In the present study, we set out to examine antioxidant enzymes and lipid peroxidation in patients with both active and inactive BD, as well as total antioxidant status (TAS) of plasma and non-enzymatic antioxidant parameters of albumin, glucose, uric acid and total bilirubin levels, in order to establish the usefulness of these parameters as non-invasive indicators in the diagnosis and prognosis of BD.

MATERIALS AND METHODS

Subjects

The study group included 45 patients with BD (16 men and 29 women; mean age 33 ± 8.1 years; range 20–49 years) and 20 healthy control subjects (7 men and 13 women; mean age 33 ± 6.2 years, age range 23–44 years). Thirty patients were in an inactive period (10 men and 20 women; mean age 33 ± 8.3 years; age range 21–49 years) while 15 were in an active period (6 men and 9 women; mean age 32.2 ± 8.0 years; age range 20–47 years).

The diagnosis of BD was made according to the criteria of the International Study Group for Behçet's disease (13). Due attention was exercised to ensure that the study patients did not have any other systemic disease or were not receiving any systemic medication.

Biochemical procedures

Following 12 h of fasting, 3 sets of venous blood samples were collected from both patient and control groups: (1) a 10 ml heparinized sample for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), TAS; (2) a 1 ml sample with K3 EDTA for haemoglobin, and (3) a 2 ml sample without anticoagulants for albumin, glucose, uric acid and total bilirubin.

Plasma MDA concentrations were determined using the methods of Yoshioka et al. (14) and the results were calculated in nmol/l using a molar absorptivity for MDA of $1.56 \times 10^5 \times \text{m}^{-1} \times \text{cm}^{-1}$.

Measurement of erythrocyte SOD activity was performed following the methods of McCord & Fridovich (15) using the Ransod Kit of Randox Company and Cobas Mira (Roche) autoanalyzer. By dividing the value obtained (U/ml) by, for example, the value for haemoglobin (g/ml), erythrocyte SOD activity was represented in U/gHb.

Measurement of GSH-Px activity was performed following the methods of Paglia & Valentine (16) using the Ransel Kit of the Randox Company and the RA-XT (Technicon) autoanalyzer. The result was presented in U/l. Measurement of TAS was performed using the Ransod Kit and RA-XT (Technicon) autoanalyzer (17) and the results presented in mmol/l. Levels of serum albumin, glucose, uric acid and total bilirubin were determined by a photometric method (the Olympus AU5200 autoanalyzer).

Statistical analysis

Significance of differences was evaluated using the Mann-Whitney U test and relationships among the variables were assessed by the mean of Pearson's product moment (*r*) correlation coefficients.

RESULTS

Parameters of BD in both the active and inactive phase and in control subjects are given in Table I. Plasma MDA concentration was significantly higher in the inactive and active patient groups and in the total patient population

compared to the control group ($p < 0.001$). Plasma MDA concentration was significantly higher in the active patient group than in the inactive patient group ($p < 0.01$). In contrast, there were no significant differences in erythrocyte SOD level.

We observed that whole blood GSH-Px activity showed no statistically significant differences between the control group and the active, inactive and total patient groups, whereas GSH-Px activity of the active patient group was significantly ($p < 0.05$) lower than that of the inactive patient group. Plasma TAS values were significantly lower in the active, inactive and total patient groups than in the control group (for each group, $p < 0.001$). However, there were no significant differences between active and inactive patient groups. Haemoglobin levels were reduced in the inactive and total patient groups compared to the control group ($p < 0.05$). Serum albumin levels were significantly lower in the active, inactive and total patient groups than in the control group ($p < 0.01$, $p < 0.001$ and $p < 0.001$, respectively). No significant differences were observed between the groups in terms of serum glucose, uric acid and total bilirubin levels.

Negative correlations in the order of 0.254–0.316 were found between plasma MDA levels and GSH-Px, SOD, TAS, uric acid and albumin levels in the group with BD ($p < 0.05$).

