Original Article

Serum Interleukin-2, Interleukin-6, Tumour Necrosis Factor-Alpha and Nitric Oxide Levels in Patients With Behçet's Disease+

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Abstract

Introduction: Behçet's disease (BD) is a chronic systemic disorder characterised by oral and genital ulcerative lesions, ocular and cutaneous manifestations. Cytokines are the major mediators of immunologic and inflammatory reactions. Nitric oxide is reactive nitrogen intermediate which plays a key role in pathogenesis of many inflammatory and autoimmune skin diseases. The study was conducted to determine serum interleukin-2 (IL-2), interleukin-6 (IL-6), tumour necrosis factor (TNF)-alpha and nitric oxide levels in relation to the pathogenesis of Behçet's disease. Materials and Methods: Serum IL-2, IL-6, and TNF-alpha levels were measured with test kits by enzyme-linked immunosorbent assay (ELISA) method, while serum nitric oxide levels were determined with a test kit by colorimetric method. Serum IL-2, IL-6, TNF-alpha and nitric oxide concentrations in 27 patients with Behçet's disease and in 16 healthy controls were determined by extrapolation from their standard curves. The significance of the mean differences between the 2 groups was assessed by the Mann-Whitney U test. Results: The serum levels of IL-2, IL-6, TNF-alpha, and nitric oxide concentrations in patients with BD were significantly higher than those of the controls (P <0.001). Conclusion: Our results suggest that elevated levels of IL-2, IL-6, TNF-alpha, and nitric oxide in Behçet's disease appear to be related to the disease.

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Key words: Cytokine, Skin disease, Vasculitis

Introduction

Behçet's disease (BD) is characterised by oral aphthous lesions, genital ulcerations and eye inflammation. The disease was first described by Hulusi Behçet in 1937.[1] Despite the diverse inflictions in different organ systems, vasculitis is perceived as the common basic pathological process in BD. The exact cause is unclear but viral, genetic, immunological and environmental factors have been implicated in the pathogenesis of BD.[2]

BD is often considered within the context of autoimmune diseases, since immunoglobulins, immune complexes, complement and acute phase proteins were reported to be increased.[3-5] Abnormalities of neutrophils and endothelial cells have been suggested to be responsible for many of the clinical manifestations of BD. Considering the evident activation of immune system in BD, pro-inflammatory cytokines and mediators may effect the course of the disease.[4]

Cytokines are known to predominate in diverse immunological mechanisms. These proteins which are produced by various cell types, are important mediators of immuno-inflammatory reactions.[6-8] One such regulating cytokine is tumour necrosis factor (TNF)-alpha, which exerts multiple stimulatory effects on T cells by binding to specific receptors and increases the expression of human leukocyte antigens (HLAs) and high-affinity interleukin (IL-2) receptors and postulated as a first messenger for priming immune cells.[6,8] Interleukin-6 (IL-6) is a

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multifunctional cytokine that is involved in the regulation of the immune response, haematopoiesis and inflammation.[9-13]

Nitric oxide (NO) is an inorganic-free radical gas produced in the vascular endothelium by the iso-enzyme nitric oxide synthase (NOS) using L-arginine as substrate. Two isoforms of NOS have previously been clearly described. The first one is inducible NOS (iNOS); it is induced in macrophages and liver cells by endotoxin and cytokines. It is independent of calcium concentration physiologically. The second form is constitutive NOS (cNOS), which is dependent on calcium and calmodulin. cNOS releases NO physiologically in the regulation of many cell functions and communication. cNOS has previously been described in the nervous system and vasculature, and later in melanocytes, endothelial cells, fibroblasts and keratinocytes.[14-18] The iNOS is calcium-independent and synthesise NO in longer duration and greater amounts. It has also been implicated in the pathogenesis of numerous inflammatory and autoimmune diseases.[14,15,19]

Thrombosis is also frequently seen in BD. The major factor responsible for the increased frequency of thrombosis is thought to be endothelial dysfunction.[20] Releasing NO by the endothelium promotes vasodilatation and inhibits inflammation, thrombosis, and vascular smooth muscle cell proliferation.[21] It has been noted that a higher intrinsic level of baseline NO production in the post-capillary microvascular endothelium may reflect both the contribution of venular-derived NO to the control of arteriolar tone and, its key role in local thrombosis control.[22]

NO has been postulated in several pathological processes related to immunological, cardiovascular and neurological disorders, infections and cancers.[14,15]

The aim of this study was to determine the serum IL-2, IL-6, TNF-alpha, and NO levels, as well as their correlations with each other in patients with BD.

Materials and Methods

Twenty-seven patients (12 males and 15 females) with active BD were included in the study. Sixteen healthy volunteers constituted the control group. The diagnosis of BD was based on the International Behçet's Study Group Criteria.[23]

The patients were examined when their diseases were in the active stage and blood samples were taken to measure serum IL-2, IL-6, TNF-alpha and NO levels between 8 am and 10 am. Venous blood was collected in vacutainer tubes and centrifuged at 1500 g for 5 minutes; then the serum samples were stored at -80°C until assayed.

Serum value of IL-2 (h-Interleukin-2-ELISA; Roche Diagnostics GmbH, Sandhoferstr, Mannheim, Germany) and IL-6 (h-Interleukin-6-ELISA; Roche Diagnostics GmbH, Sandhoferstr, Mannheim, Germany), TNF-alpha (hTNF-alpha; BioSource International, Inc. Camarillo, California, USA) were determined with test kits by enzyme-linked immunosorbent assay (ELISA) method. Serum NO (Nitric Oxide Colorimetric Assay; Roche Diagnostics GmbH, Sandhoferstr, Mannheim, Germany) levels were also determined with a test kit by colorimetric method.

