The Serum Vitamin C Levels in Behçet's Disease

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Behçet’s disease (BD) is a chronic inflammatory disorder of unknown etiology, and recognised as a multi-system vasculitis. It has been postulated that an imbalance of the oxidant and antioxidant systems related to the disease are important in its pathogenesis. Previous publications have reported increased levels of enzymatic antioxidant defence systems in patients with BD. The non-enzymatic antioxidant systems, including vitamin C and uric acid, were looked for in the present study. For this aim, the serum malondialdehyde (MDA), an end product of lipid peroxidation, and vitamin C and uric acid, as endogenous antioxidants, were determined in 20 patients with BD (11 in active and 9 in inactive periods) and 20 healthy subjects. The MDA level was significantly higher in both the active and inactive period patients compared with the control group (p<0.001, p<0.05, respectively). The MDA level was also significantly higher in the active period patients compared with the inactive period patients (p<0.05). The vitamin C levels were significantly lower in both the active and inactive period patients compared with the control group (p<0.001, p<0.05, respectively). There was no significant difference in the vitamin C level between the active and inactive period patients (p>0.05). There was also no significant difference in uric acid levels between the groups (p>0.05). In the patients group, a negative correlation was found between the levels of serum MDA and vitamin C (r=-0.517; p<0.05). Our results indicate that decreased vitamin C and increased MDA levels reflect the increased levels of oxidative stress in BD patients, and this situation may be important in relation with its pathogenesis.

Key Words: Behçet’s disease, lipid peroxidation, vitamin C

INTRODUCTION

Behçet’s disease (BD) is a chronic inflammatory disorder that is mainly characterised by recurrent aphthous ulceration, genital ulceration, skin lesions and uveitis. BD is now recognised as a multi-system vasculitis, which can also affect all types and sizes of blood vessels, joints, the lungs and the central nervous and gastrointestinal systems. The etiopathogenesis of BD is unknown, but it has long been postulated that immunological abnormalities, which are possibly induced by microbial pathogens in genetically susceptible individuals, are important in its pathogenesis. It has also been shown that various functions of polymorphonuclear cells in peripheral blood, such as chemotaxis, phagocytosis, superoxide radical anion (O₂⁻) generation and lysosomal enzyme activity, are increased in BD. Decreased enzyme activity in antioxidant systems, and increased levels of free radicals, may play a role in tissue damage. Lipid and lipoprotein profiles, and their relationship to atherogenesis in patients with BD, have been described in previous studies.

Vitamin C (ascorbic acid, AA) acts as a cofactor in the enzymatic biosynthesis of collagen and carotinoid, and catecholamine and peptide neurotransmitters. AA also prevents the injurious effects of oxidants by reducing reactive oxygen and nitrogen species to stable molecules. A decreased serum vitamin C concentration in humans is associated with neurological problems, and eventually causes scurvy. As AA loses electrons in biosynthetic or antioxidant reactions, it becomes oxidized to the short-lived ascorbyl radical and then to dehydroascorbic acid (DHA). DHA and AA have distinct effects on cell functions, as becomes obvious under conditions characterized by oxidative stress.
In this study, vitamin C and uric acid, as endogenous antioxidants, and MDA, as an index of lipid peroxidation, were investigated in the serum in patients with BD in relation to disease activity.

MATERIALS AND METHODS

Subjects

The study group consisted of 6 male and 14 female BD patients, aged 19-55 (average, 29.60 ± 2.14) yr, and 8 male and 12 female healthy control subjects, aged 22-54 (average, 31.63 ± 2.16). The disease duration of the patients, defined as the time since the diagnostic criteria had been fulfilled, was between 1-20 yr (average, 4.85 ± 1.03 yr). The diagnosis of BD was made according to the criteria from the International Study Group (ISG) for BD. There is no accepted specific clinical activity scoring system or laboratory screening profile for BD. Therefore, the clinical activity of disease was evaluating by the physical manifestations, such as oral aphthous, genital ulceration, uveitis and vasculitis. At the time of the study the patients who had at least three ISG criteria and erythrocyte sedimentation rate (ESR) greater than 20 mm/h were considered to be in the active stage of the disease. All of the active period patients had oral ulcers (100%), 10 had genital ulcers (91%), 5 had uveitis (45%), 2 had vasculitis (18%) and 7 had arthritis (64%). All of the inactive period patients had oral ulcers (100%), 7 had genital ulcers (78%), 2 had uveitis (22%), 1 had vasculitis (11%) and 3 had arthritis (33%). The clinical properties of the BD patients are given in Table 1. The acute phase reactants, such as ESR and polymorphonuclear leukocyte (PMN) count, were also evaluated. At the time of the study the patients were receiving neither systemic steroids nor lipid-lowering drugs. None had received either mineral or vitamin drugs.

