Frequency of HLA-Class I Allel in Patients with Spondyloarthritis

Spondiloartropatili Hastalarda HLA Sinif I Allel Sıklığı

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ABSTRACT

Spondyloarthritis (SpA) is a group of multi-systemic diseases, whose pathogenesis is not known, characterized by spinal inflammation, peripheral arthritis, and with a lower frequency by extra-articular involvement. Breverton and Schlosstein introduced the relationship between HLA-B27 and the disease. Along with HLA-B27, the relationship of the disease with other HLA molecules was also shown in studies. Taking this information as a starting point and knowing that the disease is related to ethnic differences, we aimed to investigate the role of the HLA-A and –B alleles in Turkish patients with SpA. Typing of the patients (n=784) was performed by the complement-dependent lymphotocxity method. The HLA-A and –B tissue groups of the control group (n=1060) were determined by using serological or molecular methods. The frequency of HLA-B27 in patients was determined as 27%. When B27-negative patients were compared with B27-negative controls, HLA-A29 was found significantly higher in the patients (p: 0.0003, pc: 0.004). Although HLA-B60 was found significantly higher in the patients (p: 0.02), a statistical significance could not be obtained after performing the Bonferroni correction method (pc>0.05). When B27-positive patients and controls were compared, HLA-A3 (p<0.0005, pc<0.008), HLA-B35 (p<0.0001, pc<0.003), HLA-B51 (p<0.0001, pc<0.003), and HLA-B52 (p<0.0001, pc<0.03) were found significantly higher in the control group, while HLA-B27 allele is related with the development of the disease. It has been shown in other studies that other HLA molecules together with ethnic differences may have an effect in liability to and protectiveness from the disease.

Keywords: HLA, Spondyloarthritis, HLA-B27

ÖZ

Spondiloartropati (SpA), spinal inflamasyon ve periferal artrit ile daha az oranda da eklem dışı tutulmalara karakterize, patogenezi henüz tam olarak bilinen multisistemik bir grup hastalıktır. Genetik faktörlerin hastalığın gelişiminde önemli rolü vardır. 1973 yılında Breverton ve Schlosstein HLA-B27 ile hastalığı ilişkisini ortaya çıkarmıştır. HLA-B27 ile birlikte diğer HLA moleküllerinin (DR1, DR4, DR8, DR15, A24, B39 ve B60) hastalığa ilişkisi yapılan çalışmalarla gösterilmiştir. Bu bilgilerden yola çıkarak SpA’lı Türk hastalarında HLA-A ve –B alelleriin rolünü araştırmaya amaçladık. Hastalardan (n=784) heparinli kan örnekleri alındı. Lenfosit izolasyonu takiben kompleman bağımlı lenfotoksisite yöntemi ile ticari kitler (Biotest-HLA-ABC tıpleme plaga, 144X2, USA) kullanılarak tıpleme yapıldı. Kontrol grubunun (n:1060) HLA-A ve –B doku grupları, serolojik veya moleküler yöntemler kullanılarak tespit edildi. Hastalarda HLA-B27 sıklığı %27 olarak saptandı. B27 negatif hastalar ise B27 negatif kontrollerle karşılaştırıldığında, hastalarda HLA-A29 anlamlı olarak yüksek bulundu (p<0.0003, pc<0.004, OR:2.6, CI:1.5-4.4). HLA-B60 hastalarda anlamlı olarak yüksek (p<0.02, OR:0.5, CI:0.2-0.9) bulunmasına rağmen Bonferroni doğrulama testi sonrası istatistiksel anlamalı olmamadı (pc>0.05). B27 pozitif hastalara kontrolleri karşılaştırıldığında HLA-A3 (p<0.0005, pc<0.008, OR:0.4, CI:0.3-0.7), HLA-B35 (p<0.0001, pc<0.003, OR:0.3, CI:0.2-0.4), HLA-B51 (p<0.0001, pc<0.003, OR:0.3, CI:0.2-0.6) ve HLA-B52 (p<0.001, pc<0.03, OR:0.4, CI:0.002-0.7) kontrol grubunda, HLA-B27 (p<0.0001, pc<0.003, OR:0.52, CI:36.2-74.7) ise hasta grubunda anlamlı olarak yüksek bulundu. Patogenezi tam olarak bilinen SpA’ların gelişiminde genetik faktörlerin rolü büyütür. HLA-B27 alelinin hastalığın gelişimi ile ilişkili olduğu bilinmektedir. Etnik farklılıklarla birlikte diğer HLA moleküllerinin de hastalığa karşı yatkınlık ve koruyuculuk etkisi olabileceği yapılan çalışmalarla gösterilmiştir.

