Lipid Peroxidation in Behçet’s Disease in Active and Remission Periods

Abstract: Recent few studies revealed that increased levels of oxygen free radicals might play a role in tissue damage. There are some studies in the literature proposing that increased levels of oxygen radicals might play a role in tissue damage, as also seen in Behçet’s disease (BD). The aim of the present study was to measure plasma malondialdehyde (MDA) (as an index of lipid peroxidation) levels in 23 patients with BD (13 men, 10 women with a mean age of 34.5 ± 7.2 years) both in active and in remission periods. Twenty-one healthy volunteers (11 men, 10 women) formed as the control group. It was found that mean plasma MDA levels were significantly higher both in the active (11.147 ± 2.452 nmol/mL) and remission (8.056 ± 1.916 nmol/mL) periods in patients with BD than in the controls (4.978 ± 1.431 nmol/mL) (p<0.001 for both). It was concluded that the plasma antioxidant defence system was significantly more impaired in active phase than in remission phase of BD.

Key Words: Behçet’s disease, lipid peroxidation

Introduction

Behçet’s disease (BD) is named eponymously for the Turkish dermatologist who described a symptom complex of oral and genital ulceration and iritis (1). Recent studies have shown that the disease is a multisystem disorder that affects skin, mucous membranes, eyes, joints, central nervous system and blood vessels (2, 3). Vasculitis is also defined as a major feature of the disease (4). The exact cause of BD is still unknown, but the list of possible agents in the pathogenesis of this disorder is long and includes genetic factors, viral infections, allergies to bacteria and several immunologic abnormalities (5). In addition to such factors, increased levels of oxygen free radicals may play a role in tissue damage in BD (6-8). Oxygen free radicals can lead to lipid peroxidation reactions as a result of which malondialdehyde (MDA) is produced (8, 9).

There are very few reports in the literature regarding antioxidants and lipid peroxidation in BD (6), and up to now, only one study has been done related to plasma MDA measurement in both at active and remission periods of this disease (10). This was the aim of the present study.

Materials and Methods

Twenty-three patients with BD admitted to the Department of Dermatology at Ataturk University were included in this study. The diagnosis was made according to international criteria for classification of BD (1). Patients were examined in both active and remission periods, and their blood samples were taken for the measurement of plasma MDA levels. At both periods; patients were subjected to a complete history and physical examination, complete blood counts, total and differential white blood cell counts, erythrocyte sedimentation rate, C-reactive protein, liver function tests, creatinine and urinalysis. Venous blood was collected in heparinized tubes and centrifuged at 2000xg for 10 minutes. Plasma aliquots were stored at -70°C until assayed. Each sample was studied in duplicate. MDA was estimated according to the method of Satoh (11). Briefly, 2.5 mL of 20% trichloracetic acid and 1 mL of 0.67% thiobarbituric acid were added to 0.5 mL of serum. The mixture was then heated in boiling water for 30 min. The resulting chromogen was extracted with 4 mL of n-butyl alcohol and the absorbance of the organic phase was determined at 530 nm. Malondialdehyde-bis-diethyl-acetal was used as a standard and the values were presented as nmol/mL.
Twenty-one healthy subjects matched with the patients for age and sex formed the control group. The results were given as mean ± SD values. The significance of the mean differences between groups was assessed by the Mann Whitney-U test. A value of p<0.05 was considered significantly different.

**Results**

This study included 13 male and 10 female patients with ages ranging from 18 to 56 years (mean: 34.5 ± 7.2 years) and a control group (11 men and 10 women) with ages ranging from 20 to 60 years (mean: 36.2 ± 6.8 years). The duration of BD ranged from 2 to 15 years with a mean age of 8.4 ± 2.3 years. Oral and genital ulcerations were present exclusively in all patients with lesions at various sites of various degrees of severity. Fifteen patients had ocular findings that were interpreted as BD. Six patients had papulopustular eruption on the trunk. Five patients had mild to moderate arthralgia and arthritis. Two patients had gastrointestinal complications. One patient suffered from headaches (Table 1).

