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Serum haptoglobin levels in ocular Behçet disease and acute phase proteins in the course of Behçet disease

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PURPOSE. *Changes in concentrations of acute phase proteins in the serum of patients might be significant in the pathogenesis of Behçet disease. This report investigates the association between ocular disease activity and serum haptoglobin levels in patients with Behçet disease, and summarizes the current understanding of the correlation between acute phase proteins and Behçet disease based on both personal studies and data from the literature.*
METHODS. *Thirty patients with Behçet disease with ocular involvement and 15 healthy subjects were included in the study. Of the 30 patients, 14 had acute uveitis and 16 had inactive ocular involvement at the time of enrollment.*
RESULTS. *There was a significant difference in haptoglobin levels between the patients with active ocular disease and controls (p=0.0005). There was also a significant difference in haptoglobin levels between the patients with inactive ocular disease and control subjects (p<0.0001). However, no significant difference was observed among patients with active versus inactive uveitis with regard to serum haptoglobin levels.*
CONCLUSIONS. *Higher serum haptoglobin levels in patients with Behçet disease compared to control subjects were obtained. However, elevated serum haptoglobin levels do not seem to be a risk factor for uveitis activity. Behçet disease is generally diagnosed by physical examinations and no laboratory marker has been widely accepted for follow-up of disease activity. (Eur J Ophthalmol 2008; 18: 787-91)*

KEY WORDS. *Acute phase protein, Behçet disease, Haptoglobin, Uveitis*

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INTRODUCTION

Inflammation is a complex process accompanied by a large number of changes. Acute phase proteins (APPs) are soluble molecules whose blood concentrations increase during certain inflammatory states. Acute phase reactants have long been used as clinical indicators of active disease. Although these proteins are also called acute phase reactants, their levels are also elevated during chronic inflammatory states. Acute phase proteins include C reactive protein (CRP), serum amyloid A (SAA), α1-antitrypsin, α2-macroglobulin, α1-acid glycoprotein, ceruloplasmin, and haptoglobin. Some coagulation factors (e.g., fibrinogen) and complement proteins are also included in APPs (1).

Acute phase proteins are generally produced by the liver and released into the blood. However, many of these molecules are also made by macrophages or produced locally in tissues (1, 2). Acute phase proteins are believed to have a part in response to inflammation. For example, CRP can stimulate the classical complement pathway, and α1-antitrypsin can neutralize some proteases released during acute inflammatory conditions (2).

Behçet disease (BD) is a chronic multisystem inflammato-

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ry disorder with unknown etiology (3). Erythrocyte sedimentation rate (ESR), CRP, and cytokines have been proposed as disease activity markers in BD (4-8). Haptoglobin is a plasma protein that has been described as an acute phase reactant. To our knowledge, the level of haptoglobin has not yet been studied in BD. The aim of this study was to determine serum haptoglobin levels, in addition to their correlations with ceruloplasmin, CRP, and α1-antitrypsin, in patients with active and inactive ocular BD. This article also summarized the current understanding of the correlation between APPs and BD based on both personal studies and data from the literature.

METHODS

Thirty patients with BD with ocular involvement were included in this study. All patients included in the study met the international diagnostic criteria for BD (9). A complete ophthalmic examination was performed by ophthalmologists with an interest in BD. The patients were divided into two groups according to the activation of eye involvement to investigate the association between ocular disease activity and haptoglobin levels. Of the 30 patients, ocular involvement was active in 14 patients and inactive in 16 patients at the time of enrollment. Patients who had received systemic steroids or immunosuppressive agents at any time 4 weeks prior to the initiation of the study were excluded. Fifteen healthy control subjects were also included in the study. Informed consent was obtained from all participants.

A venous blood sample was taken from each patient and control subject. The blood samples were centrifuged to obtain serum. All serum samples were immediately stored at –80°C until use. Serum haptoglobin, ceruloplasmin, CRP, and α1-antitrypsin levels were measured in all samples. The levels of all parameters were measured by the immuno-turbidimetric method using commercial kit (Roche Mannheim, Germany) on P 800 autoanalyzer (Roche Mannheim, Germany).

Statistical analysis was performed using SPSS 13.0 software for Windows. Differences between groups were evaluated using the Kruskal-Wallis test for the global comparison and Mann-Whitney *U* test for paired comparison. Spearman correlation analysis was used for evaluating the correlation between different parameters in all groups. Data are presented as mean ± SD. A value of $p<0.05$ was considered significant.

