Serum Cytokine Levels in Behçet's Disease

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Background: The aim of this study is to investigate and compare the serum levels of various cytokines in patients with Behçet's Disease and healthy controls. **Methods:** Thirty-five patients with Behçet's disease and 29 age and sex-matched healthy controls were included in the study. The patients were separated into groups with active and inactive disease. Serum IL-2, IL-6, IL-8, IL-10, IL-17A, and IFN- γ levels were determined using the enzyme-linked immunosorbent assay method. Cytokine levels of the two patient groups and healthy controls were compared using SPSS 15.0. **Results:** Ten patients with active disease

Key words: Behçet's disease; interleukin-8

and 25 patients with inactive disease were present. Serum IL-8 levels of active BD patients were higher compared to inactive patients ($P = 0.048$) and healthy controls ($P = 0.02$). IL-8 levels were correlated with the duration of symptoms ($r = 0.490$, $P = 0.003$) and time passed since diagnosis ($r = 0.579$, $P < 0.001$). **Conclusion:** Behçet's disease involves complex interactions of cells of the immune system, mainly T lymphocytes and neutrophils. Further studies on the cytokine profile in Behçet's disease will aid in elucidation of its pathogenesis. J. Clin. Lab. Anal. 29:317–320, 2015. ©

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Behçet's disease (BD) is a chronic systemic inflammatory disorder, standing out from autoimmune and autoinflammatory diseases due to its unique properties. It is characterized by recurrent episodes of oral and genital ulcers, uveitis, and various systems involvement (1,2). The onset of BD usually occurs in the third or fourth decade, and it is rare in children or patients over the age of 50. Turkey has the highest prevalence of BD in the world, with up to 421 per 100,000 persons affected. Behçet's disease shows a male preponderance in Middle Eastern countries and the Mediterranean basin; however, women are more commonly affected in Japan and Korea (1). The most strongly associated genetic factor to BD, HLA-B51, accounts for less than 20% of the genetic risk, which shows that there remain other factors to be discovered (3,4).

Cytokines are low-molecular weight polypeptides taking part in cell communication and are involved in an array of different immunologic mechanisms. They are im-

portant mediators of immunologic and inflammatory reactions (5). Furthermore, the discovery of new T-cell subtypes and the frequent identification of novel cytokines has hastened research into the pathogenesis of BD. The aim of this study was to investigate and compare the serum levels of various cytokines in patients with BD and healthy controls. The study was carried out at the Rheumatology, Dermatology, and Ophthalmology outpatient clinics of the Dicle University Hospital from February 2013 to May 2013. Thirty-five patients with BD and 29 age and sex-matched healthy controls were included. All patients were

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TABLE 1. Criteria Present at Sample Collection

	Active BD	Inactive BD
Oral ulcers	80%	48%
Genital ulcers	40%	8%
Eye involvement	70%	48%
Pathergy	90%	56%
Erythema nodosum	10%	0
Pseudofolliculitis	40%	40%
Big vessel involvement	0	0
Arthritis	0	4%

BD, Behçet's disease.

screened according to the International Study Group for Behçet's Disease criteria (6) and the presence at the time of the study and history of relevant symptoms were recorded in detail. Pathergy tests of all patients were performed. Active disease was defined as the presence at sample collection of three International Study Group for Behçet's Disease criteria (6). Patients who had been diagnosed as BD in the past who had less than three active criteria at the time of the study were considered to have inactive disease. Erythrocyte sedimentation rate and C-reactive protein were not regarded as criteria for active disease. All patients were receiving at least one form of treatment at the time of collection of the samples. Treatment modalities were as follows: Colchicine (12, 18.8%), disease modifying antirheumatic drugs (azathioprine, cyclosporine, methotrexate), alone (4, 6.3%) or in combination with colchicine (15, 23.4%) or corticosteroids (4, 6.3%). Patients on treatment with IFN or anti-TNF treatments were not included in the study. Approval of the Ethics Committee of Dicle University Faculty of Medicine was obtained and all subjects gave written informed consent. Venous blood samples were obtained and immediately centrifuged. Serums were kept at -80°C until laboratory testing. The serum interleukin (IL)-2, IL-6, IL-8, interferon (IFN)- γ (Invitrogen, Camarillo, CA) and IL-10 (Invitrogen, Frederick, MD, CA) and IL-17A (Biosource Europe S. A. Belgium) levels were determined using the enzyme-linked immunosorbent assay method according to the manufacturer's protocols.

Statistical analyses were performed using SPSS 15.0. Student's *t*-test and Mann-Whitney U test were used for the analysis of normally distributed and nonnormally distributed data, respectively. A *P*-value <0.05 was considered significant.

When grouped according to activity, there were ten patients with active BD (seven males and three females) and 25 patients with inactive BD (11 males and 14 females). The clinical manifestations present in active and inactive patients are shown in Table 1. The mean ages of the groups were 31.90 ± 3.51 years, 33.80 ± 10.85 years, and 29.97 ± 12.43 years in active BD, inactive BD and controls, re-

spectively ($P > 0.05$). In active and inactive patients, mean duration of symptoms (8.40 years and 6.42 years) and mean time passed since diagnosis (6.56 years and 3.68 years) were statistically similar.