Analytical procedure

Measurement of serum MDA concentration

Lipid peroxidation was determined by the thio- barbituric acid (TBA) reactivity method. A5 MDA, an end product of fatty acid peroxidation, reacts with TBA to form a coloured complex that has a maximum absorbance at 532 nm. For this purpose, the blood samples were centrifuged for 5 min at 1800 × g. One ml of the serum was transferred to another tube, with the addition of 0.075 ml 0.1 M EDTA and 0.25 ml 1% TBA in 0.5 M NaOH. The contents of the tubes were mixed and kept in a boiling water bath for 15 min. The absorbance at 532 nm was read after the cooling the tubes to room temperature. Butylated hydroxytoluene (BHT), an antioxidant, was added to prevent MDA formation during the assay. The addition of BHT to standard MDA did not affect the colour development with TBA. MDA values (nmol) were calculated from the absorbance coefficient of the MDA-TBA complex at 532 nm, (1.56 × 10³ cm⁻¹ mol⁻¹) and were expressed in nmol/ml.

Measurement of serum ascorbic acid

Determination of the total ascorbate was performed after the oxidation of ascorbic acid to dehydroascorbic acid, and reacted with acidic dinitrophenylhydrazine to form a red bis-hydrazine, which was measured at 520 nm using a spectrophotometer. All vitamin C standards were prepared fresh daily.

Measurement of serum lipid, lipoprotein and uric acid levels

The serum total cholesterol was measured by a cholesterol oxidase enzymatic method, the triacylglycerol by a glycerol oxidase enzymatic method and the high-density lipoprotein cholesterol (HDL-C) by the cholesterol oxidase enzymatic method, in the supernatant obtained after precipitation with phosphotungstic acid-magnesium chloride. The uric acid was measured by the uricase enzymatic method, using a Roche-Hitachi PP Moduler analyzer, with Roche original reagents. The low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. Determination of the apolipoproteins A1 and B were performed by immunoturbidimetric methods, using a Hitachi 911 analyzer, with Boehringer Mannheim original reagents.
Table 1. Clinical Properties of Patients with Behçet's Disease

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<tr>
<th>Patients Number</th>
<th>Age/Sex</th>
<th>Duration (years)</th>
<th>Oral Ulcer</th>
<th>Genital Ulcer</th>
<th>Eye Lesion</th>
<th>Acneiform Nodules</th>
<th>Erythema Nodosum</th>
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M, Male; F, Female.

Statistical analysis

All data were expressed as the mean ± SE. The Kolmogorov-Smirnov Goodness of Fit Test was used to control the normal distribution of parameters. The parametric distribution data were then compared with a One-way ANOVA test and non-parametric distribution data with the Kruskal-Wallis test. A Spearman test was performed for correlation.

RESULTS

The concentration of the biochemical parameters related to the disease activity in the BD patients and control subjects are shown in Table 2. The PMN-leukocyte, Apo B and total cholesterol levels were significantly different between the active period patients and the control subjects (p<0.001, p<0.05, p<0.05, respectively). The ESR was significantly higher in both the active and inactive period patients compared with the control group (p<0.001, p<0.05, respectively). The ESR was also significantly higher in the active period patients compared with the inactive period patients (p<0.05). The MDA levels were significantly higher in both the active and inactive period patients compared with the control group (p<0.001, p<0.05, respectively). In addition, the
Table 2. Some Biochemical Parameter Values in the Patients during the Active or Inactive Periods and in the Control Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Active period X ± SE* (n=11)</th>
<th>Inactive period X ± SE* (n=9)</th>
<th>Control subjects X ± SE* (n=20)</th>
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<tr>
<td>PMN leukocytes/ml</td>
<td>9.6 ± 0.7^d</td>
<td>7.4 ± 0.7</td>
<td>5.6 ± 0.4</td>
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<td>ESR (mm/h)</td>
<td>49.50 ± 4.8^d</td>
<td>20.0 ± 5.2^b</td>
<td>3.44 ± 0.4</td>
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<tr>
<td>Vitamin C (mg/dl)</td>
<td>0.31 ± 0.0^d</td>
<td>0.82 ± 0.2^b</td>
<td>1.55 ± 0.1</td>
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<td>Uric acid (mg/dl)</td>
<td>4.77 ± 0.5</td>
<td>4.38 ± 0.4</td>
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<td>MDA (nmol/ml)</td>
<td>3.91 ± 0.3^c</td>
<td>3.22 ± 0.2^b</td>
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<td>Cholesterol (mg/dl)</td>
<td>139.88 ± 12.5^b</td>
<td>149.77 ± 4.3</td>
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<td>Triacylglycerols (mg/dl)</td>
<td>92.11 ± 15.2</td>
<td>118.11 ± 18.2</td>
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<td>HDL-C (mg/dl)</td>
<td>39.55 ± 2.89</td>
<td>38.71 ± 2.7</td>
<td>46.00 ± 2.9</td>
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<tr>
<td>LDL-C (mg/dl)</td>
<td>81.77 ± 10.2</td>
<td>84.57 ± 4.8</td>
<td>101.36 ± 5.4</td>
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<tr>
<td>Apo A (mg/dl)</td>
<td>100.30 ± 7.5</td>
<td>105.12 ± 7.7</td>
<td>112.26 ± 3.9</td>
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<tr>
<td>Apo B (mg/dl)</td>
<td>75.40 ± 6.7^b</td>
<td>95.75 ± 8.1</td>
<td>97.79 ± 4.5</td>
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^p<0.001, ^p<0.05. Significantly different from those of control subjects.
^p<0.05, ^p<0.001. Significantly different from those of inactive period.
*Standard error.

