# Serum Interleukin-23 Levels and Relationship with Clinical Symptoms of Behçet's Disease

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**ABSTRACT**

***Background:*** *Behçet's disease (BD) is a chronic, multisystemic vasculitis of unknown etiology.*

***Objectives:*** *This study was performed to evaluate the relation between IL-23 and clinical symptoms of BD.*

***Methods:*** *The study included twenty BD patients and twenty healthy control subjects matched for age and gender. Serum IL-23 levels were measured using human IL-23 enzyme-linked immunosorbent assay (ELISA) kit.*

***Results:*** *There was no significant difference in terms of demographic characteristics of BD patients and healthy controls. The mean serum IL-23 levels of BD and control groups were 273.39±225.37 pg/ml and 166.49±177.50 pg/ml respectively, and there was no significant difference between the groups. In BD group, 10 patients had active uveitis, 10 patients had active arthritis, and 14 patients had positive pathergy test. None of the patients had a major vascular involvement. Serum IL-23 levels were not related to uveitis, arthritis and pathergy test.*

***Conclusion:*** *IL-23 is not associated with clinical symptoms of BD.*

**Keywords:** Behçet's disease, clinical symptoms, interleukin-23

## 1. INTRODUCTION

BD is a multisystemic, chronic inflammatory vasculitis with unclear etiopathogenesis that is defined with recurrent oral ulcers, genital ulcerations and hypopyon iridocyclitis triad (Behçet, 1937).

In individuals with genetic predisposition, the disorders occurred in humoral and cellular immunity as a result of various environmental factors, the microorganisms in particular, and the resulting inflammation is held responsible for the pathogenesis of BD (Akpolat et al., 1992; Gül, 1997; Hegab and Al-Mutawa, 2000). The disease is believed to be occurred by the increase in immune complexes, lymphocyte chemotaxis and over-stimulation of B cells due to the resulting immunological inflammation (Alpsoy, 2003; Alpsoy and Akman, 2007; Borlu, 2007).

The immunological studies on BD was first started in 1963 by Oshima et al (Oshima et al., 1963) with the identification of an increase in levels of serum gamma globulin and the demonstration of the presence of autoantibodies against the oral mucosa in the circulatory system of BD patients. Since then, the studies continued to increase up to date by gaining a significant momentum. Today, studies on the immunopathogenesis of BD are focused on cellular immunity and T lymphocytes which are considered to play a key role in the pathogenesis (Kawai et al., 1978). Since there is no any specific laboratory evidence associated with the clinical activity and diagnosis of BD, diagnosis is based on clinical findings and follow-up of disease. Elucidating immunopathogenesis of BD will be significant in the diagnosis and follow-up of BD. Cytokines are prominent mediators of the immuno-inflammatory reactions (Sayinalp et al., 1996). In previous studies it was suggested that the Th 1-type immune response was dominant in BD. Among pro-inflammatory cytokines associated with BD, the Th-1 family of cytokines such as interferon-γ (IFN-γ), IL-12

and tumor necrosis factor-α (TNF-α), IL-17 and IL-18 are reported to be increased in BD (Hamzaoui et al., 2002; Raziuddin et al., 1998). Other factors are suggested to play a role in the pathogenesis of BD. IL-23 is pointed out to be a necessary mediator for organ-specific inflammatory autoimmune diseases (Yen et al., 2006). IL-23 allows the formation and growth of active $CD_4^+$ cells, and enables the activated $CD_4^+$ cells to produce IL-17, IL-17F, IL-6 and TNF (Langrish et al., 2005). In addition, it has also been demonstrated that IL-23 is able to increase and stabilize the Th17 cells in disease models and humans. It is thought that, IL-23 may contribute to the formation and development of BD by increasing the neutrophil migration (Langrish et al., 2005; Yen et al., 2006). The studies investigated serum IL-23 levels and relations between disease markers in BD is summarized in Table 1 (Chi et al., 2008; Ferrante et al., 2010; Habibagahi et al., 2010; Jiang et al., 2011;Lew et al., 2008; Liang et al., 2011;Na et al., 2013). In this study serum IL-23 levels was investigated in BD.

