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ORIGINAL ARTICLE

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Seroprevalence of *Borrelia burgdorferi* in patients with Behçet's disease

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Abstract *Objective* Turkey is one of the countries where Behçet's disease is most prevalent. Although its pathogenesis is not defined clearly, infectious agents are thought to play a role in the etiology. In one study of a group of uveitis patients, including those with Behçet's disease, increased seropositivity to *B. burgdorferi* was reported by enzyme-linked immunosorbent assay (ELISA). The seroprevalence of *B. burgdorferi* has been found to be as high as 36% in some rural areas of Turkey, although Lyme disease caused by *B. burgdorferi* is quite rare. In this study, we investigated the seroreactivity to *B. burgdorferi* antigens in patients with Behçet's disease and compared it with that of healthy and disease controls. *Materials and methods* This study was conducted in Izmir in western Turkey. *B. burgdorferi* immunoglobulin (Ig)M and IgG antibodies were tested by ELISA in the sera of patients with Behçet's disease ($n = 30$), rheumatoid arthritis patients as disease controls ($n = 31$), and healthy controls ($n = 31$). Positive results were confirmed by Western blotting. *Results* The difference in *B. burgdorferi* seropositivity between the groups was not significant by any method. Seroreactivity to *B. burgdorferi* antigens by ELISA was detected in 26.7% of the patients with Behçet's disease, 35.5% of those with rheumatoid arthritis, and 19.4% of the healthy controls. Immunoblots were positive in 13.3% of the Behçet's disease patients, 22.6% of the rheumatoid arthritis patients, and 12.9% of healthy controls. *Conclusion* These results suggest no association between Behçet's disease and *B. burgdorferi* infection.

Keywords Behçet's disease · *Borrelia burgdorferi* · Lyme disease · Seroprevalence

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Introduction

Behçet's disease is a chronic, inflammatory, multisystemic disorder presenting with recurrent oral and genital aphthous ulcerations and uveitis often leading to blindness. It is most common in Turkey (80–370 cases per 100,000) and the underlying cause is unknown. This disorder may represent aberrant immune activity triggered by exposure to an agent, perhaps infectious, in patients with a genetic predisposition to the disease [1, 2].

In a study of uveitis patients including those with Behçet's disease, increased seropositivity for *Borrelia burgdorferi* was reported by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA), [3]. The seroprevalence of *B. burgdorferi* has been found to be as high as 36% in some rural areas of Turkey, although Lyme disease caused by *B. burgdorferi* is quite rare [4].

In this study, we investigated the seroreactivity to *B. burgdorferi* antigens in patients with Behçet's disease and compared it with that of healthy and disease controls to determine a possible relationship between this spirochete and Behçet's disease.

Materials and methods

The study was conducted in Izmir, a city in western Turkey. The study group included 30 patients with Behçet's disease (16 male, 14 female, mean age 36.8 years, range 20–59) who fulfilled the criteria of the International Study Group for Behçet's disease [5]. Thirty-one rheumatoid arthritis patients (25 female, six male, mean age 57.5 years, range 40–72) fulfilling the 1987 American College of Rheumatology criteria for the disease [6] and 31 normal healthy

Table 1 Demographic and clinical features of study and control groups

	Behçet's disease patients ($n=30$)	Rheumatoid arthritic controls ($n=31$)	Healthy controls z ($n=31$)
Mean age (years)	36.8	57.5	45.8
Female/male	16/14	25/6	18/13
Mean disease duration (years)	7.6	8.9	–
Eye involvement	14	2	–
Arthritis (active)	15 (9)	31 (28)	–
History of tick bite	5	6	2
History of erythema migrans	–	1 (?)	–
Rheumatoid factor positivity	–	23	–

subjects (18 female, 13 male, mean age 45.8 years, range 23–74) were included as controls. The demographic and clinical characteristics of the study and control groups are shown in Table 1.

Sera obtained from patients and controls were divided into two tubes and kept separately at −20°C until used. Firstly, each sample was tested by enzyme immunoassay for *B.burgdorferi* IgM and IgG antibodies in the sera. Borrelia IgG and IgM ELISA (Gull, Germany) based on a mixture of soluble antigen preparation originating from selected strains representing *B. burgdorferi sensu stricto* and *B. afzelii* was used. Absorbency values within ±5% of the cutoff value were accepted borderline, those above being positive and those below negative. When borderline results were obtained, the test was repeated.

