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Effect of Colchicine on Th1 and Th17 Cytokines, Cytokine Receptors, and Chemokine Gene Expression Profiles in Behçet's Disease

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Abstract Objective: Colchicine has been used in recent years as an effective drug for controlling attacks in Behçet's disease. In the present study, we investigated expression levels of IL1R, IL2R, IL12RB, IL23R, IL17, CXCR3, CXCR10 and IL8 genes in patients with active and inactive Behçet's disease. We also evaluated how colchicine use in patients with active and inactive disease affected these genes and evaluated their role in the etiopathogenesis of the disease. Methods: Thirty-five patients who were diagnosed with Behçet's disease according to the International Working Group criteria (28 with active disease, 7 inactive) and were taking colchicine were enrolled in the study. Twenty healthy subjects were included as a control group. Expression levels of the IL1R, IL2R, IL12RB, IL23R, IL17, CXCR3 ,CXCR10 and IL8, genes were evaluated. Results: Expression levels of CXCR3 and IL23R were significantly lower in patients with active Behçet's disease when compared with the inactive disease and control groups. However, the differences in CXCR3 and IL23R expression between the inactive Behçet's patient group and the control group were nonsignificant. Expression levels of the other genes did not differ statistically between the active Behçet's patients, inactive Behçet's patients, and control subjects. Conclusion: While the expression levels of the CXCR3 and IL23R genes in active Behçet's patients were statistically lower than controls, there was no statistical difference between active and inactive Behçet's patients or controls in terms of IL1R, IL2R, IL17, IL12RB, CXCR10 and IL8, gene expression levels. This study may form the basis for further studies to determine the molecular mechanism of colchicine in the treatment of Behçet's disease.

Keywords: Behçet's disease, Chemokine, Cytokine, Th1, Th17

1. Introduction

Behçet's disease (BD) is a chronic systemic inflammatory condition characterized by recurrent mucocutaneous findings and manifests with ocular, articular, vascular, neurological, pulmonary, and gastrointestinal multisystem involvement.

The etiopathogenesis of BD is poorly understood. However, it is currently thought that environmental factors trigger the immune system in genetically predisposed individuals, and that neutrophils, endothelial damage, and thrombotic tendency also contribute to the etiopathogenesis. [1-5] BD occurs all over the world and in nearly all races, but is more common in the northern hemisphere, particularly in countries along the historic silk road. [6-8]

There are two groups of genes implicated in BD pathogenesis: those located within the major histocompatibility complex (MHC) locus, and those not in the MHC locus (non-MHC genes). HLA-B51, MCH class I chain related gene A (MICA), and tumor necrosis factor (TNF) are MHC genes. Of these, the HLA allele HLA-B51 is most strongly associated with BD. [9,10]

The major cell types involved in the pathogenesis of BD are Th1, Th17, gamma delta (γδ) T cells, cytotoxic T cells, and regulatory T cells (Tregs). Data from previous studies indicate that the Th1/Th2 ratio is skewed toward Th1 cells in BD. Likewise, Th1 type cytokines are predominant in BD patients. According to the literature, TNF-alpha (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-1, IL-8, IL-12, IL-18, soluble IL-2R and TNF receptor (TNFR) are elevated in BD. It has also been determined that these cytokines and receptors are associated with clinical activity in BD. Increased IFN-γ levels have also been reported in patients who are in remission. [12-14]

Th17 cells have been implicated in many autoimmune and inflammatory diseases. IL-23 stimulates IL-17 secretion from Th17 cells, increasing T cell expression and induces the production of numerous pro-inflammatory mediators such as IL-1, IL-6, TNF-α, and nitric oxide synthase (NOS) by stimulating fibroblasts, endothelial cells, macrophages, and epithelial cells. It has been suggested that Th17 cells and IL-17 may be especially important in acute attacks of BD. IL-17 promotes the release of cytokines such as TNF, IL-1, IL-6, and IL-8 from monocytes, epithelial and endothelial cells, which causes neutrophil migration and activation and results in neutrophil-mediated inflammatory response. As the IL-17 pathway may play an important role in the communication between lymphocytes and neutrophils in BD patients, cytokines associated with this pathway may be important targets for treatment. IFN-α is shown to increase IL-10 levels in peripheral blood mononuclear cells (PBMCs) and inhibit IL-17 expression. Numerous studies have indicated that the IL-23/IL-17 pathway may be a new therapeutic target in the treatment of chronic inflammatory diseases. [15-21]

