Serum Mannose-Binding Lectin Levels Are Decreased in Behçet's Disease and Associated with Disease Severity

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ABSTRACT. ***Objective.*** To investigate serum levels of mannose-binding lectin (MBL), a complement-like protein of collectin family, in patients with Behçet's disease (BD).

Methods. MBL levels were measured in sera of 130 patients with BD, 64 patients with recurrent oral ulcerations (ROU), and 105 healthy controls (HC) with ELISA.

Results. Patients with BD had significantly lower median serum MBL levels compared to HC (1857 vs 3136 ng/ml, p = 0.001). No significant difference was observed in median serum MBL levels between BD and ROU (2309 ng/ml, p = 0.252). Low MBL levels (≤ 500 ng/ml) were present in a higher proportion of BD patients compared to HC (29% vs 16%, p = 0.021). A severe disease course (total clinical severity score ≥ 4) was more frequently observed in BD patients with very low serum MBL levels (≤ 100 ng/ml) (19% vs 6%, p = 0.046). When serum MBL levels were analyzed separately according to gender, the frequency of vascular disease was higher in men with very low serum MBL levels (80% vs 42%, p = 0.042).

Conclusions. MBL deficiency might contribute to the pathogenesis of BD and affect its clinical course. (J Rheumatol 2005;32:287-91)

Key Indexing Terms:
MANNOSE-BINDING LECTIN BEHÇET'S DISEASE
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Behçet's disease (BD) is a multisystemic disorder with mucocutaneous, ocular, musculoskeletal, vascular, gastrointestinal, and central nervous system involvement[1]. The main pathological finding in BD is an immunological abnormality leading to an occlusive vasculitis with activated Th1 cells, neutrophils, gamma-delta T cells and possibly natural killer (NK) cells taking part in the immune-mediated inflammation[2]. Although etiopathogenesis is still unknown, infectious agents, immune mechanisms, and genetic factors such as HLA-B51 are implicated[3]. Clinical observations such as increased oral manifestations after dental treatment procedures, hypersensitivity to skin tests with streptococcal antigens, dominance of atypical streptococcal species in the oral flora of patients with BD, and recent reports of clinical benefit from antibacterial treatments suggest a role for microorganisms in the pathogenesis of BD[4-6].

Mannose binding lectin (MBL), a member of the collectin family, is a macromolecule with similar functional characteristics of IgM/IgG and C1q and is an important constituent of the innate immune system. It binds to surface carbohydrate structures on gram-positive and gram-negative bacteria as well as some yeasts, viruses, and parasites. MBL then activates the complement system via MBL-associated serine proteases 1 and 2, which lead to the cleavage of the complement components C2 and C4. The latter cleavage products C2a and C4b form a C3 convertase, which initiates the complement cascade by cleaving C3 protein. Activation of the lectin pathway of the complement system is triggered directly by microbial recognition and therefore functions independent of adaptive immune response, while the classical complement pathway is activated by the binding of C1q to the antigen-antibody complex and thus depends on antibody responses[7-9].

Plasma concentration of MBL is genetically determined by a series of alellic dimorphisms located both in the structural MBL gene and its promoter region in chromosome 6. Deficiency of MBL with the common opsonic defect was

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first identified in humans in association with persistent infections of children with no other known immunologic abnormality. Subsequent studies supported the role of MBL as an important mediator of opsonophagocytosis[9,10]. Association of MBL gene polymorphisms with meningococcal disease, and infectious complications in children undergoing treatment for malignancies have also been observed[11-13]. Low serum MBL levels and MBL gene mutations are also implicated in the pathogenesis of some autoimmune disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)[9,10].

With this background, we investigated the role of MBL deficiency as a contributing factor to the pathogenesis of BD. We also evaluated the association between serum MBL levels, clinical manifestations, and disease course.

MATERIALS AND METHODS

Patients and controls. One hundred thirty consecutive patients with BD (M/F: 59/71), fulfilling the International Study Group Criteria, were recruited from the Behçet's disease clinics of Marmara and Cerrahpasa Medical Schools in Istanbul[14]. The mean age (range) of the patient group was 34.5 ± 10.9 (14-63) and mean age of disease onset was 28.4 ± 9.2 (11-60) years. All patients had oral ulcers. Genital ulcers were present in 85%, skin lesions in 97%, positive pathergy reaction in 69%, ocular disease in 36%, arthritis in 34%, vascular disease in 29%, and neurologic involvement in 4%. Twenty-seven percent of patients were positive for HLA-B51. Seventy-nine percent of patients had active clinical manifestations at the time of the study and 31% were receiving immunosuppressive treatment.