Sera determined positive by ELISA were retested with *Borrelia* Western blot IgG and IgM (Gull, Germany) for confirmation. Interpretative criteria were used according to the kit instructions based on studies using sera obtained from European locales [7]. For IgM, criteria were the presence of one of the 23-kDa (Osp C) and 39-kDa bands. For IgG, four of the following seven bands were required: 21 kDa, 23 kDa (Osp C), 37 kDa, 39 kDa, 41 kDa, 45 kDa, and 93 kDa. Although bands at 36 kDa, 37 kDa, 45 kDa, and 93 kDa exhibited high specificity for IgM and bands at 18 kDa, 36 kDa, 58 kDa, and 66 kDa for IgG, they were not included in the current criteria since they did not significantly increase sensitivity, as suggested by the manufacturer.

Statistical analysis

All results are given as means ± standard deviations. Statistical evaluation was performed by the χ^2 and ANOVA tests. P values lower than 0.05 were accepted as statistically significant.

Results

Seroreactivity to *B. burgdorferi* antigens was detected by ELISA in eight patients (26.7%) with Behçet's disease, 11 (35.5%) with rheumatoid arthritis, and six (19.4%) healthy controls. Of these, seven patients (23.3%) with Behçet's disease, six (19.4%) with rheumatoid arthritis, and three (9.7%) healthy controls were positive for IgM, and three patients (10%) with Behçet's disease, six (19.4%) with rheumatoid arthritis, and three (9.7%) healthy controls were positive for IgG (Table 2).

Positive results by ELISA were further tested by Western blotting. Seropositivity was observed in four patients (13.3%) with Behçet's disease, seven (22.6%) with rheumatoid arthritis, and four (12.9%) healthy controls. Of these, two (6.7%) Behçet's disease patients and three (9.7%) rheumatoid arthritis patients were positive for IgM, and three (10%) Behçet's disease patients and four (12.9%) rheumatoid arthritis patients were positive for IgG. Both IgM and IgG immunoblots were positive in the sera of two (6.5%) healthy controls (Table 2). The difference in *B. burgdorferi* seropositivity between groups was not significant by any method ($P>0.05$) (Table 2).

Table 2 Patients and controls with positive serological tests ($P>0.05$)

Serological test	Behçet's disease patients (%) ($n=30$)	Rheumatoid arthritic controls (%) ($n=31$)	Healthy controls (%) ($n=31$)
Micro-ELISA			
IgM	7 (23.3)[a]	6 (19.4)[b]	3 (9.7)
IgG	3 (10)[a]	6 (19.4)[b]	3 (9.7)
Total positive	8 (26.7)	11 (35.5)	6 (19.4)
Western blot			
IgM	2 (6.7)[b]	3 (9.7)	2 (6.5)
IgG	3 (10)[b]	4 (12.9)	2 (6.5)
Total positive	4 (13.3)	7 (22.6)	4 (12.9)

[a]Two patients IgG- and IgM-positive
[b]One patient IgG- and IgM-positive

Analysis of the immunoblots revealed no statistically significant difference in the numbers of seroreactive bands for either IgM or IgG between groups ($P>0.05$). The mean numbers of IgM bands of reactivity on Western blot were 2.2 for the Behçet's group, 1.8 for the rheumatoid arthritis group, and 2.5 for healthy controls. For IgG, they were 6.7, 8.2, and 6, respectively. There was no statistically significant relationship between rheumatoid factor (RF) positivity and *B. burgdorferi* seropositivity in the rheumatoid arthritis group ($P>0.05$).