Compared to healthy controls, Th17 levels are associated with disease activity in patients with BD. In one study, levels of IL-17 were significantly elevated in BD, particularly during periods of active disease, and serum IL-23 concentration was reported to increase with BD activity. [16-18]

In genetically predisposed individuals, upregulation of IL-12 receptors has been shown to promote IFN-γ expression in Th0 cells and skew the Th1/Th2 balance towards Th1 dominance. Upregulation of IL-23 receptor, which shares a common p40 subunit with IL-12, induces Th0 transformation to Th17 and stimulates Th17 cells to release proinflammatory cytokines such as TNF-α, IL-1, and IL-6. [17]

Chemokines and receptors are instrumental in many biological processes. Chemokines are involved in the interactions between leukocytes and endothelial cells, chemotaxis, T and B cell maturation, developing immunity, intracellular communication between T and B cells, and primary immune response. During inflammation, chemokines and chemokine receptors mediate transendothelial migration from within blood vessels across the endothelial cell wall, and control cell traffic to the site of inflammation.

IL-8 is the most important member of the CXC family and is the main chemokine that induces neutrophil chemotaxis to the site of infection/inflammation. In addition to neutrophil chemotaxis, IL-8 also promotes neutrophil adhesion and degranulation, and enhances their microbicidal effect. [22]

CXC chemokines likely play an important role in the neutrophil activation that occurs in the etiopathogenesis of BD. In particular, the CXCR3 chemokine receptor is expressed on active Th1 cells, and studies have provided evidence that CXCR3 is the primary inflammatory chemokine receptor involved in BD. Recent studies have determined that serum CXCL10 level is associated with disease activity in BD. [23,24]

There is no definitive treatment for BD. The cause of the inflammation has yet to be determined. Until recently, the principal strategy of BD management was to induce immunosuppression by disrupting immune system pathways in a broad, nonspecific way using drugs such as corticosteroids and cyclosporin. However, colchicine therapy is now regarded as the main treatment for BD because it reduces the severity, duration, and frequency of attacks or provides complete remission

for most patients. Some studies have shown that genital and ocular lesions respond well to colchicine, and that articular and cutaneous lesions also improve with colchicine. [25]

In the present study we investigated how colchicine affects gene expression by conducting expression profile analysis of IL1R, IL2R, IL12R, IL23R, IL17, CXCR3, CXCR10 and IL8, mRNA from peripheral blood samples of patients with active BD (exhibiting at least one sign of BD) and patients with inactive BD.

2. Materials and Methods

2.1. Material Collection

This research was carried out at the Department of Dermatology and the laboratory of the Department of Medical Biology in Atatürk University Faculty of Medicine. The study was approved by the local ethics committee and included a total of 35 patients diagnosed with BD according to the International Study Group criteria and receiving colchicine, and 20 healthy individuals as a control group. Twenty-eight of the BD patients (21 males, 7 females; mean age 37.4 ± 41.8 years) had active disease (11 with positive pathergy test, 14 with oral aphthae, 4 with skin lesions, and 11 with uveitis). The other 7 BD patients exhibited no signs of the disease during the study period (inactive disease). Eleven of the 35 BD patients were HLA-B51 allele carriers. All patients were using 0.5 mg/kg colchicine. Patients with known rheumatologic or autoimmune diseases other than BD were not included in the study. The patients underwent detailed clinical examinations and the findings were recorded. Active disease was defined as the presence of one or more of the following signs: oral aphthae, genital ulcers, erythema nodosum, arthritis, ocular findings, thrombosis, neurological involvement, or gastrointestinal involvement. Written informed consent forms were obtained from all patients.