MDA level was significantly higher in the active period patients compared with the inactive period patients (p<0.05). The vitamin C levels were significantly lower in both the active and inactive period patients compared with the control group (p<0.001, p<0.05, respectively). There was no significant difference in the vitamin C levels between the active and inactive period patients (p >0.05). There was also no significant difference in the uric acid levels between the groups (p>0.05). In the patients group, there was a significant negative correlation between the levels of serum MDA and vitamin C (r=-0.517; p<0.05) (Fig. 1).

DISCUSSION

Activated oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical, produced by the partial reduction of oxygen, are highly unstable and extremely reactive. The short half lives of many of these species make them highly toxic toward tissues.19 Oxygen radicals are capable of reacting with unsaturated lipids and initiating the self-perpetuating chain reactions of lipid peroxidation in membranes.20 The import-

Fig. 1. The correlation of serum MDA and vitamin C levels in Behçet’s disease.
of oxidative stress, in a variety of lipid systems, such as plasma, organs and cell membranes. Previous studies have shown a significant increase in serum and erythrocyte lipid peroxidation, characterised by elevated MDA levels, in BD patients, which was consistent with the present study. Lipid peroxides alter the cell membrane fluidity by increasing the incorporation of cholesterol, oxidised fatty acids and low-density lipoproteins. The most common factors affecting the initiation and development of atherosclerosis are endothelial cell damage and the oxidation of lipoproteins, especially low-density lipoprotein, which continuously enter and exit the artery wall. The presumed site of LDL oxidation, in vivo, is in the sub-endothelial interstitial matrix. LDL may be exposed more frequently to cell-derived oxidants, thus it may be less protected by antioxidants compared to circulating LDL. The potential for prolonged contact with LDL is one reason the endothelium is prone to oxidative disturbances. Antibodies directed against oxidised LDL are found in the serum of most people, but are increased in disorders associated with oxidative stress. Increased autoantibodies to an epitope of oxidised LDL have been described in patients with BD. Endothelial damage and increased PMN leukocyte activity, in BD patients, may result in a pro-oxidation environment in the sub-endothelial region. Thus, the process causing atherosclerosis may be initiated by the oxidation of LDL. The significant differences in lipid peroxidation between the active and inactive period patients indicate that BD patients in the active period may be much more susceptible to atherosclerotic events than those in the inactive period and the control subjects.

The plasma lipid and lipoprotein levels in BD patients have been described in various previous studies. High total cholesterol and LDL-C and low HDL-C levels were predominant in BD patients. In the present study, significantly decreased Apo B and cholesterol levels were found in the BD patients compared to the control group. Because of the small size of the active and inactive patient groups in the present study, the lipids levels obtained might be different from previous studies.