**Table 1.** Changes in the levels of IL-23 in Behçet's Disease

| | **Serum** | **EN like lesions** | **Uveitis** | **GIS involvement** | **Disease activity** |
|---|---|---|---|---|---|
| **IL-23** | + [15,16,17], - [18] | | + [19,20] | **-** [21] | +[19] |
| **IL-23 p19** | | + [18] | | | |

GIS: gastrointestinal system, EN: erythema nodosum, +: increased in Behçet's disease (BD) or in active BD, -: decreased in Behçet's disease (BD) or in active BD, IL-23 p19: IL-23 p19 mRNA expression in peripheral blood mononuclear cells (PBMCs). References are given inside the brackets.

## 2. MATERIALS AND METHODS

*2.1. Patients*

Twenty BD patients (male/female:13/7) fulfilling the criteria of International Study Group for BD and twenty healty subjects (male/female:14/6) were included to the study (International Study Group for Behçet's Disease, 1990). The demographic characteristics, clinical and laboratory findings were recorded by the same researcher. The patients with active disease had been using 1.5 mg/day colchicine and the patients with inactive disease had been using 0.5 mg/day. All patients had been stopped using other drugs such as corticosteoroids, cyclosporin, methotrexate, azathioprine etc. for 2 months.

*2.2. Biochemical analysis*

Blood samples were collected at early morning hours after night fasting. Serum IL-23 levels were measured in BD patients and healthy controls. Serum IL-23 levels were measured using human IL-23 enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, Vienna).

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

*2.3. Statistical analysis*

Data were analyzed using SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA). Results were expressed as mean±standard deviation. Differences were considered as significant when p value was less than 0.05 in 95 % confidence of interval. The associations between baseline clinical symptoms and serum IL-23 levels were analyzed with independent samples T test for categorical variables because they followed normal distribution by Kolmogorov-Smirnov test. Logistic regression was used to estimate the independent association of IL-23 and clinical symptoms.

## 3. RESULTS

There was no significant differences in terms of gender between groups (p=0.40). Mean ages was 38.5±9.5 years and mean disease duration was 7.5±5.2 in patient group. Mean ages of controls was 40.2±10.2 years and there was no significant difference between groups (p=0.25).

The mean serum IL-23 levels in the patient and control groups were 273.39±225.37 pg/ml and 166.49±177.50 pg/ml, respectively, and no significant difference were observed between the groups (p=0.10). In BD group, 10 patients (50%) had active uveitis, 10 patients (50%) had active arthritis, and 14 patients (70%) had positive pathergy test. None of the patients had major vascular involvement (Table 2). As shown in Table 2, serum IL-23 levels were not significantly different in patients with and without uveitis, arthritis and pathergy test.

A multinomial regression analysis was conducted using uveitis, arthritis, and pathergy test as dependent variables and serum IL-23 levels as independent variable (Table 3). The regression analysis did not reveal serum IL-23 levels as an independent predictor for uveitis, arthritis and positive pathergy test symptoms of BD. Regression analysis couldn't be performed for major vascular involvement symptom because the conditions for this analysis couldn't be assumed.

**Table 2.** Association between serum Interleukin-23 (IL-23) levels and clinical symptoms in Behçet's Disease (BD) group

| | | IL-23 (pg/ml) | | |
|---|---|---|---|---|
| | | **Patients with positive clinical parameter** | **Patients with negative clinical parameter** | **p value** |
| **Clinical symptoms** | **Uveitis** | 267.38±155.57 (n=10, 50%) | 279.49±287.57 (n=10, 50%) | 0.90 |
| | **Arthritis** | 312.92±297.89 (n=10, 50%) | 233.96±122.38 (n=10, 50%) | 0.44 |
| | **Major vascular involvement** | - (n=0, 0%) | 273.39±225.37 (n=0, 0%) | ND |
| | **Pathergy test** | 249.84±222.79 (n=14, 70%) | 328.49±242.22 (n=6, 30%) | 0.48 |

Results are expressed as mean ± standart deviation, ND: Not done, n: number of patients, %: percentage of patients have clinical finding are presented in the brackets

**Table 3.** Multinomial Logistic Regression Model for the Association Between IL-23 Levels and Clinical Symptoms of BD

| **Clinical symptom** | **β** | **p value** |
|---|---|---|
| **Uveitis** | 0.001 | 0.90 |
| **Arthritis** | 0.043 | 0.431 |
| **Major vascular involvement** | ND | ND |
| **Positive pathergy test** | 0.036 | 0.471 |