2.2. Gene Expression Analysis

Total RNA was isolated using MagNA Pure LC Instrument from the cells of venous blood samples. RNA concentration was determined using a NanoDrop spectrophotometer (MaestroNano).RNA sampleswere stored at -80 ℃ until used. Total RNA (1 µg) served as a template for first-strandcDNA synthesis in a 20 µl reaction using the Tanscriptor First Strand cDNA synthesis kit (Roche).). Each reaction included 4 µL buffer, 2 µL deoxynucleotide mix, 0.5 µL Reverse Transcriptase, 0.5 µL RNase inhibitor, 1 µL distilled water, 10 µL RNA, and 2 µL Random Primer. The reactions were incubated at 25 °C for 10 minutes, 55 ℃ for 30 min, and 85 ℃ for 5 min. Quantitative real-time PCR (qPCR) wasperformed using the Roche 480 Light Cycler for the IL1R, IL2R, IL17, IL12RB, IL23R, CXCR3, CXCR10 and IL8 genes. All samples were analyzed in duplicate. Using the Roche LightCycler 480 relative quantification assay method,. The cycle threshold for each sample was calculated and relative mRNA abundance determined based on that of the β-actin and G6PDH control. SPSS version 17 software was used for data analysis. P values less than 0.05 were accepted as statistically significant.

3. Results

The mRNA expression levels of IL23R and CXCR3 were significantly lower in the BD patients compared to controls ($p<0.001$), whereas no significant differences were observed in IL1R, IL2R, IL12RB, IL17, CXCR10 or IL8 (Table 1). There were no significant differences in the mRNA expression levels of any of the studied genes between patients with inactive BD and healthy controls ($p>0.001$).

4. Discussion

BD is a vasculitic, chronic inflammatory disease characterized by recurrent oral and genital aphthous ulcers, uveitis and skin lesions. It can affect all types and sizes of vessels and involve multiple systems such as the central nervous system, lungs, and gastrointestinal system. Although the exact cause of BD is unknown, an unidentified infectious agent may play a role. This is supported by the presence of neutrophil hyperfunction and an increased CD8/CD4 ratio. Immunodeficiencies triggered by genetic predisposition and certain environmental factors seem to play a role in the etiopathogenesis of BD.

Th1, Th17, γδ T cells, cytotoxic T cells, and Treg cells are the major players in BD pathogenesis. Some studies have also emphasized the influence of Th2 autoreactive T cells in the etiopathogenesis. [26-30]

Table 1. Comparison of messenger RNA (mRNA) expression levels of the IL1R,IL2R, IL12RB,IL23R, IL17, CXCR3 CXCR10 and IL8genes in the active, inactive, Beh çet's disease and control groups

mRNA Expression	Aktive group n=28 median (min-max)	İnactive group n=7 median (min-max)	Control group n=20 median (min-max)	P value
IL1R	8.71 (1.22-22.18)	6.53 (4.11-17.57)	6.99 (3.14-15.36)	>0.001
IL2R	13.41 (1.12-9.56)	14.74 (1.81-8.54)	10.21 (6,37-15.41)	>0.001
IIL12RB	2.12 (0.55-7.22)	3.67 (1.21-8.01)	4.08 (2.30-8.10)	>0.001
IL23R	3.02 (0.11-5.74)	17.99 (4.41-10.80)	20.38 (5.45-12.65)	<0.001
IL17	1.01 (0.23-1.71)	1.41 (2.59-3.09)	0.99 (0.12-1.99)	>0.001
CXCR3	6.53 (0.88-5.77)	15.94 (2.44-8.07)	19.33 (2.29-10.00)	<0.001
CXR10	5.11 (1.10-9.79)	7.44 (2,04-12.21)	5.99 (0.94-7.54)	>0.001
IL8	4.09 (0.24-2.31)	5.49 (0.99-5.08)	6.68 (1.60-9.09)	>0.001

Th1 cytokines have been associated with disease activity. Elevated TNF-α, IFN-ɣ, IL-8, IL-12, IL-18, soluble IL-2 receptor (sIL-2R) and TNFR-75 levels have been reported in BD. Correlations were also observed between IL-8, IL-12, sIL-2R, and TNFR-75 levels and clinical activity of BD. Furthermore, it has been shown that BD patients have more δ T cell in the blood and that these cells intensely secreted TNF-α and IFN-ɣ. [11,16]

There is a paucity of serum level, polymorphism, and expression data in the literature regarding the relationship between BD and IL1R. However, Ertenli et al. analyzed cytokine levels in synovial fluid in BD patients and determined that IL-1Ra levels were high. In their polymorphism study, Öz ġimen et al. showed that there was no significant difference between Turkish BD patients and healthy controls in terms of IL-1Ra and IL-1α gene polymorphism.[31,32] In the present study, we observed no significant differences between the active BD group, inactive BD group, and control group in our molecular analysis of IL1R mRNA expression.

