

Comparison of serum levels of IL-6, IL-8, TNF- α , C reactive protein and heat shock protein 70 in patients with active or inactive Behçet's disease

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Aim: The aim of this study was to elucidate serum levels of IL-6, IL-8, TNF- α , CRP, and HSP 70 in patients with active or inactive Behçet's disease.

Materials and methods: The study included 50 patients who met the International Study Group criteria for Behçet's disease. Of these, 26 had active disease and 24 had inactive disease. The control group was comprised of 25 age- and sex-matched healthy participants. Serum levels of IL-6, IL-8, TNF- α , CRP, and HSP 70 were measured.

Results: In patients with active disease, significantly higher mean serum levels of IL-6, IL-8, TNF- α , and CRP were found compared to patients with inactive disease or in controls (overall, $P < 0.05$). In patients with active disease, the mean serum level of HSP 70 was significantly higher than that of the control group ($P = 0.02$). In patients with inactive disease it was higher compared to the controls but with a significance level close to 0.05 ($P = 0.044$). However, HSP 70 levels did not differ significantly between patients with active disease and patients with inactive disease ($P = 0.93$).

Conclusion: The results of our study suggest that serum IL-6, IL-8, TNF- α , and CRP levels are increased in patients with active Behçet's disease. HSP 70 levels in both active and inactive disease groups were higher than in controls, though it was significant only in the active group. This suggests that HSP 70 has a role in the chronic nature of Behçet's disease, with HSP 70 expression possibly not falling to normal levels in the inactive phase of the disease.

Key words: Behçet's disease, chemokine, C-reactive protein, cytokine, heat shock protein, interleukin

Aktif ve inaktif Behçet hastalarında serum İL-6, İL-8, TNF- α , C reaktif protein ve ısı şok protein 70 düzeylerinin karşılaştırılması

Amaç: Bu çalışmanın amacı, aktif ve inaktif Behçet hastalarında serum İL-6, İL-8, TNF- α , CRP ve HSP 70 düzeylerini araştırmaktır.

Yöntem ve gereç: Çalışmaya Uluslararası Çalışma Grubunu kriterlerine göre Behçet hastalığı tanısı almış 50 hasta alındı. Bunların 26'sı aktif ve 24'ü inaktif hastalığa sahipti. Kontrol grubu yaş ve cinsiyet olarak çalışma grubuna benzeyen 25 sağlıklı bireyden oluşmaktaydı. Serum İL-6, İL-8, TNF- α , CRP ve HSP 70 düzeyleri ölçüldü.

Bulgular: Ortalama serum İL-6, İL-8, TNF- α ve CRP düzeyleri aktif hastalığı olanlarda, inaktif hastalığı olanlar ve kontrol grubuna göre anlamlı şekilde yüksek bulundu (hepsinde, $P < 0,05$). Ortalama serum HSP 70 düzeyi aktif hastalığı olanlarda kontrol grubuna göre anlamlı şekilde yüksek iken ($P = 0,02$), inaktif hastalığı olanlarda kontrol grubuna göre sınırda anlamlı yüksek bulundu ($P = 0,044$). Bununla birlikte aktif ve inaktif hastalar arasında anlamlı fark tespit edilmedi ($P = 0,93$).

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Sonuç: Aktif Behçet hastalarında serum IL-6, IL-8, TNF- α ve CRP düzeyleri artmaktadır. HSP 70 düzeyi aktif hastalarda belirgin olmak üzere hem aktif hem de inaktif hasta grubunda kontrol grubuna göre yüksek bulunmaktadır. Bu da, HSP 70'in Behçet hastalığının kronik seyirinde rol aldığını ve inaktif hastalık fazında HSP 70 düzeyleri normal değerlerine kadar yeterince düşmediğini göstermektedir.

Anahtar sözcükler: Behçet hastalığı, kemokin, C-reaktif protein, sitokin, ısı şok protein, interlökin

Introduction

Behçet's disease is a recurring inflammatory disorder characterized by 4 major findings: oral aphthous ulcers, ocular lesions, skin lesions, and genital ulcerations, with inflammation also occasionally occurring in other tissues and organs, such as the cardiovascular system, central nervous system, gastrointestinal tract, lungs, kidneys, and joints (1). The causal mechanism of Behçet's disease has not been determined, but genetic, infectious, and immune mechanisms have been proposed (1).