Total clinical severity score (TCSS) (according to Krause, *et al*[15]) was determined as follows: 1 point each for mild symptoms (oral ulcers, genital ulcers, typical skin lesions, arthralgia, recurrent headaches, epididymitis, mild gastrointestinal symptoms, pleuritic pains, and superficial vein thrombosis), 2 points each for moderate symptoms (arthritis, deep vein thrombosis, anterior uveitis, and gastrointestinal bleeding), and 3 points each for severe disease manifestations (posterior/panuveitis, retinal vasculitis, arterial thrombosis or aneurysms, major vein thrombosis, neuro-Behçet's, and bowel perforation). Patients were categorized into severe (severity score ≥ 4) and mild (severity score < 4) disease activity groups. Oral health of patients with BD was investigated as described[16].

Sixty-four patients with recurrent oral ulcers (ROU) (M/F:28/36, mean age: 34.7 yrs) and 105 age and sex matched healthy controls (HC) were also studied as control groups. The study was approved by the Ethical Committee of Marmara University Medical School, and informed consent was obtained from all patients and controls.

Detection of serum MBL concentrations. Serum MBL levels were measured by MBL oligomer ELISA (Antibody Shop, Copenhagen, Denmark). Briefly, diluted serum samples were incubated in microwells precoated with a specific antibody against MBL carbohydrate-binding domain. Unbound components were removed by washing at each step. Wells were incubated with biotinylated antibody to MBL, washed again, incubated with horseradish peroxidase-conjugated streptavidin, and then with a chromogenic substrate. The reaction was stopped with sulfuric acid, and the optical density was read at 450 nm in an ELISA reader.

Statistical analysis. Mann-Whitney U test was used to compare serum levels of MBL in different study groups. BD and control groups were divided into 2 groups according to low and very low (≤ 500 and ≤ 100 ng/ml, respectively) serum MBL levels and non-parametric comparisons were performed by chi-square test. Comparison of clinical variables in BD patients with different serum MBL levels were tested by chi-square test. Contributing factors for severe BD (HLA-B51, sex, disease duration, and serum MBL values) were analyzed using logistic regression analysis. SPSS statistical package was used (version 10.0 for Windows) for statistical analysis.

RESULTS

Distribution of serum MBL concentrations in study groups. A wide range of serum MBL levels were observed in all groups (BD: 11-5756 ng/ml, ROU: 48-6152 ng/ml, HC: 0-8320 ng/ml). Median serum MBL concentrations in BD patients were significantly lower compared to HC (Figure 1) (BD: 1857 vs HC: 3136, p = 0.001). Low serum MBL levels (≤ 500 ng/ml) were detected in a significantly higher subset of patients with BD (29%, 38/130) compared to HC (16%, 17/105) (BD vs HC, p = 0.02). The difference between BD and ROU was not statistically significant (17%, 11/64) (BD vs ROU, p = 0.27). However, the percentage of patients with very low serum MBL levels (≤ 100 ng/ml) were similar among the 3 groups (BD: 15% vs HC: 12% and ROU: 14%, p = NS). In contrast, the percentage of patients with serum MBL levels over 3000 ng/ml was significantly higher in HC than BD and ROU (BD vs HC, p < 0.001) (ROU vs HC, p < 0.05). The median serum MBL level in ROU (2309 ng/ml) was not different compared to HC (p = 0.074) and BD (p = 0.252).

Association of serum MBL concentrations with the clinical manifestations of BD and gender. Median serum MBL levels were 2424 (11-5756) in men and 1534 (48-5390) in women with BD (p > 0.05). The number of patients with very low serum MBL levels were also similar regardless of gender. When the association of clinical manifestations with serum MBL levels was analyzed separately according to gender, it was observed that the frequency of vascular disease was higher in men with very low serum MBL levels (80% vs 42%, p = 0.042). Although increased frequencies of some clinical manifestations (vascular, ocular, musculoskeletal, and neurologic involvement) and intensive treatment regimens were observed in the very low serum MBL