RESULTS

Of the 30 patients in this study, 19 were male (63%) and 11 were female (37%). There were 10 men (67%) and 5 women (33%) in the control group. The mean age was 33.5±8.4 in patients with active ocular BD, 35.1±7.0 in patients with inactive ocular BD, and 33.9±9.0 in the control group.

Laboratory findings are outlined in Table I. Alpha 1-antitrypsin levels were not significantly different between groups. Haptoglobin, ceruloplasmin, and CRP levels were found to be significantly higher (for each, $p<0.0001$) among the patients with BD when compared with healthy control subjects. There was a statistically significant difference in haptoglobin ($p=0.0005$), ceruloplasmin ($p=0.0004$), and CRP ($p=0.0008$) levels between the patients with active ocular disease and controls. There was also a statistically significant difference in haptoglobin ($p<0.0001$), ceruloplasmin ($p<0.0001$), and CRP ($p=0.0002$) levels between the patients with inactive ocular disease and control subjects. However, there were no significant differences in haptoglobin, ceruloplasmin, or

TABLE I - LABORATORY FINDINGS IN PATIENTS WITH OCULAR AND NON-OCULAR BEHÇET DISEASE AND CONTROLS

	Patients with ocular Behçet disease (BD)			Control subjects
	All ocular BD	Active ocular BD	Inactive ocular BD	
Number	30	14	16	15
Haptoglobin (mg/dL)	276.1±130.8	275.8±87.5	276.2±146.3	92.1±37.4
CRP (mg/dL)	12.9±19.1	16.1±22.7	11.8±18.3	0.6±0.7
Alpha 1-antitrypsin (mg/dL)	175.0±43.3	177.2±12.3	174.2±50.7	163.8±22.4
Ceruloplasmin (mg/dL)	30.6±6.7	32.0±3.0	30.1±7.6	17.9±1.8

CRP levels between patients with active versus inactive ocular involvement.

In patients with active ocular BD, there was no significant correlation between the parameters. However, there were significant and positive correlations between haptoglobin–ceruloplasmin (r=0.72, p=0.002), haptoglobin–CRP (r=0.64, p=0.007), and haptoglobin–α1-antitrypsin (r=0.61, p=0.012) in patients with inactive ocular BD. In the control group, no correlation was observed between haptoglobin and ceruloplasmin, α1-antitrypsin, or CRP levels.

DISCUSSION

Acute phase proteins regulate immune responses and function as mediators or inhibitors of inflammation. The elevated serum levels of certain APPs are of diagnostic relation and also of prognostic value (2). The synthesis of many APPs is induced by several cytokines. In response to injury, macrophages and monocytes secrete a number of cytokines into the bloodstream (1, 10). The most notable inflammation-associated cytokines include tumor necrosis factor α (TNF-α), IL-1, and IL-6. To date, increased serum levels of cytokines such as TNF-α, IL-1, IL-2, IL-6, IL-8, and IL-10 have been proposed as disease activity markers in patients with BD (5, 11-17). However, recent studies have shown contradictory results concerning the serum level of IL-6 and BD activity. Sayinalp et al reported that serum IL-6 levels were not elevated in patients with BD (18).

The changes in concentrations of APPs in serum of patients might be significant in the evaluation of disease activity. Since the synthesis of acute phase reactants is affected variously by inflammation, we evaluated serum concentrations of haptoglobin among the APPs in patients with BD with ocular involvement. Haptoglobin is a plasma glycoprotein that binds extracorpuscular hemoglobin. It is also one of the APPs and its plasma level is elevated in a variety of inflammatory states (2). Serum haptoglobin levels have been determined to correlate both with disease and angiogenic activity in patients with systemic vasculitis (19). It has been suggested that the increased levels of haptoglobin found in chronic inflammatory conditions may play an important role in tissue repair (19). Our study, which is the first investigation of haptoglobin in BD, revealed that haptoglobin levels were significantly higher in patients with active and inactive ocular BD when compared with controls. However, there was no significant difference between patients with active versus inactive ocular involvement. Therefore, elevated serum haptoglobin levels do not seem to be a risk factor for uveitis activity.