IL-2R is the target of many IL-2 inhibitors used in the management of BD. The high IL-2 levels seen in many diseases are associated with immune system hyperactivation. Evereklıoğlu et al. reported that serum sIL-2R levels in active BD patients ranged from 347 to 1076 U/ml, and stated that immunosuppressive agents such as hydrocortisone, cyclosporine, and cyclophosphamide commonly used in the treatment of BD reduce IL-2 synthesis. Clinically, high levels of sIL-2R are associated with strong activation of the immune system, such as in atopic dermatitis and psoriasis. Evereklıoğlu et al. also reported that serum sIL-2R levels were significantly higher in patients with BD compared to healthy controls, and showed that serum sIL-2R levels were significantly higher in patients with active BD than in patients with inactive BD. In the present study, our analysis of IL2R mRNA expression level revealed no significant differences between BD patients using colchicine and the inactive disease and control groups. [31-34]

Th17 cells have been implicated in many autoimmune and inflammatory diseases. IL-23 is one of the main cytokines that stimulate IL-17 expression in Th17 cells. The IL-23 receptor, IL23R, is expressed on the surface of Th17 cells and macrophages. IL-23 shares a p40 subunit with IL-12, and alteration or upregulation of the IL-23 receptor induces Th0 transformation to Th17, and causes release of TNF-α, IL-1, and IL-6 proinflammatory cytokines from Th17 cells [16].

Th17 cells are known to play a key role in IL-17 production in neutrophil inflammation and autoimmune diseases, and the disease-associated alleles may increase IL-23 receptor expression or signaling compared to protective alleles. While there is strong evidence that disease-associated variants affect susceptibility to BD via IL23R, it is also possible that the variants have an alternative or additional role in modulating expression of IL12RB2, another gene near the variants. The IL12RB2 gene codes for IL-12 receptor beta2, a subunit of the IL-12 receptor. IL-12 has an important role in Th1 responses by T cells and NK cells, NK cell cytotoxicity, and IFN-γ production. IL-12R β2 has been reported to be essential for high affinity IL-12 binding. It has the ability to signal IL-12 and IL-12 and has an important role in Th1 cell differentiation. [17,35]

Single nucleotide polymorphisms in the IL12R and IL23R genes have been implicated in the pathogenesis of BD. In genetically predisposed individuals, an increase in IL-12 receptors has been shown to promote IFN-γ expression in Th0 cells and skew the Th1/Th2 balance towards Th1 dominance. The IL23R–IL12RB2 locus was identified in two early genome-wide association (GWA) studies as having genome-wide significance. One of these studies was by Mizuki et al., conducted in a Japanese cohort including 612 individuals with BD and 740 unaffected control subjects. They conducted IL23R-IL12RB2 rs12119179 SNP analysis and obtained significant results in addition to Turkish and Korean genome-wide studies. However, other studies in Middle Eastern Arab, Greek, Korean, and British populations failed to replicate these variants The susceptibility of the major allele of rs924080 was confirmed in the Iranian population in another recent study. On the other hand, two low-frequency missense variants of IL23R, p.Arg381G and p.Gly149Arg, inhibit receptor response to IL-23 stimulation and were shown to be protective against BD. Yu et al. reported in their study that they confirmed the association of IL10/rs1800871 and IL23R-IL12RB2/rs924080 with BD in the Chinese population. In the present study, there was a statistically significant decrease in IL23R mRNA expression in the active patient group, but no difference was observed between the active BD, inactive BD, and control groups in terms of IL12R expression. [36-47]