Since NO is a very labile molecule, its direct measurement in the biological samples is very difficult. In aqueous solution, NO reacts with molecular oxygen and accumulates in the serum as NO_2^- and NO_3^- ions. Therefore, NO_2^- and NO_3^-, the last stable oxidation products of NO, can be readily measured in biological fluids and have been used in vitro and in vivo as indicators of NO production.[24] In the first step, nitrate is converted into nitrite using nitrate reductase. The second step is the addition of Griess reagent, which converts nitrite into a deep purple azocompound; photometric measurement of the absorbance of 540 nm determines the nitrite concentration. NO levels in patients with BD and the samples taken from the healthy controls were determined by extrapolation from a standard curve of sodium nitrate.

The results were given in mean ± standard deviations. The significance of the mean differences between the two groups was assessed using the Mann-Whitney U test. The relationship between the parameters was determined with correlation analysis. A value of $P < 0.05$ was considered statistically significant.

Results

The BD group included 12 males and 15 females between the ages of 18 and 51 years (26 ± 5 years). The control group comprised 8 males and 8 females with ages 20 to 40 years (31 ± 3 years). Oral-genital ulcerations and arthralgia, the classical findings of BD, were present in all of the patients. The clinical characteristics of the patients are summarised in Table 1.

Compared to the controls, there was a significant increase in the serum levels of IL-2, IL-6, TNF-alpha and NO in BD patients ($P < 0.001$). No significant correlation was found between any cytokine and NO level in the patient group or in the control group. The results are summarised in Table 2.

Discussion

BD is a chronic, multi-systemic disorder, characterised by a relapsing inflammatory activation.[2] Although the aetiopathogenesis of the disease has not yet been clarified, several mechanisms such as genetics, infections and autoimmunity have been suggested.[7,10,25-27] Treatment of BD is presently difficult, partly because of the multi-

Table 1. Clinical Manifestations in Patients with Behçet's Disease

Clinical data	BD (%) (n = 27)
Oral ulcers	100
Genital ulcers	100
Ocular disease	63.7
Cutaneous lesions	55.3
Positive pathergy test	54.1
Arthralgia	100
Arthropathy	22.8
Gastrointestinal symptoms	4.1
Venous disease	18.8
Central system involvement	8.2

BD: Behçet's disease

Table 2. Serum IL-2, IL-6, TNF-alpha and Nitric Oxide Levels in the BD and Control Groups

Parameters	Mean ± SD of control group (n = 16)	Mean ± SD of BD group (n = 27)	*P*
IL-2 (pg/mL)	141 ± 141	315 ± 163	<0.001
IL-6 (pg/mL)	22.8 ± 13	74.4 ± 70.3	<0.001
TNF-alpha (pg/mL)	10.88 ± 5.72	20.5 ± 12.3	<0.001
Nitric oxide (mmol/L)	184.8 ± 43.3	327 ± 171	<0.001

BD: Behçet's disease; IL-2: interleukin-2; IL-6: interleukin-6; TNF-alpha: tumour necrosis factor

systemic nature of the disease. Recent treatment modalities are encouraging, and it is probable that further treatment modalities that interfere with cell signalling processes may be the future direction for the clinical management of BD.

It was reported that the number of IL-2 producing CD4+ cells in BD patients with active uveoretinitis was significantly compared to inactive cases and controls.[10] The authors concluded that Th1 cells may play an important role in the immunopathogenesis of ocular inflammation of BD.[10]

Abnormal IL-6 production has been implicated in some autoimmune diseases and chronic inflammatory reactions, in which the overproduction of IL-6 might lead to abnormal B cell differentiation and antibody production.[11,28,29] The present study showed that IL-6 levels in patients with BD were significantly higher compared to the controls. Likewise, IL-2 and TNF-alpha levels were also increased in patients with BD. It is probable that the disease is associated with the secretion of proinflammatory mediators by direct activation of circulating monocytes.[4,28] Previous studies have also reported increased serum IL-2, IL-6, and TNF-alpha levels.[4,7,10,26,27,29] The results of the present study confirm these findings and suggests an involvement of the activation of the immune system in BD.

Considering the increased levels of the cytokines in our patient group, we suggested that these cytokines could be related to pathogenesis of the disease. These pro-inflammatory cytokines may play a role during the course of the disease and take part in tissue damage.

We found increased serum NO levels in BD patients. In previous studies, NO was also found to increase in diseases such as ocular inflammation,[30] systemic lupus erythematosus,[31] cerebral systemic lupus erythematosus,[32] inflammatory arthritis.[33] Increased NO production is believed to be associated with inflammatory processes. Hence, increased NO production is expected in patients with BD during exacerbations as an inflammatory dermatosis. Two previous studies have reported increased NO levels in BD, similar to our study.[34,35] However, in another study with BD patients, NO levels were found to be decreased.[36] They postulated that decreased NO production might have critical biological activities relevant to pathologic events during disease activity.[36] NO production appears to be increased ubiquitously in inflammatory processes and is expected in patients in the acute inflammatory stage of BD. Our results on NO are in keeping with the general observation.

In our study IL-2, IL -6, TNF-alpha and NO levels were found to be increased. However, the lack of correlation between each cytokine and NO suggests that activation of NOS and cytokine production might be by different mechanisms resulting in various clinical manifestations of the disease. To our knowledge, this is the first study investigating the possible correlations between proinflammatory cytokines and NO in BD. Whether these cytokines and NO can serve as markers of activity, treatment response and prognostication in Behçet's disease need further studies with long-term follow-up.

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