Discussion

The etiopathogenesis of Behçet's disease still remains unknown. The most likely hypothesis seems to be of aberrant immune activity triggered by an infectious agent(s) in patients with a genetic predisposition to developing the disease. Among proposed triggering infectious agents are herpes simplex virus type 1 [8, 9] and *Streptococcus* species [10, 11]. Microbial and human heat shock proteins (HSP) show marked amino acid homology. Mycobacterial HSP proteins may also be important contributors to a cross-reactive response among patients with Behçet's disease [12, 13]. In one

study of a group of uveitis patients, increased seropositivity against *B. burgdorferi* was found in the sera of patients with Behçet's disease, sarcoidosis, and Vogt-Koyanagi-Harada syndrome, and no antibodies were observed in the sera of healthy controls by ELISA and IFA. It was suggested that these disorders favor the production of antibodies that cross-react with *B. burgdorferi* proteins [3]. In a review of the literature, no other study was found that investigates the relationship between Behçet's disease and *B. burgdorferi*. In the present one, we saw that the increased seroprevalence of *B. burgdorferi* in the Behçet's disease group by both ELISA and Western blot was no different from that in control groups.

Patients with rheumatoid arthritis have been found seropositive for *B. burgdorferi* by ELISA or IFA, suggesting that *B. burgdorferi* might be involved in the etiopathogenesis of rheumatoid arthritis [14, 15]. However, subsequent studies with Western blot did not support any link between rheumatoid arthritis and this micro-organism [16, 17]. Weiss et al. [16] found no seropositivity by IgG immunoblot analysis in their series of 26 rheumatoid arthritis cases but emphasized that rheumatoid arthritis was associated with the presence of multiple antibodies to *B. burgdorferi*. They suggested that the immunologic abnormalities linked to rheumatoid arthritis may lead to cross-reactivity with several nonspecific *B. burgdorferi* antigens. In the present study, no difference was seen in the numbers of seroreactive bands between the groups. There was no association between RF positivity and *B. burgdorferi* seropositivity in the rheumatoid arthritis group. It also was previously shown that Western blot was not affected by RF activity in the absence of Lyme disease [18].

Clinicians should never diagnose Lyme disease solely on the basis of serologic tests; instead, diagnosis should be based on specific historic information and objective clinical findings [19]. Therefore, all the subjects found seropositive by Western blot testing in the present study were clinically reevaluated. None had histories of frequent tick bites, skin lesions, or neurologic or cardiac manifestations compatible with Lyme disease. Clinical findings of Western blot-positive patients with arthritis were entirely compatible with their known diseases. Thus, no diagnosis of Lyme disease was established in any seropositive subject.

The diagnosis of early Lyme disease can be made on clinical grounds alone in the presence of classic erythema migrans (EM). Another classic finding for Lyme disease is lymphocytic meningitis with facial palsy and/or radiculoneuropathy (called Bannwarth's syndrome). However, diagnostic difficulty may arise when neurologic, cardiac, or arthritic complications occur without a recent or current history of EM. Monoarticular or oligoarticular arthritis may mimic either an acute septic joint or other causes of arthritis such as reactive arthritis or osteoarthritis in adults. Rheumatoid factor-positive symmetric small joint arthritis, which is unusual for Lyme disease, could be confused with rheumatoid arthritis. Clinically, facial palsy due to Lyme disease is indistinguishable from Bell's palsy, and Lyme meningitis may mimic meningitis due to an enterovirus. Lyme encephalopathy may be confused with other causes of concentration and memory impairment. Laboratory evidence of infection, by demonstration of specific antibodies, is essential for the diagnosis of all clinical manifestations of Lyme disease except EM [19]. The diagnosis of early and late neuroborreliosis requires demonstration of intrathecal antibody production [20].

In endemic areas of the United States of America and Europe, the seroprevalence of *B. burgdorferi* in several studies ranged from 5.7% to 35% by ELISA and IFA [21, 22, 23]. A high frequency of seropositivity to *B. burgdorferi* was also reported in Asia [24, 25]. In Japan, the antibody positivity to *B. burgdorferi* in forest workers was found to be 20% by Western blot analysis [25]. There are few studies on the seroprevalence of Lyme disease in Turkey [4, 26, 27], the which ranged from 7% to 35.9% in some rural areas by ELISA and IFA. In one of them [26], positive ELISA results were also confirmed by Western blot. Although most of the subjects in our study are from urban areas of Izmir, the seroprevalence of *B.burgdorferi* is as high as that reported from other endemic areas in the world and also from some rural areas of Turkey.