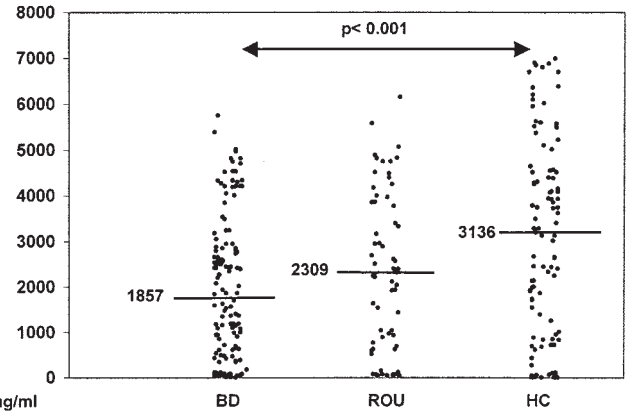

Figure 1. Serum MBL concentrations in patients with BD and ROU, and HC. Median serum MBL concentrations for BD, ROU, and HC are shown with horizontal bars. MBL: mannose-binding lectin; BD: Behçet's disease; ROU: recurrent oral ulcers; HC: healthy controls.

group, these differences did not reach statistical significance (Table 1). However, pathergy positivity was lower in this subset, especially in men compared to patients with higher serum MBL levels (44% vs 74%, p = 0.02). The prevalence of HLA-B51 positivity was not significantly different in patients with low and very low serum MBL levels compared to patients with higher serum MBL levels.

In 88 patients, we also investigated the relationship between oral health (plaque index, gingival index, sulcus bleeding index, probing depth, and attachment level) and serum MBL levels. Patients with very low serum MBL levels had higher mean plaque index score (1.8 ± 1.1 vs 1.6 ± 1.06) showing the thickness of dental plaque and higher mean gingival index score (2.3 ± 1.3 vs 1.9 ± 1.05) associated with the severity of gingivitis but differences did not reach statistical significance.

Factors associated with severity score in BD. Mean total clinical severity score was 5.2 ± 2.2 in the BD group. Frequency of patients with very low serum MBL levels were significantly higher in the severe BD group (TCSS: 6.4) compared to the group with milder disease (TCSS: 2.6) (19% vs 6%, p = 0.046). Linear regression analysis was performed to explore the independent factors (HLA-B51 status, sex, and serum MBL levels) affecting the severity score of BD patients. Male gender (p = 0.086) and serum MBL levels (p = 0.052) remained in the equation in the last step, and serum MBL levels were found to have an inverse relationship with the severity score (Table 2).

DISCUSSION

Lower serum MBL levels were observed in patients with BD compared to controls in our study. Very low serum MBL levels were associated with a higher severity score and more extensive clinical manifestations such as vascular involvement, especially in male patients with BD. In linear regression analysis, MBL was also found to be an independent factor associated with the severity of BD.

Innate immunity, which includes antimicrobial peptides, phagocytes, and the alternative complement pathway, is the first defense mechanism against infections. Interaction with and control of the adaptive immune system is also a crucial role of the innate immune system in host defenses[8]. MBL is a member of the complement system functioning as a recognition molecule in the lectin pathway and binds to mannose and N-acetylglucosamine sugar groups on different microorganisms[17]. MBL deficiency may lead to impaired opsonization and complement mediated clearance of immune complexes causing decreased protection against infections[9,10]. The role of MBL deficiency as a predisposing factor for infections such as meningococcemia was demonstrated in various studies[10-12].

Viral or streptococcal infections in association with BD have been proposed and a dysfunction of the innate immune system might predispose to persistent immune activation after infectious stimuli. MBL deficiency may lead to a decreased activity of the innate function of the immune apparatus, which might possibly increase the presentation of

Table 1. Frequencies of very low serum mannose-binding lectin (MBL) levels (≤ 100 ng/ml) according to the different clinical manifestations and gender in patients with BD.

	Behçet's Disease			Male Patients			Female Patients		
	MBL ≤ 100 n = 19 (%)	MBL > 100 n = 111 (%)	p	MBL ≤ 100 n = 10 (%)	MBL > 100 n = 49 (%)	p	MBL ≤ 100 n = 9 (%)	MBL > 100 n = 62 (%)	p
Uveitis	7/19 (37)	40/111 (36)	1.0	4/10 (40)	23/49 (47)	0.7	3/9 (33)	17/62 (27)	0.7
Erythema nodosum	14/19 (74)	70/111 (63)	0.4	8/10 (80)	25/49 (51)	0.09	6/9 (67)	45/62 (72)	0.7
Vascular involvement	8/19 (42)	30/111 (27)	0.2	8/10 (80)	21/49 (42)	0.042*	0/9 (0)	9/62 (15)	0.6
Arthritis	7/19 (37)	37/111 (33)	0.8	4/10 (40)	18/49 (37)	1.0	3/9 (33)	19/62 (31)	1.0
CNS involvement	1/19 (5)	4/111 (4)	0.5	0/10 (0)	1/49 (2)	1.0	1/9 (11)	3/62 (5)	0.4
Genital ulcers	18/19 (95)	93/111 (84)	0.3	0/10 (0)	7/49 (14)	0.6	1/9 (11)	11/62 (17)	1.0
Pathergy positivity	8/18 (44)	75/102 (74)	0.02*	4/10 (40)	36/47 (76)	0.051	4/8 (50)	39/55 (71)	0.2
Active disease	14/19 (74)	88/111 (79)	0.6	8/10 (80)	40/49 (81)	1.0	6/9 (67)	48/62 (77)	0.4
Immunosuppressive treatment	12/19 (63)	57/111 (51)	0.4	7/10 (70)	29/49 (59)	0.7	5/9 (55)	28/62 (45)	0.7
Severity score ≥ 4	16/19 (84)	67/111 (60)	0.046*	9/10 (90)	34/49 (69)	0.26	7/9 (78)	33/62 (53)	0.3