A role for autoimmunity in Behçet's disease is suggested by reports of increases in immunoglobulins, immune complexes, and complement and acute phase proteins (2,3). Given the immune activation that occurs in Behçet's disease, the course of the disease might also be influenced by pro-inflammatory cytokines (4,5). These proteins are produced by various cell types and are important mediators of immune and inflammatory responses (6).

Another class of proteins important in infection and immunity, and possibly in Behçet's disease, is that of the heat shock proteins, which are a group of proteins that are common to many organisms, they range in molecular weight from 8 kDa to 150 kDa, and are rapidly produced by most cells in response to environmental stresses (7,8). Heat shock proteins are capable of eliciting immune responses (9), have been proposed as a source of cross-reactivity that could link infection and autoimmunity (10), and may play a role in regulating immune response pathways (8). Among the heat shock proteins, heat shock protein 70 (HSP 70) has been reported to have both pro-inflammatory (11,12) and anti-inflammatory (13-17) properties. In its pro-inflammatory role, HSP 70 appears to bind with receptors on antigen presenting cells and to stimulate cytokine secretion (12), as well as facilitating antigen presentation (18). In its anti-inflammatory

role, HSP 70 decreases the release of inflammatory mediators (15-17). The administration of recombinant HSP 70 has been reported to attenuate the experimental autoimmune disease (19). Heat shock proteins appear to be involved in the etiology of Behçet's disease, but the details of this involvement are not yet clear (7,11).

Behçet's disease is generally diagnosed based on the clinical criteria established by the International Study Group for Behçet's disease (20). To date, increased serum levels of biochemical parameters, such as cytokines (3-5), lipoprotein A (21), and C reactive protein (CRP) (22) have been proposed as disease activity markers; however, no single parameter has been widely accepted as an indicator of disease activity.

Given the intricate involvement of cytokines and heat shock proteins in inflammation and possibly in Behçet's disease, as well as the lack of a marker for activity in this disease, the aim of the present study was to investigate serum levels of cytokines, CRP, and HSP 70 in patients with active or inactive Behçet's disease.

Materials and methods

This cross-sectional study included 50 patients who met the International Study Group criteria for Behçet's disease (20) (13 male, 37 female, mean age 34.8 ± 8.8 years). Of these, 26 had active disease and 24 had inactive disease. Age- and sex-matched 25 healthy participants (7 male, 18 female, mean age 34.1 ± 6.0 years) were enrolled in the study as controls.

Active Behçet's disease was defined as the presence of at least 2 of the characteristic findings according to the Behçet's Disease Current Activity Form (23). Patients who had been free of lesions for the previous 30 days or more were regarded as having inactive disease. According to these criteria, 26 patients had

active disease (19 female, 7 male, mean age 35.5 ± 8.3 years) and 24 had inactive disease (18 female, 6 male, mean age 34.0 ± 9.5 years). Patients with acute infection or any inflammatory disease other than Behçet's disease and who were on medications that may affect serum levels of cytokines, HSP-70, and CRP were not included in the study.

Venous blood samples were taken from the antecubital vein for biochemical assays. The blood samples were centrifuged at $1000 \times g$ for 15 min at $4^\circ C$ to separate the serum. Serum samples were immediately stored at $-80^\circ C$ until analysis. Serum levels of CRP were determined using a nephelometer (Image, Beckman Coulter, CA, USA). For the other parameters, serum levels were determined with enzyme-linked immunoassay kits as follows: IL-6 (Biosource International, CA, USA), IL-8 (Orgenium, Finland), TNF- α (Biosource International, CA, USA), and HSP 70 (Stressgen, Canada). IL-6, IL-8, and TNF- α were expressed as pg/mL, HSP 70 was expressed as ng/mL, and CRP was expressed as mg/mL.

Statistical analyses were performed with SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean \pm standard deviation. The data were normally distributed. Analysis of variance (ANOVA) and Tukey's Honestly Significant Differences test were used to compare the means of the 3 groups; significance was defined as $P < 0.05$.

Results

Clinical characteristics and laboratory findings for patients with active or inactive disease are summarized in Table 1.