DISCUSSION

Recent studies on the aetiopathogenesis of BD have focused on oxidative stress and the antioxidant system. It was found that the PMNLs in peripheral blood of patients with BD were activated by an unknown cause to produce excessive amounts of superoxides, and, as a

Table I. Concentrations of malondialdehyde (MDA), total antioxidant status (TAS) and non-enzymatic antioxidants and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in patients with active and inactive Behçet's disease and healthy control subjects (mean $\pm$ SD)

Variables	Active (<i>n</i> = 15)	Inactive (<i>n</i> = 30)	Total (<i>n</i> = 45)	Healthy controls (<i>n</i> = 20)
Male/female	6M/9F	10M/20F	16M/29F	7M/13F
Age (years)	32.2 $\pm$ 8.0	33.6 $\pm$ 8.3	33.1 $\pm$ 8.1	33.9 $\pm$ 6.2
MDA (nmol/ml)	4.02 $\pm$ 0.44 ^a	3.10 $\pm$ 0.32 ^b	3.41 $\pm$ 0.56 ^c	2.73 $\pm$ 0.37
GSH-Px (U/l)	6776 $\pm$ 1511 ^d	8115 $\pm$ 1778	7668 $\pm$ 1793	7556 $\pm$ 1817
SOD (U/gHb)	1004 $\pm$ 110	1094 $\pm$ 216	1064 $\pm$ 191	985 $\pm$ 178
TAS (mmol/ml)	1.19 $\pm$ 0.03 ^c	1.22 $\pm$ 0.05 ^c	1.21 $\pm$ 0.05 ^c	1.72 $\pm$ 0.27
Albumin (g/dl)	4.40 $\pm$ 0.25 ^c	4.61 $\pm$ 0.41 ^b	4.54 $\pm$ 0.37 ^c	4.93 $\pm$ 0.35
Glucose (mg/dl)	83.9 $\pm$ 9.4	81.3 $\pm$ 10.	82.2 $\pm$ 9.8	81.2 $\pm$ 6.87
Uric acid (mg/dl)	3.93 $\pm$ 1.58	4.40 $\pm$ 0.89	4.24 $\pm$ 1.16	4.50 $\pm$ 1.27
Bilirubin (mg/dl)	0.51 $\pm$ 0.16	0.52 $\pm$ 0.12	0.51 $\pm$ 0.13	0.59 $\pm$ 0.17
Haemoglobin (g/dl)	12.8 $\pm$ 0.82	12.3 $\pm$ 1.42 ^c	12.5 $\pm$ 1.26 ^c	13.3 $\pm$ 1.30

^a $p < 0.001$ compared with inactive BD and control subjects. ^b $p < 0.01$ compared with control subjects. ^c $p < 0.001$ compared with control subjects.

^d $p < 0.05$ compared with inactive BD. ^e $p < 0.05$ compared with control subjects.

M: Male, F: Female.

result, functional disorders such as chemotaxis and an increase in phagocytosis occurred (5, 18). One group of investigators stated that the tissue damage seen in BD that is associated with acute inflammatory reactions was probably associated with an excessive release of lysosomal enzymes of neutrophils into the extracellular milieu (18, 19). Another group related this to the excessive production of reactive oxygen species (ROS) by neutrophils stimulated by pathogens, endotoxins or other inflammation mediators (5, 6). Excessive amounts of reactive oxygen species cause lipid peroxidation. The source of plasma MDA concentration is the non-enzymatic oxidative lipid decomposition.

In our study we found that plasma MDA levels in all patients with BD were significantly higher than those of the control group. The levels in the active and inactive groups were significantly higher than the levels in the control group. The MDA levels in the active group were also significantly higher than the levels in the inactive group. Kose et al. (11) found that MDA levels were higher in Behçet's disease patients than in the control group. In another study, they found that levels of plasma and erythrocyte MDA in patients with BD were higher than those of the control group, but they did not compare active and inactive patients with BD (20). Kose et al. (21) also determined the antioxidant effect of ginkgo biloba extract (Egb 761) on lipoperoxidation induced by hydrogen peroxide in erythrocytes of patients with BD. They found that the erythrocyte MDA levels in patients with BD who had taken Egb 761 were decreased. They suggested that (i) oxidative damage is present in erythrocytes obtained from patients with BD, and (ii) ginkgo biloba extract, which may strengthen the antioxidant defence system, may contribute to the treatment of BD. Orem et al. (12) found that (i) MDA levels in the active group were higher than those in the inactive group, and (ii) the levels in the inactive BD group were higher than those of the control group. Evereklioglu et al. (22) determined serum cytokine and malondialdehyde levels in patients with BD and found that serum cytokine and MDA levels were significantly higher in active BD than in the inactive period. The MDA levels we observed are consistent with the results reported in the literature. Our results suggest that increased lipid peroxidation might be responsible for generalized tissue damage (especially the endothelial damage) seen in patients with Behçet's disease.

