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Investigation of human leukocyte antigen in osteoarticular brucellosis

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Background/aim: To determine the relationship between human leukocyte antigen (HLA) antigens and osteoarticular brucellosis by evaluating HLA Class I and II antigens in control subjects and patients developing osteoarticular brucellosis in Turkey.

Materials and methods: The study included 28 patients with osteoarticular involvement diagnosed with brucellosis and 100 controls. The HLA Class I and II antigens were studied in isolated DNA samples using sequence-specific oligonucleotide procedure.

Results: The mean age of the 28 patients and 100 controls was 42.3 ± 20.2 years and 50.1 ± 16.3 years, respectively. When the frequency of HLA Class I was examined, HLA-A*02 and HLA-B*27 antigens were detected in 50% and 28.57% of the patients, respectively. However, there was no significant difference when compared to the control group. Among the HLA Class I antigens, HLA-Cw*10 was determined in 35.71% of the patients and 17% of the controls; the difference was significant ($P = 0.039$).

Conclusion: In the development of brucellosis, the frequency of HLA-Cw*10 among HLA Class I antigens was observed to be increased, and HLA-Cw*10 was considered likely to cause predisposition to brucellosis. Further studies to be performed on a higher number of patients and controls could demonstrate that genetic factors play a role in the diagnosis of osteoarticular brucellosis.

Key words: Brucellosis, human leukocyte antigen, polymerase chain reaction

1. Introduction

Brucellosis is a polymorphic disease that may involve any organ in the body. Osteoarticular involvement is the most common type. Osteoarticular brucellosis can present as peripheral arthritis, sacroiliitis, spondylitis, tenosynovitis, bursitis, and osteomyelitis. Large- and medium-sized peripheral joints, sacroiliac joints, and the spinal region are the most commonly involved sites (1–3). Recent studies have supported the hypothesis that genetic predisposition associated with human leukocyte antigen (HLA) genes plays a role in the development of osteoarticular complications. In the studies conducted, mostly HLA-A*02, HLA-B*27, and HLA-B*39, which are HLA Class I antigens, have been detected (4,5). The relationship of these tissue group antigens with osteoarticular involvement of brucellosis is not exactly known. Although there are several studies on the relationship of HLA-A*02 and HLA-B*27 antigens with arthritis and arthralgia, studies on the tissue group antigens that are common in osteoarticular brucellosis in Turkey are limited. The present study aims to determine the relationship of HLA antigens with osteoarticular brucellosis by evaluating HLA antigens in patients

developing osteoarticular brucellosis and control subjects in Turkey. Moreover, whereas the literature contains data on only HLA Class I antigens, we also studied HLA Class II antigens.

2. Materials and methods

The present study included 28 patients admitted to the Department of Infectious Diseases of the Faculty of Medicine, Gazi University, in 2014 and 2015, and diagnosed with osteoarticular involvement of brucellosis. For the control group, we selected 100 healthy renal transplant donors without any disease that had been admitted to the Tissue Typing Laboratory of our hospital. DNA samples of the patients and controls were isolated from the blood samples containing ethylenediaminetetraacetic acid (EDTA), using the BioRobot EZ1 system (QIAGEN, Hilden, Germany). HLA Class I and II antigens were studied in these isolated DNA samples using the polymerase chain reaction (PCR) sequence-specific oligonucleotide (PCR-SSO-Luminex; Gene-Probe Lifecodes, Stanford, CA, USA) procedure. The samples were studied according to the method described by the manufacturer (6). Briefly, 10 μ L of DNA

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sample from each patient was pipetted into the PCR tubes. We added 15 µL of ready-to-use MasterMix provided by the manufacturer to each PCR tube, and the DNA samples were amplified in a thermal cycler using the recommended program. Next 5 µL of PCR product was aliquoted into a Costar plate to provide the hybridization of the amplified DNA samples with specific oligonucleotides on a solid medium (wells or beads). We then added 15 µL of bead mix and covered the plate with a seal. Hybridization was initiated in the thermal cycler by selecting the program recommended by the manufacturer. Various reagents (a mixture of phycoerythrin and streptavidin) were used to visualize the hybridized oligonucleotide probes and HLA alleles. At the end of the test run, the results were analyzed using Quick-Type in the Luminex-Life-Match device. The present study was approved by the Ethics Committee of Gazi University, and informed consent of the patients was obtained.

2.1. Statistical analysis

Data analysis was performed using chi-square test and Fisher's exact test. Statistical significance was assessed using Fisher's exact test. $P < 0.05$ was considered

statistically significant. Data are presented as mean \pm standard deviation. The Shapiro-Wilk test was used to assess the normality of variable distribution. Since the group sizes were vastly unequal, homogeneity of variance was checked with Levene's test. No differences in variance were found between the patient and control groups, and distribution was normal.