In patients with inactive disease, serum levels of all biochemical parameters measured were similar to those in the control group, and no significant differences were found between these groups. In patients with active disease, serum levels of IL-6, IL-8, TNF- α , and CRP were significantly higher compared to either the inactive disease group or the control group.

In patients with inactive disease, the mean serum HSP 70 concentration was just at the level of significance, being higher than in the control group. In patients with active disease, the mean HSP 70 concentration was significantly higher compared to the controls ($P = 0.02$); however, between patients with active disease and those with inactive disease, the difference in HSP 70 was not significant ($P = 0.93$).

Discussion

Behçet's disease is a chronic inflammatory disorder that can involve multiple organ systems, and is characterized by exacerbations and remissions whose mechanisms are still unclear (1,7,11). Inflammatory mediators have been investigated in

Table 1. HSP 70 and inflammatory mediator levels in patients with active or inactive Behçet's disease.

	Control (n = 25)	Inactive (n = 24)	Active (n = 26)	p (C-I)	p (I-A)	p (C-A)
Age *	34.1 ± 6.0	34.0 ± 9.5	35.5 ± 8.3		0.53	
Gender (F/M) $^{\epsilon}$	18/7	18/6	19/7		0.97	
CRP \pm SD $^{\zeta}$	0.29 ± 0.29	0.32 ± 0.20	0.49 ± 0.28	0.68	0.04	0.03
HSP 70 \pm SD $^{\zeta}$	622.11 ± 244.20	871.92 ± 560.48	909.44 ± 251.92	0.044	0.93	0.02
IL-8 \pm SD $^{\zeta}$	14.48 ± 5.15	20.26 ± 8.55	27.41 ± 12.95	0.107	0.035	<0.001
IL-6 \pm SD $^{\zeta}$	13.36 ± 0.51	14.34 ± 1.79	15.68 ± 1.99	0.077	0.008	<0.001
TNF- α \pm SD $^{\zeta}$	8.67 ± 2.01	10.56 ± 3.21	13.18 ± 4.38	0.162	0.032	<0.001

Abbreviations: C: Control group, I: Inactive disease group, A: Active disease group, F: Female, M: Male

* Student's t test,

$^{\epsilon}$ Mann-Whitney U test,

$^{\zeta}$ ANOVA test used.

patients with Behçet's disease, and some of these mediators appear to be associated with the clinical activity of the disease (24).

IL-6 is a glycoprotein secreted mainly by activated monocytes and macrophages, but it is also produced by many other cell types. It influences antigen-specific immune responses and inflammatory reactions, and has been found to be abnormally increased in some autoimmune diseases and chronic inflammatory reactions (25). IL-6 has been found increased in some studies in the serum of patients with Behçet's disease (3-5) ; however, other studies have found similar serum IL-6 levels in patients with active Behçet's disease and patients with inactive Behçet's disease, (26) and in patients with active Behçet's disease and healthy control subjects (6). Among our patients, serum IL-6 levels were significantly higher in those with active compared to those with inactive disease, which suggests that IL-6 is involved in the mechanism that determines the activity of the disease.

IL-8 is a cytokine that attracts and activates leukocytes in addition to attracting T cells and basophils. IL-8 is also involved in the conversion of mononuclear to granulocytic infiltration and in the enhanced adhesion of peripheral blood leukocytes to endothelial cells. IL-8 is mainly produced by monocytes/macrophages, T cells, granulocytes, and endothelial cells, but can also be produced by fibroblasts, keratinocytes, hepatocytes, and chondrocytes (27). In patients with Behçet's disease, some studies have found IL-8 levels to be higher in the active phase of the disease (3,4) while others have found it to be unchanged (28). This difference in findings may be due to the fact that different criteria for active disease were used in some studies. Our finding that IL-8 levels were significantly higher in patients with active versus inactive disease is consistent with the role of IL-8 in the inflammatory response and its attraction of polymorphonuclear cells into lesions, which is seen in Behçet's disease.

TNF- α is a cytokine secreted by lymphocytes and reticuloendothelial cells, and its serum levels are increased in many acute and chronic inflammatory diseases (29). As with the other cytokines, some previous studies involved patients with Behçet's disease have found TNF- α levels to be increased in the active phase relative to the inactive phase of the

disease, (3,23) while other studies have found no difference (6,27). We found TNF- α levels to be higher in patients with active disease. CRP levels were also higher in our patients with active disease relative to those with inactive disease, as would be expected given the increases in CRP that are generally seen in acute diseases.