We found that TAS of plasma was significantly lower in all Behçet groups when compared with the TAS found in the control group. Like us, Orem et al. (23) found that levels of TAS were significantly higher in healthy subjects. However, we found no significant difference between active patients and inactive patients. Unlike us, Pronai & Arimori (7) reported that the superoxide radical binding activity of plasma showed

correlation with the activity of the disease. The total antioxidant capacity (status) might be decreasing due to the release of superoxides by the PMNLs. We found that the levels of albumin, which is a non-enzymatic antioxidant molecule, were lower in the active, inactive and the total BD group compared to the control group. We believe this change in albumin, which is considered an antioxidant with lipid hydroperoxides, hypochlorous acid, heme and copper binding properties, may have contributed to this decrease in the TAS of the plasma observed in patients with BD.

The finding in our study that the levels of enzymatic antioxidant SOD showed no difference between the groups is consistent with the findings of Tüzün et al. (24) and Toker et al. (10), but it contradicts the results of Tutkak (25) and Kose et al. (21). Kose et al. found that levels of superoxide dismutase in erythrocytes increased in patients with BD. We believe that those contradictory results can be clarified by larger-scale investigations shedding light on the mechanisms of intra-erythrocyte superoxide radical formation and release in addition to erythrocyte SOD activities.

We found that the activities of GSH-Px, another enzymatic antioxidant, were lower in the active BD group compared with the inactive group. There was no difference (i) between the total BD group and the control group; (ii) between the active group and the control group, and (iii) between the inactive group and the control group. Unlike us, Kose et al. (20) observed decreased GSH-Px activities in erythrocytes in active patients compared to the controls. Blood GSH-Px was also determined in patients with various skin disorders (26). Depressed levels were observed in patients with psoriasis, eczema, atopic dermatitis, vasculitis, mycosis fungoides and dermatitis herpetiformis. Low levels of GSH-Px were also found in some patients with pemphigoid, acne conglobata, polymyositis, rheumatoid arthritis, scleroderma and systemic lupus erythematoses (26). Thus, our observations on GSH-Px levels are not particular to BD.

In our patients with BD ($n=45$), GSH-Px, SOD, the TAS, albumin and uric acid levels showed significant negative correlation with plasma MDA levels. This finding supports the role of oxidative stress in the aetiopathogenesis of BD, and also the presence of a deficiency or disorder in both the enzymatic and the non-enzymatic antioxidant parameters. Kose et al. (20) showed that there was a negative correlation between MDA and GSH-Px, but positive correlation between MDA and SOD. Our correlation results between MDA and GSH-Px are consistent with their results. However, our correlation results between MDA and SOD contradict their results.

Chambers et al. (27) showed that vascular endothelial function was impaired in BD and could be rapidly improved by vitamin C treatment. Their results

supported a role for oxidative stress in the pathophysiology of BD. Freitas et al. (28) detected circulating prooxidants (clastogenic factors) and determined malondialdehyde levels in adamantiades-BD. They suggested that the presence of clastogenic factors in plasma of patients with BD, indicating the presence of circulating prooxidants with chromosome-damaging effects, confirms an oxidative stress in adamantiades-BD. According to our study, the oxidant-prooxidant balance is changed in favour of prooxidants, and this imbalance may have a role in the aetiopathology of the disease.

Finally, plasma MDA levels may provide useful information for both the diagnosis of BD and for monitoring its activation. While plasma TAS level is useful in differentiating between the active and inactive phases of the disease, it may also be useful in diagnosis because of its significantly decreased level in BD patients. In addition, erythrocyte SOD level is not a useful indicator for the diagnosis of BD and for determining activation in patients with BD. GSH-Px levels may contribute to the other findings in the determination of activation but the differences on GSH-Px levels are not particular to BD as depressed levels of this enzyme were observed in patients with various skin disorders. While our findings cannot explain the mechanisms of changes that occur in the antioxidant system in BD, they do show an increase in the oxidative stress and a deficiency or disorder in the antioxidant system. We believe that more comprehensive studies, and of a larger scale, are needed to clarify the physiopathological aetiology and prognosis of BD.

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