3. Results

The mean age of 28 patients (16 male and 12 female), who had osteoarticular involvement and were diagnosed with brucellosis, was 42.3 ± 20.2 years, and the mean age of 100 healthy donor candidates (49 male and 51 female) was 50.1 ± 16.3 years. In accordance with the study method, all HLA Class I and II antigens were evaluated both in the patient group, with osteoarticular brucellosis, and in the control group. The frequencies of HLA Class I and II antigens in the patient and control groups are presented in Tables 1 and 2, respectively. When the frequency of HLA Class I antigens was examined, HLA-A*02 and HLA-B*27 were detected in 50% and 28.57% of the patients, respectively. However, there was no significant difference compared to

Table 1. Frequency of HLA Class I antigens in the patient and control groups.

HLA Class I antigens	Patient group n = 28	Control group n = 100	P
	n (%)	n (%)	
HLA-A*02	14 (50)	39 (39)	0.408
HLA-A*24	7 (25)	25 (25)	1.000
HLA-B*27	8 (28.57)	16 (16)	0.218
HLA-B*35	8 (28.57)	32 (32)	0.908
HLA-Cw*10	10 (35.71)	17 (17)	0.039
HLA-Cw*12	8 (28.57)	33 (33)	0.830

HLA: human leukocyte antigen.

Table 2. Frequency of HLA Class II antigens in the patient and control groups.

HLA Class II antigens	Patient group n = 28	Control group n = 100	P
	n (%)	n (%)	
HLA-DRB1*11	11 (39.28)	43 (43)	0.892
HLA-DRB1*04	10 (35.71)	23 (23)	0.265
HLA-DRB1*15	8 (28.57)	20 (20)	0.477
HLA-DQB1*03	21 (75)	70 (70)	0.779
HLA-DQB1*05	10 (35.71)	43 (43)	0.635
HLA-DQB1*06	9 (32.14)	20 (20)	0.271

HLA: human leukocyte antigen.

the control group. Among the HLA Class I antigens, HLA-Cw*10 was determined in 35.71% of the patients and 17% of the controls. The difference was found to be significant ($P = 0.039$). The frequency of HLA Class II antigens was similar in the patient and control groups.

4. Discussion

In brucellosis, which is primarily a reticuloendothelial system disease, osteoarticular involvement (spondylitis, peripheral arthritis, sacroiliitis, and osteomyelitis) is the most common (7,8). In the literature, there are several studies evaluating the relationship between osteoarticular involvement of brucellosis and various tissue groups. In the present study, the most common HLA Class I antigens were HLA-A*02, HLA-A*24, HLA-B*27, HLA-B*35, HLA-Cw*10, and HLA-Cw*12. The most common HLA Class II antigens were HLA-DRB1*04, HLA-DRB1*11, HLA-DRB1*15, HLA-DQB1*03, HLA-DQB1*05, and HLA-DQB1*06. Particularly, HLA-Cw*10 significantly more frequent in the patients with osteoarticular involvement. No significant difference was found between the patient and control groups in terms of the other tissue group antigens.

In the present study, although the frequencies of HLA-A*02 and HLA-B*27 antigens were 50% and 28.57%, respectively, no significant difference was determined in the patient group compared to the controls. The frequency of HLA-Cw*10 antigen was 35.71% in the patient group, which was significantly higher than in the control group (17%; $P < 0.039$). Studies on HLA-Cw* antigens are limited. In a single study on ankylosing spondylitis, HLA-Cw*10 antigen was found to be significant (9). To the best of our knowledge, there is no study in the literature on the relation of HLA-Cw*10 antigen with brucellosis. The present study is the first to demonstrate this relationship. However, our study has a small sample size, and further studies with larger sample sizes are required in the long

term. Although there are studies demonstrating the relation between HLA-B*27 antigen and osteoarticular brucellosis in the literature, we did not find a significant difference between the patient and control groups in terms of the frequency of HLA-B*27 antigen. There are limited studies on the HLA Class II antigens, and their relation with osteoarticular brucellosis has not been reported yet. Consistently with reports in the literature, no relation was found between HLA Class II antigens and osteoarticular brucellosis in the present study.

There are many studies investigating the genetic factors that play a role in susceptibility to infectious diseases, and several of these studies focus on HLA. The relation of host HLA with bacterial, viral, fungal, and parasitic infections has been widely investigated (10,11). The type and strength of this relationship vary depending on the racial/ethnic characteristics of the population. Certain types of HLA antigens are more frequent in some diseases. Studies on patients with rheumatic diseases can be given as examples (12,13). Osteoarticular involvement is a frequent and treatable complication of brucellosis. It has been thought that the pathogenesis of osteoarticular involvement might be infectious or reactive, and it has been suggested that osteoarticular complications might be more prevalent in patients carrying HLA-B*27 antigen in endemic regions (14). In other studies investigating the relationship between osteoarticular involvement and genetic predisposition, the frequency and severity of osteoarticular complications were determined to be significantly higher in individuals carrying HLA-A*02 and HLA-B*39 antigens (15,16).

In conclusion, high frequency of HLA-Cw*10 antigen was determined to be associated with the development of osteoarticular brucellosis. Studies performed on a higher number of patients and controls can confirm that genetic factors (HLA antigens) may play a role in diagnosing osteoarticular brucellosis.

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