Heat shock proteins, especially HSP 60/65, have been implicated in the etiology of Behçet's disease, for example as antigens providing for cross-reactivity against infectious agents and host tissues (11). However, specific roles of different heat shock proteins in Behçet's disease are unclear. For example, antibodies against HSP 70 were found in patients with Behçet's disease, but serum levels of these antibodies were not correlated with serum HSP 70 levels (30). This raises the question of whether the occurrence of HSP 70 in the course of Behçet's disease might be a reaction against the pathogenesis rather than a contributor to it.

The possibility that HSP 70 expression is an anti-inflammatory response is supported by several studies. In a study of experimental autoimmune uveoretinitis in mice, Kitamei et al. (13) found that induction of HSP 70 expression reduced the histopathological severity of the disease. In humans taking Diclofenac, Yanaka et al. (14) administered the drug geranylgeranylacetone to induce HSP expression, and observed a reduction in the damage to gastric mucosa caused by the former drug. In experimental stroke in mice overexpressing HSP 70, Zheng et al. (15) found that the inflammatory transcription factor nuclear factor κ B was inhibited. In vitro studies also appear to attribute an anti-inflammatory role for HSP 70. In enterocyte-like cells, Malago et al. (16) used Salmonella to induce IL-8 expression, and concluded that IL-8 down-regulation might be in part mediated by the production of HSP 70. In fibroblast-like synoviocytes, Luo et al. (17) used TNF- α to induce production of IL-6, IL-8, and monocyte chemoattractant protein-1 and found that HSP 70 could downregulate these cytokines.

In conclusion, we found that IL-6, IL-8, TNF- α , and CRP levels were significantly higher in patients with active Behçet's disease than in those with inactive disease. These molecules might therefore be useful in monitoring the effect of therapy in patients with

Behçet's disease. HSP 70 levels in both active and inactive disease groups were higher than in controls, though it was significant only in the active group, which suggests that HSP 70 has a role in the chronic nature of Behçet's disease, with HSP 70 expression in

the inactive phase possibly remaining slightly higher than the baseline levels before the original onset of the disease. Further studies are warranted to elucidate the role of these parameters on management and prognosis of Behçet's disease.