Ceruloplasmin binds and carries copper present in plasma (2). It is also an APP synthesized and secreted by hepatocytes and cytokine stimulated macrophages that elevates moderately in inflammation (20, 21). It has been shown that ceruloplasmin may be useful as a serum marker for indolent or recurrent inflammation (21). Plasma ceruloplasmin levels have been shown to be higher in patients with BD than those in controls (22, 23). In the present study, elevated serum levels of ceruloplasmin have been shown to be associated with ocular BD. However, there was no statistically significant difference between patients with active versus inactive ocular involvement.

C-reactive protein, the best known of APPs, is synthesized by the liver, mainly under the regulation of the proinflammatory cytokines, especially IL-6. Other cytokines such as IL-6 and TNF-α may contribute to hepatic synthesis of CRP. It is thought to assist in complement binding to foreign and damaged cells and increases phagocytosis by macrophages. It is also believed to play an important role in innate immunity, as an early defense system against infections. Marked rises in CRP reflect the presence and intensity of inflammation. However, an elevation in CRP is not specific for certain diseases. In the literature, CRP has been reported to be elevated in patients with BD (5, 7, 24-28). Although significant associations have been reported between elevated APP levels and disease activity in BD (5-8, 28), no association of CRP and sedimentation rate with the activity of the BD has been found in a previous study (15). We found significantly higher CRP levels in patients with BD with ocular involvement compared to healthy controls. However, there was no significant difference in CRP levels between patients with active versus inactive ocular involvement.

Alpha 1-antitrypsin, also called alpha 1-proteinase inhibitor, is a glycoprotein. It protects tissues from enzymes of inflammatory cells. In the acute phase reaction, a large concentration raise is required to limit the damage caused by activated neutrophil granulocytes and their enzyme elastase, which breaks down the connective tissue fiber elastin (1). Wakefield et al reported significantly higher serum α1-antitrypsin levels in patients with retinal vasculitis compared to controls. Of the 25 patients with retinal vasculitis included in that study, 8 had BD (29). In another study, α1-antitrypsin levels have been found to be raised

in patients with BD when compared with controls (21). In addition, higher levels of α1-antitrypsin have been reported in active period of BD than in inactive period and in control subjects (13, 30). In contrast, no differences have been found in α1-antitrypsin between patients with BD and controls in another study (26). In our study, α1-antitrypsin levels were not elevated in patients with BD compared to controls.

Serum amyloid A is a high-density lipoprotein-associated apolipoprotein. Although the actions of SAA are largely unknown, it has several roles, including the transport of cholesterol and the recruitment of immune cells to inflammatory sites (1, 10). Serum amyloid A levels of patients with active BD have been found to be significantly higher than in patients in remission and controls (31).

Alpha 1-acid glycoprotein has been proposed to possibly coat damaged tissue and thereby decrease potential antigenicity (1). Patients with collagen diseases had significant increases of α1-acid glycoprotein in their serum and on the surface of peripheral leukocytes compared with controls. Interleukin-1 beta and TNF-α stimulated the production of α1-acid glycoprotein RNA message in peripheral blood mononuclear cells (32). The level of this glycoprotein was significantly increased predominantly in the ocular type of BD (25).

Alpha 2-macroglobulin is able to inactivate a wide variety of proteinases. It functions as an inhibitor of coagulation by inhibiting thrombin as well as an inhibitor of fibrinolysis by inhibiting plasmin. Alpha 2-macroglobulin levels have been found to be significantly increased in patients with active BD than in those in inactive stage of BD or control subjects (13). The erythrocyte sedimentation rate is an indirect measurement of plasma APP concentrations. It can be considerably influenced by the size, shape, and number of erythrocytes, and also depends largely on the plasma concentration of fibrinogen. A positive relationship has been reported between erythrocyte sedimentation rate and BD activity (13, 28). This indirect method is no longer needed to assess plasma levels of fibrinogen, which can now be determined directly. Fibrinogen aids wound healing by causing endothelial cell adhesion, spreading, and proliferation. Fibrinogen levels have been reported to be significantly raised in patients with BD compared to healthy population (23, 24, 33-35).

The acute phase response is an important pathophysiologic phenomenon. Despite the lack of diagnostic specificity, acute phase changes express the presence and intensity of an inflammatory process. However, BD is generally diagnosed by physical examinations and no laboratory marker has been widely accepted for follow-up of disease activity.

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