β:standardized regression coefficient, ND: Not done

## 4. DISCUSSION

Behçet's disease (BD) is a systemic vasculitis of unknown etiology that has a long-term trend with attacks. The widely acceptable hypothesis for the development of the disease today; is considered it as an irregular immune response developed in genetically predisposed individuals and triggered by autoantigens such as heat shock proteins (HSP) and/or by environmental antigens such as viral, bacterial, etc. The etiopathogenesis of the disease can be classified under three main sub-headings, in the light of the studies conducted in recent years; genetic factors, infectious agents and immunological changes. As a result of advances in molecular biology in recent years, the new information obtained on the structure and functions of the immune system elements indicate that the immune system plays a key role in the beginning or in the course of BD. The levels of cytokines, which have important functions in inflammation of BD, have specially increased in the active stage of the disease. Previous studies have demonstrated that chemokines, cytokine receptors and cytokines such as IFN-γ, TNF-α, TNFR75, IL-1, IL-2, sIL-2R, IL-6, IL-8, IL-12, IL-17, IL-18 and IL-22 originated from various cells had been increased in serum and/or plasma (Alpsoy et al., 1998; Hamzaoui et al., 1990; Hamzaoui et al., 2002;Lew et al., 1993; Sayinalp et al., 1996; Sugita et al., 2013;Turan et al., 1997).

It has also been reported that serum samples from patients with BD have caused proinflammatory activation of macrophages from peripheral blood. Additionally IL23R, IL12RB2 and IL10 are identified as Behçet's disease susceptibility loci in genome-wide association studies (Remmers et al., 2010). These recent reports suggest that Th1/Th17-type immune responses play a critical role in Behçet's disease and should be instrumental in the pathogenesis of disease symptoms. As a result, a large number of elements of the immune system are involved in the inflammatory attacks of the disease.

IL-23 is a heterodimeric cytokine having a specific p19 subunit and a p40 subunit shared with IL-12 which has additional inflammatory effects apart of IL-12 (Oppmann et al., 2000). IL-23 is pointed as the necessary mediator for organ-specific autoimmune diseases, but not IL-12. One of the remarkable differences between IL-23 and IL-12 is that, IL-23 stimulates the activated $CD_4^+$ cells and enables the activated $CD_4^+$ cells to produce IL-17, IL-17F, IL-6 and TNF whereas IL-12 stimulates the inactivated $CD_4^+$ cells [14]. Prior studies suggested that the IL-23/IL-17 axis plays a dominant role in the progression of chronic autoimmune inflammation in the central nervous system and joints (Langrish et al., 2005) and IL-23 plays the main role in some inflammatory autoimmune diseases (Chi et al., 2008; Yen et al., 2006). It has been demonstrated that IL-23-deficient rats were resistant to experimental autoimmune encephalomyelitis and collagen induced arthritis, the importance of this cytokine in the autoimmune pathogenesis is considerable. Additionally, it has also been demonstrated that IL-23 can increase and stabilize the Th17 cells in disease models and humans (Yen et al., 2006; Langrish et al., 2005). It is thought that, IL-23 may contribute to the formation and development of BD by increasing neutrophil migration.

Therefore, in this study the role of IL-23 in BD was investigated. The mean serum IL-23 levels in the patient and control groups were 273.39±225.37 pg/ml and 166.49±177.50 pg/ml, respectively, and there was no significant difference between the groups ($p>0.05$). correlation analysis showed no any significant relation between serum IL-23 levels and the clinical markers of disease activation such as active uveitis, peripheral arthritis, and pathergy test ($p>0.05$).

In a study conducted on BD patients, IL-23 p19 mRNA levels were significantly higher in the patients with BD having erythema nodosum-like skin lesions compared to healthy controls, but there was no significant difference between IL-23 p40 mRNA levels in groups and serum levels of IL-12 and IL-23 in BD and healty controls. The researchers have suggested that IL-23 p19 mRNA expression is associated with the formation of erythema nodosum-like lesions in patients with active BD. The researchers also compared serum IL-23 levels in a pairwise fashion at initial presentation and follow-up visits after beginning oral colchicine treatment within subgroups and no significant differences in IL-23 levels after oral colchicines therapy was reported. Therefore IL-23 could not be considered as a serological marker for reflecting the disease activity (Lew et al., 2008). Although serum IL-23 levels BD group and control group of cited study is very similar to those determined in our study, the insignificant difference of our study may be due to the limitation that we had small patient samples.

In conclusion, our results indicate that serum IL-23 levels were not related to uveitis, arthritis and pathergy test in patients with BD. Studies to be conducted in large series examining IL-23 levels on tissue samples and IL-23 p19 and p40 mRNA levels can provide useful information about the relation of IL-23 and clinical symptoms BD.

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