It has been reported that increased IL-17 may be associated with the clinical activity of BD, and plays a particularly important role in the active phase of the disease .IL-17 increases production of additional proinflammatory cytokines such as TNF, IL-1, IL-6, and IL-8 in monocytes, epithelium, and endothelium, thus promoting neutrophil migration and activation, resulting in a neutrophil-mediated inflammatory response. However, a serum study showed no significant differences in serum IL-17A between BD patients and controls. [16,19,48-50]

Chi et al. reported that mRNA expression levels of the IL-23, IL-17, and IFN-γ genes were elevated in active BD patients with uveitis. They also observed significantly higher frequencies of IL-17-producing T cells from PBMCs in BD patients with active uveitis. The results of another expression study by Wei Chi et al. showed that cyclosporin A significantly inhibited the production of IL-17 and IFN-γ both in vivo and in vitro, which was believed to account for its inhibitory effect on intraocular inflammation in BD.[51,52] In our expression study, we observed no statistically significant difference between IL-17 mRNA expression levels in the active BD patients using colchicine, patients with inactive BD, and healthy controls.

Chemokine receptors have been used as cell surface markers to distinguish type 1 and type 2 T cells, but Th1/Tc1 cells characterized as CXCR3- and CCR5-expressing. However, chemokines and receptors can also be classified based on function, with chemokines grouped as inflammatory or homeostatic Inflammatory chemokines are often upregulated in nonlymphoid tissues under inflammatory conditions and are key factors in effector T lymphocyte recruitment. [53]

Houman et al. determined by flow cytometry that in active BD, CXCR3 was the predominant cytokine receptor expressed in CD3+ T cells. In a comparative expression analysis of BD uveitis and non-BD uveitis, Kim et al. found that the expression of CXCR1 and CCR5 did not differ significantly, but CXCR3 expression was higher in the CD4+ cells of non-BD patients and in the CD8+ cells of BD patients. In addition, intraocular CXCL8 and CXCL10 levels were higher the BD patients compared to the non-BD patients. [53-59]

Lee et al. reported that serum CXCL10 levels were associated with disease activity. They also observed that CXCR3-positive cells were present at the same frequency in the peripheral blood of BD patients and controls, but were significantly more prominent in the cutaneous lesions of BD patients. Therefore, they concluded that the CXCL10/CXCR3 axis had a role in the development of BD, especially mucocutaneous lesions, and suggested that serum CXCL10 level may serve as a marker of disease activity in BD. In the present expression study, there was a statistically significant decrease in CXCR3

mRNA expression in the active patient group, whereas the expression of CXCR10 did not differ between the patient and control groups. [23,60]

IL-8 is involved in neutrophil activation, chemotaxis, and angiogenesis. There are numerous studies in the literature investigating IL-8 polymorphisms and serum levels, and several have reported that IL-8 is elevated in the active phase of BD. Gür-Toy et al. determined that IL-8 level was more closely associated with disease activity than acute phase reactants . Durmazlar found that IL-8 levels were higher in BD patients with vascular involvement compared to those with oral or genital ulceration or uveitis. Their results may lead to the use of IL-8 level as a marker of disease severity. [61-65]

While Alireza Sadeghi et al. and Durmazlar et al. were unable to establish an association between IL-8 and Behçet's uveitis in their studies, Bardak et al. and Nalbant et al. found that IL-8 levels were significantly lower in these patients. Inanır et al. showed in a serum study that IL-8 levels were higher in active patients than in inactive patients. Karadağ et al. also demonstrated that serum IL-8 levels were significantly higher in patients with active Behçet's disease compared to those with inactive disease and the control group. In addition, Zouboulis et al. reported that IL-8 levels were higher in patients with active BD, especially those with oral and neurological findings. Bardak et al. also observed higher IL-8 and TNF-α levels in BD patients during periods of active uveitis compared to the same patients during disease inactivity, as well as healthy controls. [63-68]

According to our expression study, there was no statistically significant difference in IL8 mRNA levels between the active BD group, the inactive BD group, and control group.

In summary, this study investigated whether the molecular mechanism of colchicine may influence the expression of IL1R, IL2R, IL12RB, IL23R, IL17, CXCR3, CXCR10 andIL8 genes. We determined that two genes (IL223R and CXCR3) were downregulated in patients with active BD. These data may provide a basis for future studies.

Conflict of Interest

None

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