In all of the patients with active and remissive BD, mean plasma MDA was found to be above the normal range. When compared to controls, plasma levels of MDA were significantly higher in both active and remission periods in BD patients (p<0.001, for both). Additionally, there was a significant difference between the MDA values of patients in active and remission phases (p<0.001). The data of the present study are presented in table 2.

<table>
<thead>
<tr>
<th>Finding</th>
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<tbody>
<tr>
<td>Oral and genital ulceration</td>
<td>23</td>
</tr>
<tr>
<td>Ocular findings</td>
<td>15</td>
</tr>
<tr>
<td>Papulopustular eruption</td>
<td>6</td>
</tr>
<tr>
<td>Arthritis and arthralgia</td>
<td>5</td>
</tr>
<tr>
<td>Gastrointestinal complaints</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
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</table>

**Table 2.** MDA in plasma of patients with BD active and remission periods, and of controls.

<table>
<thead>
<tr>
<th>Cases</th>
<th>MDA levels (nmol/mL) (mean ± SD)</th>
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<tbody>
<tr>
<td>Controls (n: 21)</td>
<td>4.978 ± 1.431*</td>
</tr>
<tr>
<td>Patients at active period of BD (n: 23)</td>
<td>11.147 ± 2.452*</td>
</tr>
<tr>
<td>Patients at remission period of BD (n: 23)</td>
<td>8.056 ± 1.916*</td>
</tr>
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* p< 0.001; controls vs patients in both active and inactive period, and patients in active vs remission period.

**Discussion**

There is growing evidence that oxygen-derived free radicals play a role in the pathogenesis of various inflammatory diseases. Leukocyte infiltration is a common feature of inflammatory disorders, and the resultant oxygen-derived free radicals may account for the alternation in vascular and mucosal permeability. With increasing frequency, inflammatory cells, particularly polymorphonuclear cell (PMN) leukocytes, are being implicated as mediators of tissue-destructive events in inflammatory diseases. Inflammatory cells attracted to the site of injury are capable of greatly increasing reactive oxygen species during respiratory burst. Antioxidant mechanisms exist for the control of the effects of reactive oxygen species (ROS). However, there is evidence to suggest that the continual production of free radicals within the inflammed region may result in the exhaustion of effective antioxidant control (6,12-14). In other words, the antioxidant mechanism fails, and then the severity of destruction increases, because ROS can react with vital biological substances, such as proteins, nucleic acids and polyunsaturated fatty acids. The reaction of ROS with polyunsaturated fatty acids leads to the formation of MDA, which is a marker of lipid peroxidation (15,16).

BD is a chronic inflammatory disease of unknown aetiology. The stimulated neutrophils seen in BD have been considered to be correlated with the pathogenesis of BD. In particular, oxidative species such as \( \text{O}_2^- \), which are reported to be increased in BD, have been proposed as possible mediators for tissue damage occurring in BD (7,8,17). In a study by Pronai et al. (18), it was determined...
that the superoxide scavenging activity (SSA) of PMN were lower in BD patients, and there were negative correlations between the SSA of PMN. Their $O_2^-$ generation by PMN in vivo might be responsible for the decreased SSA of PMN in BD, and PMN might be able to release more $O_2^-$ in tissue. Consequently, increased generation of $O_2^-$ may lead to more lipid peroxidation in the tissues of patients with BD.

As yet, although there have been some studies on antioxidative enzymes and lipid peroxidation in BD (6, 7), there is only one study on MDA measured both during active and remission periods of BD (10). Freitas et al. reported that thiobarbituric acid reactive substances (expressed as nanomoles MDA per milliliter) were significantly higher in BD patients than in healthy controls; however, they did not group BD patients at active or at remission periods (19). Orem et al. studied lipid peroxidation in 18 BD patients and detected that lipid peroxidation was higher in the active period than in the inactive period of the disease and in control subjects (10).

The main finding of the study was that plasma MDA level was higher in both active and remission periods of BD than in healthy controls, and additionally, that patients in the active period had much higher plasma MDA levels than the values obtained in the remission period. Our results confirm the previous studies (10, 19). These data indicate that lipid peroxidation becomes more prominent in active phase of BD. However, it is uncertain whether increased lipid peroxidation, or in other words, the impaired antioxidant defence system, is a cause or a consequence of this disease. Detailed studies are needed to clarify the situation.

References