Serum IL-8 levels of active BD patients (2.04 ± 0.33 pg/ml) were higher compared to inactive patients (1.81 ± 0.28 pg/ml) ($P = 0.048$) and healthy controls (1.82 ± 0.20 pg/ml) ($P = 0.02$). IL-8 levels were correlated with the duration of symptoms ($r = 0.490$, $P = 0.003$) and time passed since diagnosis ($r = 0.579$, $P < 0.001$). Serum levels of IL-2, 6, 10, 17A, and IFN- γ were similar in all study groups ($P > 0.05$). Evaluation for an association between individual clinical manifestations and serum levels revealed no significant difference with all the measured cytokines.

Higher levels of IL-8 were detected in patients with active BD in this study. IL-8, also known as neutrophil activating factor, is a major cytokine which strongly attracts and activates leukocytes. It is involved in the conversion of mononuclear to granulocytic infiltration and causes increased adhesion of peripheral blood leukocytes to endothelial cells, thus linking immune system activation with endothelial alterations in BD. It is mainly produced by macrophages, T cells, granulocytes, and endothelial cells. Small amounts can also be produced by fibroblasts, keratinocytes, hepatocytes, and chondrocytes (4, 7, 8). Various studies have shown the levels of IL-8 to be significantly elevated in active BD compared with inactive BD (7, 9). Despite this, there are studies reporting no difference between IL-8 levels and active and inactive disease (10).

IL-8 has been shown to be associated with different individual clinical manifestations in BD, that is, oral lesions, skin lesions, ocular manifestations in one study (8). Such an association was not detected in the present study. However, the levels of IL-8 were correlated with duration of symptoms and time passed since diagnosis. This might be due to the longer duration of inflammation in these patients. Very high increases in IL-8 mRNA expression have been observed within lesions of BD. This may reflect the important in situ recruitment of both polynuclear and mononuclear cells. Since IL-8 is a strong activator of neutrophil function, this finding may support the implication of polynuclear cell hyperfunction in the pathogenesis of BD lesions (11). Levels of IL-8 may be useful in following disease activity, or in predicting patients with more severe disease.

Other than IL-8, increased Th1-associated cytokines such as IFN- γ , IL-12, and TNF- α , as well as IL-2 and IL-6 have been documented in BD patients and it is thought that the immune response in BD is skewed toward the Th1 path (11). However, treatment with IFN- α and anti-TNF agents have only partially prevented the progression of BD (12). This shows that, as in the case of allergy and other autoimmune disorders, only the

Th1-Th2 paradigm cannot fully account for the full mechanism of the disease (13). The presence of a high Th17/Th1 ratio has been shown in BD, which indicates that Th17 cells, a recently identified T-cell population, may be the missing piece of the puzzle (14). IL17A is a powerful proinflammatory mediator secreted by Th17 cells (15).

Increased serum IL-17A levels have been shown in active BD patients, suggesting that Th17 cells and the IL-17 pathway has an important role in acute attacks of the disease (16, 17). Other studies have demonstrated increases in IL-23 and 27, also Th17 cytokines, rather than IL-17A (18).

IL-6 is a key cytokine in BD as it plays a role in differentiation of CD4⁺ T cells to Th17 cells (15, 19). Other cytokines thought to play a part in the pathogenesis of BD are IL-10, a potent suppressor of inflammatory cytokines, IL-12, a potent immunoregulatory cytokine, IL-18, a proinflammatory cytokine (4, 20).

The main limitation of this study was that all of the patients had been diagnosed in the past and were already receiving treatment. Medications, such as glucocorticosteroids and immunosuppressives reduce transcription of the proinflammatory cytokines (IL-8, TNF- α , IL-1 etc.). Colchicine has been shown to significantly decrease levels of IL-6,8 and TNF- α in patients with mucocutaneous BD to a normal level when used with levamisole (21). Cyclosporine can significantly inhibit the intraocular inflammation of BD patients and the expression of IL-17 and IFN- γ (22). IFN- α has been shown to inhibit IL-17 expression and increase IL-10 production by peripheral blood mononuclear cells and CD4⁺ T cells in vitro (23). Anti-TNF- α therapy suppresses effector T cell differentiation in BD patients with uveitis (24). Therefore, it is difficult to assess the true levels of cytokines in studies including patients taking such medications (7).

We did not find it appropriate to stop the use of medications for sample collection. Another limitation was that due to the setting of the study, the patient group was mostly composed of patients with mucocutaneous and/or ocular involvement, in whom systemic inflammation might have been less severe. Results of cytokine studies in BD should not be evaluated singly and should be viewed as a whole. Inconsistency of the results of various studies on serum cytokine levels in BD may be due to differences in patient characteristics, admission criteria, setting of the studies, and even ethnic differences.

In conclusion, BD is a disease involving complex interactions of cells of the immune system, mainly T lymphocytes and neutrophils. Various alterations in cytokine signaling seem to play a major role in its pathogenesis. Further research should focus on the immune interactions in BD.

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