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EXPERIMENTAL / LABORATORY STUDIES

Analysis of *Borrelia Burgdorferi* Antibody Seroprevalence in Patients with Behçet's Disease Using 3 Different Serological Assays

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Abstract: Behçet's disease is a chronic multisystemic inflammatory vasculitis of unknown etiology. Various infectious agents have been implicated as potential triggering factors in the pathogenesis of the disease. The present study was designed to evaluate the seroprevalence of Borrelia burgdorferi antibodies in Turkish patients with Behçet's disease.

For this purpose, 90 consecutive patients with Behçet's disease and 50 healthy individuals were enrolled into a prospective study. The IgM and IgG antibodies to *B. burgdorferi* in sera were determined by 3 different serological techniques, namely indirect immunofluorescence assay (IFA), enzyme linked immunosorbent assay (ELISA) and Western blotting (WB).

Although there was a tendency in the patient group toward a higher seropositivity rate using the ELISA and WB tests, statistical analysis revealed no difference in antibody prevalence between the patient and control groups for each technique performed ($P > 0.05$ for all 3 assays).

The findings of the present study do not support a potential role for *B. burgdorferi* in the etiopathogenesis of Behçet's disease, at least in the geographical region of central Anatolia.

Key Words: Behçet's disease, seroprevalence, *B. burgdorferi*

Introduction

Behçet's disease (BD) is a chronic multisystemic inflammatory disorder characterized by periodic exacerbations and progressive deterioriation. The disease most often presents with recurrent orogenital aphthous ulcerations, uveitis, and skin manifestations like erythema nodosum, folliculitis and acneiform lesions. Progressive disease may involve the arthritic, vascular, intestinal and neurological systems (1,2). The etiology and pathogenesis of BD are unknown, but a complex interaction of genetic and enviromental factors seems to be responsible for immunological dysregulation and inflammation. Various infectious agents have been postulated as triggering factors for BD (3,4).

Lyme disease (LD) is an infectious multisystemic disorder caused by a spirochete, *Borrelia burgdorferi*. The disease may affect several organ systems such as the skin, joints, the central nervous system and the cardiovascular system (5,6). Because of the wide spectrum of clinical manifestations in symptomatic infection, LD can mimic several other systemic disorders. Of interest is the fact that LD has been initially described in children with juvenile rheumatoid arthritis (7).

Recently, a role for *B. burgdorferi* has been proposed in the etiopathogenesis of BD (8). The aim of this study was to evaluate the seroprevalence of *B. burgdorferi* antibodies among Turkish patients with BD and to compare the results with those of healthy subjects.

Materials and Methods

Patient and control groups

Serum samples from 90 patients with BD and 50 adult volunteer blood donors were collected between July 1998 and December 2002. Patients and controls were from the same geographical area. The diagnosis of BD was based on the criteria of the International Study Group for Behçet's Disease (9). Disease activity was based on clinical findings at the time of venipuncture. Patients lacking any clinical signs were categorized as having inactive disease (42 patients; 46.7%). Patients with major oral aphthae and genital ulcerations, arthritis, ocular or neurologic manifestations and/or vascular manifestations constituted the group with active disease (48 patients; 53.3%). Clinical symptoms of patients with active BD are shown in Table 1. At the time of the study, 69 patients were taking colchicine and/or non-steroidal anti-inflammatory drugs. Nine patients were additionally receiving second line drugs, namely prednisolone, salazopyrin, azathioprine or cyclosporine. Seven patients with inactive disease and 5 patients who had received their initial diagnosis recently were taking no drugs. Fifty healthy individuals of a similar gender ratio and age range were assigned as the control group.

Serological Assays for *B. burgdorferi* Antibodies

Serum IgM and IgG antibodies to *B. burgdorferi* were measured by 3 serological techniques: enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA) and Western blot (WB) test. Test procedures were performed according to the manufacturer's protocol (Euroimmun, Luebeck, Germany). Appropriate positive and negative serum samples, as provided by the manufacturers, served as internal controls in all serological assays to improve the reliability of the test procedures.

Table 1. Clinical symptoms of patients with Behçet's disease (n = 90).

Symptom *	N (%)
Oral ulcer	90 (100)
Genital ulcer	81 (90.0)
Papulopustular lesions	38 (42.2)
Erythema nodosum	30 (33.3)
Arthritis	28 (31.1)
Arthralgia	61 (67.8)
Eye lesions	22 (24.4)
Deep vein thrombosis	14 (15.6)
Neurological involvement	2 (2.2)

*All symptoms observed during the course of the disease.

ELISA: In the first reaction step, diluted patient samples were incubated with wells coated with antigen extracts of *B. burgdorferi* and *Borrelia afzelii*. To detect the bound Borrelia-specific IgG or IgM antibodies, a second incubation was carried out using enzyme-labeled anti-human antibodies. Values above the recommended cut-off value of 20 relative units (RU)/ml were considered positive.

IFA: In order to prevent false positive IgM results, immunoabsorption was applied before testing with IFA. A smear of *B. burgdorferi* covering the reaction areas of a BIOCHIP slide was incubated with a diluted serum sample. In a second step, the attached Borrelia-specific IgG or IgM antibodies were stained with fluorescein-labeled anti-human antibodies and positive reactions were visualized under immunofluorescence microscope. Titers > 1/ 10 and > 1/ 100 were considered positive for IgM and IgG immunoreactivities, respectively.