References

- Zierhut M, Mizuki N, Ohno S, Inoko H, Gül A, Onoé K et al. Immunology and functional genomics of Behçet's disease. *Cell Mol Life Sci* 2003; 60: 1903-22.
- Lehner T, Adinolfi M. Acute phase proteins, C9, factor B, and lysozyme in recurrent oral ulceration and Behçet's syndrome. *J Clin Pathol* 1980; 33: 269-75.
- Bardak Y, Aridogan BC. The demonstration of serum interleukin 6-8, tumor necrosis factor-alpha, complement, and immunoglobulin levels in Behçet's disease with ocular involvement. *Ocul Immunol Inflamm* 2004; 12: 53-58.
- Evereklioglu C, Er H, Türköz Y, Cekmen M. Serum levels of TNF-alpha, sIL-2R, IL-6, and IL-8 are increased and associated with elevated lipid peroxidation in patients with Behçet's disease. *Mediators Inflamm* 2002; 11: 87-93.
- Akdeniz N, Esrefoglu M, Keleş MS, Karakuzu A, Atasoy M. Serum interleukin-2, interleukin-6, tumour necrosis factor-alpha and nitric oxide levels in patients with Behçet's disease. *Ann Acad Med Singapore* 2004; 33: 596-9.
- Sayinalp N, Ozcebe OI, Ozdemir O, Haznedaroğlu IC, Dündar S, Kirazlı S. Cytokines in Behçet's disease. *J Rheumatol* 1996; 23: 321-2.
- Direskeneli H, Saruhan-Direskeneli G. The role of heat shock proteins in Behçet's disease. *Clin Exp Rheumatol* 2003; 21(4 Suppl 30): S44-8.
- Pockley AG. Heat shock proteins as regulators of the immune response. *Lancet* 2003; 362: 469-76.
- Murray PJ, Young RA. Stress and immunological recognition in host-pathogen interactions. *J Bacteriol* 1992; 174: 4193-6.
- Kaufmann SHE. Heat shock proteins and the immune response. *Immunol Today* 1990; 11: 129-36.
- Direskeneli H. Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. *Ann Rheum Dis* 2001; 60: 996-1002.
- Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *Int Immunol* 2000; 12: 1539-46.
- Kitamei H, Kitaichi N, Yoshida K, Nakai A, Fujimoto M, Kitamura M et al. Association of heat shock protein 70 induction and the amelioration of experimental autoimmune uveoretinitis in mice. *Immunobiology* 2007; 212: 11-8.
- Yanaka A, Zhang S, Sato D, Tsuchi M, Suzuki H, Shibahara T et al. Geranylgeranylacetone protects the human gastric mucosa from diclofenac-induced injury via induction of heat shock protein 70. *Digestion* 2007; 75: 148-55.
- Zheng Z, Kim JY, Ma H, Lee JE, Yenari MA. Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke. *J Cereb Blood Flow Metab* 2008; 28: 53-63.
- Malago JJ, Koninkx JF, Tooten PC, van Liere EA, van Dijk JE. Anti-inflammatory properties of heat shock protein 70 and butyrate on Salmonella-induced interleukin-8 secretion in enterocyte-like Caco-2 cells. *Clin Exp Immunol* 2005; 141: 62-71.
- Luo X, Zuo X, Zhou Y, Zhang B, Shi Y, Liu M et al. Extracellular heat shock protein 70 inhibits tumour necrosis factor-alpha induced proinflammatory mediator production in fibroblast-like synoviocytes. *Arthritis Res Ther* 2008; 10: R41.
- Moseley PL. Heat shock proteins and the inflammatory response. *Ann N Y Acad Sci* 1998; 856: 206-13.
- Todryk SM, Gough MJ, Pockley AG. Facets of heat shock protein 70 show immunotherapeutic potential. *Immunology* 2003; 110: 1-9.
- International Study Group for Behçet's Disease. Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
- Gürbüz O, Ozdemir Y, Coşar CB, Kural G. Lipoprotein (a) in Behçet's disease as an indicator of disease activity and in thrombotic complications. *Eur J Ophthalmol* 2001; 11: 62-5.
- Müftüoğlu AU, Yazici H, Yurdakul S, Tüzün Y, Pazarlı H, Güngen G et al. Behçet's disease. Relation of serum C reactive protein and erythrocyte sedimentation rates to disease activity. *Int J Dermatol* 1986; 25: 235-9.
- Bodur H, Borman P, Ozdemir Y, Atan C, Kural G. Quality of life and life satisfaction in patients with Behçet's disease: relationship with disease activity. *Clin Rheumatol* 2006; 25: 329-33.

24. Pay S, Simşek I, Erdem H, Dinç A. Immunopathogenesis of Behçet's disease with special emphasize on the possible role of antigen presenting cells. *Rheumatol Int* 2007; 27: 417-24.
25. Hirano T. Interleukin-6 and its relation to inflammation and disease. *Clin Immunol Immunopathol* 1992; 62: 60-5.
26. al-Dalaan A, al-Sedairy S, al-Balaa S, al-Janadi M, Elramahi K, Bahabri S et al. Enhanced interleukin 8 secretion in circulation of patients with Behçet's disease. *J Rheumatol* 1995; 22: 904-7.
27. Luscinskas FW, Kiely JM, Ding H, Obin MS, Hébert CA, Baker JB et al. In vitro inhibitory effect of IL-8 and other chemoattractants on neutrophil-endothelial adhesive interactions. *J Immunol* 1992; 149: 2163-71.
28. Sahin S, Akoğlu T, Direskeneli H, Sen LS, Lawrence R. Neutrophil adhesion to endothelial cells and factors affecting adhesion in patients with Behçet's disease. *Ann Rheum Dis* 1996; 55: 128-33.
29. Balkwill FR. Tumour necrosis factor. *Br Med Bull* 1989; 45: 389-400.
30. de Smet MD, Ramadan A. Circulating antibodies to inducible heat shock protein 70 in patients with uveitis. *Ocul Immunol Inflamm* 2001; 9: 85-92.