Cross-reacting antibodies may be at least partially responsible for the high seroprevalence of *B.burgdorferi* by ELISA. These antibodies can occur in patients with other borrelial, spirochetal, viral, parasite, and autoimmune diseases. It is estimated that 5% or more of the normal population must test positive for Lyme disease by ELISA due to cross-reacting antibodies elicited by other infections or by the immune response to normal flora [14, 28]. Western blot allows detection of antibodies to individual components of the organism and can therefore be much more specific than ELISA [19]. It was suggested that our subjects with *B.burgdorferi* antibodies detected by Western blot may have subclinical infection. It is known that subclinical infection contributes to seroprevalence of *B. burgdorferi*, particularly in areas with high risk of tick exposure [22, 23, 29]. Most infections were found to remain inapparent in a study done in Switzerland [23]. The ratio of apparent to inapparent infection was shown to be 1:1 in two United States studies [22, 29]. Despite of the rarity of clinical Lyme disease, seroprevalence to *B. burgdorferi* as high as 36% in a rural area [4] and the finding that 80.4% of ticks collected in the same area were found to be *Ixodes ricinus* [26] suggest that some endemic areas may exist in Turkey.

The clinical manifestations of Lyme disease may differ in Europe and America, possibly due to the differences in genospecies in these regions. It has been suggested that some *B. burgdorferi* strains are more pathogenic than others, possibly because of the presence of certain not yet identified plasmids [30]. In general, European isolates have been more diverse than American isolates [31]. Possibly, geographic differences in the

pathogenicity of *B. burgdorferi* strains or in the distribution of pathogenic strains may lead to the low prevalence of clinical Lyme disease, despite the high prevalence of asymptomatic positive Lyme serology in our region. Host factors may also modify the clinical course [32].

Many patients with Lyme disease do not remember being bitten by ticks, although it takes 48 h or more after tick attachment for the organism to spread to the host. Nymphal *Ixodes* ticks are round and very small [19], which may explain why all the seropositive subjects in the present study have no history of tick exposure.

Although immunoblot is more sensitive and more specific than ELISA, there is still some debate about its interpretation. It is difficult to determine accurately the molecular weight of the bands and identify the antigenic proteins, due to variations between different strains of *B. burgdorferi* [33, 34, 35]. The United States Centers for Disease Control and Prevention (CDC) recommended that an IgM immunoblot be considered positive if two of the following bands are present: 24 kDa (Osp C), 39 kDa, and 41 kDa. For IgG, five of the following ten bands are required: 18 kDa, 21 kDa, 28 kDa, 30 kDa, 39 kDa, 41 kDa, 45 kDa, 58 kDa, 66 kDa, and 93 kDa [36]. The CDC criteria are highly specific, but their sensitivity may decrease when used in other areas of the world [37]. In Europe, where there is less expansion of the antibody response, no single set of criteria for the interpretation of immunoblots results in high levels of sensitivity and specificity in all countries [38]. Hauser et al. [39] proposed the following criteria for Western blot on the basis of the data obtained with European Union Concerted Action on Lyme Borreliosis (EUCALB) sera:

- For IgG, at least two bands among p83/100, p58, p43, p39, p30, OspC, p21, p17, and p14 must be present for *Borrelia afzelii* (PKo) and at least one band among p83/100, p39, p30, OspC, p21, and p17b are required for *Borrelia garinii* (PBi)
- For IgM, at least one band among p39, OspC, and p17 or a strong p41 band for PKo and at least one band among p39 and OspC or a strong p41 band for PBi must be present

Interpretation of the Western blot test results in the present study was done according to other criteria [7] based on studies using sera obtained from European locales.

Standardization of criteria for interpreting Western blot in Europe was the subject of a recent study by EUCALB. This multicenter study identified a subset of eight bands, although with variations in significance. The set of European rules formulated from these bands provides a framework for Western blot interpretation that may be adapted to the characteristics of Lyme disease in local areas [38].

In conclusion, these results suggest no association between Behçet's disease and *B. burgdorferi* infection. However, the high prevalence of *B. burgdorferi* antibodies in patient and control groups suggests that Lyme disease should be considered in patients with appropriate clinical findings in the Izmir region of Turkey.

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