* significant difference.

Table 2. Factors affecting the severity score according to linear regression analysis of patients with BD.

	Unstandardized Coefficients		Standardized Coefficients		
Severity Score	B	Standard Error	Beta	t	p
Sex	1.432	0.806	0.290	1.777	0.086
Serum MBL levels	–4. E-04	0.000	–0.330	–2.021	0.052
Constant	3.772	1.299		2.903	0.007

R: 0.44; R^2: 0.20; Adj R^2: 0.14.

exogenous antigens to the host. Longterm bacterial stimulation might then induce abnormal adaptive immune responses, such as tissue lymphocyte infiltrations and Th1 type cytokine profile with augmented expression of interleukin-12 and interferon-γ[4,5].

We also observed an association of MBL deficiency with a more severe course in BD. Similar observations of MBL deficiency associated with the disease course have been reported in RA populations from Denmark, China, and Iceland, where associations were observed with more severe radiographic disease and extraarticular manifestations[18-20]. These results suggest that in addition to being a susceptibility factor, MBL deficiency can have a disease-modifying effect in autoimmune and inflammatory disorders. In this context, Ogden, *et al*[21] recently suggested that complement system deficiencies might predispose to autoimmune disorders such as SLE and RA by insufficient clearance of apoptotic or necrotic cells. Apoptotic cells including neutrophils are recognized and removed by professional phagocytes or neighboring semi-professional cells such as fibroblasts and epithelial cells. Recent studies showed the role of collectins, including MBL, surfactant protein-A, and SP-D in apoptotic cell clearance. Deficiencies of collectins upregulate dendritic cell maturation towards a proinflammatory profile with increased antigen presentation to T cells[22,23]. In this respect, an abundance of neutrophils are observed in BD lesions such as folliculitis, pathergy infiltrates, or ocular hypopyons[4,24]. If MBL deficiency leads to impaired clearance of apoptotic neutrophils, local, prolonged accumulation of apoptotic debris might trigger adaptive proinflammatory pathways, increasing local tissue destruction in BD.

The only contrasting observation in our data is the lower frequency of pathergy positivity in the group with very low serum MBL levels, as pathergy reaction is generally reported to be associated with a more severe course of BD. As most patients with very low serum MBL levels were active with higher severity scores, immunosuppressive treatment might have influenced pathergy positivity in this subset.

We observed a trend towards lower serum MBL levels in patients with recurrent oral ulcerations compared to HC, but still higher than in patients with BD. MBL deficiency can be another factor in ROU pathogenesis impairing innate defenses against microorganisms and facilitating ulcer development. ROU, a common disorder with 5-10% prevalence in the general population, possibly has a multifactorial pathogenesis similar to BD[25]. In this context, we have recently reported impaired oral health both in BD and ROU, possibly associated with oral ulcer development, decreased dental care, and increased oral microbial flora[16]. Although our results are not conclusive, we also observed a trend towards impaired oral health in the patient subset with very low MBL levels in our study.

In conclusion, patients with BD were observed to have lower serum MBL levels associated with a more severe disease course. MBL deficiency might contribute to the pathogenesis of BD by impairing innate immune responses against microorganisms or by modifying inflammatory mechanisms through decreased apoptotic cell clearance. Further studies investigating serum MBL levels in different populations and its role in anti-infectious and antiinflammatory mechanisms such as apoptotic clearance are required to clarify the role of MBL in BD pathogenesis. As plasma concentration of MBL is genetically determined, the association of our findings with MBL genotypes should also be determined.

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