WB: The test kit contains test strips with electrophoretically separated antigen extracts of *B. burgdorferi*. In the initial reaction step, the blot strips were incubated with diluted patient serum. To detect the bound Borrelia-specific IgG or IgM antibodies, a second incubation was carried out using enzyme-labeled anti-human antibodies. The results of the WB test were interpreted as negative, borderline or positive, according to the manufacturer's information sheet. Borderline and positive IgG or IgM WB test results were considered indicative of seropositivity for *B. burgdorferi* antibodies.

Statistical Analysis

Statistical analysis was performed on PC using SPSS for Windows, version 11.0. Chi-square test was utilized to compare numerical data. A P value ≤ 0.05 was considered significant.

Results

The study group consisted of 90 patients with BD (47 females, 43 males) with a mean age of 35.99 ± 9.09 and an age range of 16-54 years. The control group of 50 healthy individuals had a comparable age range (18-50 years) and sex ratio (26 females, 24 males).

IFA, ELISA and WB test results for the patient and control groups are shown in Table 2. Although there was a tendency in the patient group toward a higher seropositivity rate using ELISA and WB, statistical analysis revealed no significant difference between the patient and control groups in terms of *B. burgdorferi* IgG or IgM seropositivity using ELISA, IFA or WB.

Discussion

Lyme disease (LD), is a tick-borne treatable disease, endemic in some parts of the world. The disease usually presents with a pathognomonic skin lesion, known as erythema chronicum migrans. After the dissemination of the microorganism through blood, the disease secondarily involves several organ systems such as the skin, nervous system, joints and heart. The rising incidence and expanded clinical spectrum make LD an attraction and a focus of investigations in all medical disciplines. Although the definitive diagnosis of LD requires the isolation of the microorganism by culture or demonstration by polymerase chain reaction, this is not always feasible. Therefore, diagnosis usually relies on compatible clinical findings and confirmation by serological assays. Centers for Disease Control and Prevention recommends a dual serology approach for the serodiagnosis of *B. burgdorferi* infection where WB is utilized as the confirmatory serological test (10,11).

BD occurs endemically in the eastern Mediterranean and in Middle and Far Eastern countries, the population deriving from the ancient Silk Road. The highest prevalence has been reported in Turkey (4). The geographical restriction of the disease has been considered as indirect evidence for a role of infections in its etiopathogenesis. Several infectious agents have been speculated to dysregulate the immune system in the genetically susceptible population of the Silk Road, leading to the development of BD. Included in this list are Streptococcus spp., *Chlamydia pneumonia*, herpes simplex virus and parvovirus B19 (3,12,13).

The first report speculating a potential role for *B. burgdorferi* in patients with BD was that by Isogai et al. (8). The authors investigated the seropositivity rate for *B. burgdorferi* antibodies in a Japanese population of 127 patients with uveitis, including those with BD. However, a recent serological study from the western part of Turkey by Önen et al. (14) yielded no significant association between BD and *B. burgdorferi* infection. The authors analyzed *B. burgdorferi* seroprevalence in BD and detected seropositivity in 26.7% and 13.3% of patients and 19.4% and 12.9% of healthy controls using ELISA and WB, respectively.

In the present study, 3 different serological methods were employed to elucidate an association between BD and *B. burgdorferi* infection. Although LD is rarely encountered in Turkey, there is evidence that the Ixodes ticks harboring *B. burgdorferi* microorganisms exist in the country (16). The reported seroprevalence rates for *B. burgdorferi* antibodies in the general Turkish population vary between 2% (in urban areas) and 36% (in rural areas) depending on the geographical region (15,16). In our study, the seroprevalence rates for both patient and control groups were within the range determined by previous Turkish studies. Although a

Table 2. Antibodies to *Borrelia burgdorferi* in patients with Behçet's disease and healthy adult sera as determined by indirect fluorescent antibody assay (IFA), enzyme-linked immunosorbent assay (ELISA), and Western immunoblotting (WB).

	No. and (%) of positive serum samples					
	IFA		ELISA		WB*	
	IgG	IgM	IgG	IgM	IgG	IgM
Patient Group (90)	9 (10.0%)	1 (1.1%)	5 (5.6 %)	10 (11.1%)	8 (8.9%)	0
Control Group (50)	7 (14.0%)	2 (4.0%)	0 (0.0 %)	2 (4.0%)	1 (2.0%)	0
p	0.476	0.258	0.090	0.150	0.111	-

*Positive in 2 and borderline in 6 patients with Behçet's disease; and borderline in 1 control subject.

higher seroprevalence rate was detected in patients with BD using ELISA and WB, comparison with the control group did not reveal a statistically significant difference.

The serologic diagnosis of LD is a challenge. Although serologic tests, including WB, have several well-defined limitations, they are frequently employed in seroprevalence studies and may even be used as diagnostic tests in patients with compatible clinical findings (10,17). In the present study, we aimed at determining the seroprevalence of *B. burgdorferi* antibodies in patients with BD using serological testing. False positivities in serological diagnosis of LD were reported in patients with several infectious and chronic inflammatory disorders. In our study, the total number of positive results with any of the techniques utilized was rather low. Thus, we think that the main statistical findings of this seroprevalence study would not have been affected even in the presence of false positive results.

In conclusion, these findings do not support a potential role for *B. burgdorferi* infection in the etiopathogenesis of BD, at least in the geographical region of central Anatolia.

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