Under normal conditions, 3-5% of the oxygen taken up by cells undergoes univalent reduction, leading to the formation of free radicals. However, the tissue concentrations of free radicals are limited by a system of enzymatic and non-enzymatic antioxidants and free radical scavengers that have developed and been conserved during the evolution of aerobic life. Previous publications have reported the increased levels of enzymatic antioxidant defence systems in BD patients. Therefore, the non-enzymatic antioxidant systems, including reducing ascorbic acid and uric acid, were looked for in the present study. Vitamin C is supremely effective in protecting plasma lipoproteins, and other susceptible molecules, from peroxidation during exposure to a wide spectrum of water and lipid soluble free radicals. Decreased vitamin C levels and increased MDA levels were found in both the active and inactive period BD patients compared to those in the control subjects. It has also been demonstrated that there was an inverse correlation between serum levels of vitamin C and MDA in BD patients. According to Freij, et al., vitamin C plays a pivotal role as a chain-breaking antioxidant, and plasma lipid peroxidation is prevented only as long as vitamin C is present. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing and antioxidant agent, it directly reacts with various lipid hydroperoxides, and prevents the oxidative modification of the cytolomic and membrane components of cells. No previous study has been found that reporting the vitamin C levels in BD patients. However, Chambers, et al. have reported that the vascular endothelial function is impaired in patients with Behçet’s syndrome, and can be rapidly improved by treatment with vitamin C used as an antioxidant. These authors have suggested that their results may be mediated by increased oxidative stress, and provide a rationale for the use of antioxidant vitamins for the reduction of vascular complications in BD. Under the pathological conditions characterized by oxidative stress, AA is oxidized by reactive oxygen species at rates that overwhelm the ability of cells to regenerate the vitamin. For example, inflammation of the skin during wound healing markedly raises the extracellular concentration of dehydroascorbic acid (DHA). The model consists
of applying a bacterial endotoxin (lipopolysaccharide) and the inflammatory cytokine interferon-γ (IFNγ) to primary cultures of astrocytes. Lipopolysaccharide and IFNγ induce nitric oxide synthase isoform 2, increase intracellular levels of reactive oxygen species (ROS) and decrease the rate of intracellular AA conversion from extracellular AA or DHA.43 The oxidations produced during inflammatory reactions may directly alter the mechanisms by which cells recycle DHA. For instance, prior exposure of astrocytes to peroxyl radicals decreases their subsequent conversion of intracellular AA from extracellular DHA.44

In humans, uric acid is the major product of the catabolism of the purine nucleosides, adenosine and guanosine. Purine nucleotides are degraded by a pathway where the phosphate group is lost by the action of 5′-nucleotidase. Adenosine is then deaminated to inosine by adenosine deaminase (AD). Inosine is hydroxylated to yield its purine base, hypoxanthine. Hypoxanthine is oxidized successively to xanthine, and then to uric acid, by xanthine oxidase (XO). The dehydrogenase (type D) form of XO requires nicotinamide adenine dinucleotide (NAD) and produces uric acid and reduced nicotinamide adenine dinucleotide (NADH). During hypoxia/ischemia, this form is increasingly converted to the oxidase (type O) form, which requires oxygen, and produces uric acid and superoxide radical (O2) during its reoxygenation.45,46 A paper regarding the serum XO and AD activity in BD has recently been published.46 These investigators suggest that increased AD and XO activities may provide an additional benefit in the diagnosis and subtyping of BD. Kose, et al.29 also found that the AD activity was increased in BD, providing some evidence for a potential role of T lymphocyte activation. Albumin and uric acid, along with ascorbic acid, account for the major contributions (>8 5%) to the total antioxidant capacity in human plasma.47,48 due largely to their high concentrations relative to other antioxidants in blood, such as bilirubin, α-tocopherol and β-carotene. Contrary to what is traditionally considered a metabolically inert and waste compound of no physiological significance, uric acid can be oxidized following the nonenzymatic degradation, and has proven to be a selective antioxidant, capable, especially, of reacting with hydroxyl radicals and hypochlorous acid. Uric acid may be found in all tissue compartments, with the exception of the lipid phase. Thus, measuring the levels of specific antioxidant molecules, such as plasma albumin, bilirubin and uric acid, can yield valuable information, and low levels of such antioxidants may provide suggestive evidence of oxidative stress.49 In the present study, although the serum uric acid level was reduced in BD patients compared to the control group, this difference was not statistically significant. Further studies however, are required to explain the importance of uric acid as an antioxidant in BD patients.

In conclusion, this study suggests that there is an imbalance between the MDA and vitamin C levels in inflammatory Behçet’s disease. Also, the decreased vitamin C and increased MDA levels reflect increased levels of oxidative stress in BD patients, and this situation may be important in its pathogenesis. Further studies however, are required to explain the clinical importance of such alterations in Behçet’s disease.

REFERENCES