Anahtar Kelimeler: HLA, Spondiloartropati, HLA-B27
INTRODUCTION

Spondyloarthritis (SpA) is a heterogeneous multisystemic disease which is characterized by spinal inflammation, peripheral arthritis and extra-articular involvement which include ankylosing spondylitis (AS) and reactive arthritis (ReA). Its pathogenesis has not yet fully known, however, genetics is an important factor in this disease group. Gender, onset of age and ethnic differences affect the clinical presentation of the disease (1-5).

An association between HLA-B27 and AS was first reported in 1973 (6,7). There is a strong association between HLA-B27 and SpA. Studies showed that HLA-B*2705 is the most common subgroup (1,8-11). The frequency of HLA-B27 is 70-90% in patients with AS (12).

HLA-B27 positive individual’s chance of having these diseases is 30 to 100 times higher than HLA-B27 negative (8). Studies showed that not only B27, but other HLA molecules (DR1, DR4, DR8, DR15, A24, B7, B39 and B60) are associated with SpA (13-20). The HLA-B27 molecule is related with uveitis, familial Mediterranean fever (FMF) and Behcet’ disease (22-24).

In this study, we aimed to investigate the association of HLA class I in Turkish patients with SpA.

MATERIAL AND METHOD

Patient and control group:

5248 patients (F/M: 2646/2602, mean age: 37±14 years) with spondyloarthropathy preliminary diagnosis and 1060 healthy controls (F/M: 441/619, mean age: 36±14 years) who are unrelated were included for HLA Class I typing between 2000 and 2018 in this study. All tests of the patient and control groups were carried out by XXX, accredited by the European Federation of Immunogenetics (EFI-European Federation for Immunogenetics).

HLA-B27 typing:

We used two different methods for HLA typing:

1. Molecular method:

DNA (n=4464) was extracted from the whole peripheral blood with EDTA (25). HLA genotyping was performed by PCR-SSP (26) with Olerup HLA B27 typing kit. The results were evaluated as negative or positive.

2. Serologic method:

Lymphocytes (n=784) were isolated from the whole peripheral blood with heparine. HLA typing was performed by complement dependent cytotoxicity (CDC) with Biotest,144X2, USA HLA-ABC typing plate (27).

HLA-A and –B tissue type of the control group was detected by serologic or molecular method.

Statistical Analysis

Statistical analyses were performed by SPSS 12.0 software. Frequencies and percentage (%) ratios of HLA were calculated. Chi-square and Fisher’s exact test were applied to compare the number of cases and controls who were positive for a specific antigen. p values less than 0.05 were considered as significant. Corrected p value (p_c) was obtained by multiplying the p value by the number of antigens tested for each locus (16 for HLA-A, 32 for HLA-B) according to Bonferroni’s correction.

RESULTS

Demographic characteristics of the patients (n:5248; mean age: 37±14 [range:2-87] years; F/M: 2646/2602) and controls (n:1060; mean age: 35±14 [range:1-86] years; F/M: 441/619) are shown in Table 1.

HLA-B27 positive individual was detected in 4% of the control group while 27% of patients (n=5248) were positive. Frequency of HLA-B27 in the patient group was more than seven times that of the control group, and this difference was highly statistically significant (p<0.0001, OR:0.11, CI:0.08-0.15) (Table 1). 27% of HLA-B27 negative patients were male while 62% of HLA-B27 positive patients, and this difference was highly statistically significant (p<0.0001, OR:1.95, CI:1.7-2.2). Diagnosed patients with spondyloarthropathy were 84% and 16% of patients who have similar clinical findings (FMF %1, uveitis %7 and Behcet’s disease %8).

We performed HLA Class I typing by CDC in 15% of patients (n:784; mean age: 34±14 [range:4-77] years).
years; F/M: 390/394) and HLA B27 was found positive in 27% (n:211) of this patients.

We divided the patients into two groups (Group I: ReA ve AS, Group II: Behcet disease, FMF, Uveitis). We detected 27% HLA-B27 in both groups. When we compared B27 negative patients and controls, A29 was found significantly high in patients (p:0.0003, pc:0.004, OR:2.6, CI:1.5-4.4). Although HLA-B60 was found significantly high (p:0.02, OR:0.5, CI:0.2-0.9) in patients, the difference remained significant after Bonferroni correction (p_c >0.05).

When we compared B27 positive group I patients and controls, HLA-A3 (p:0.0005, pc:0.008, OR:0.4, CI:0.3-0.7), HLA-B35 (p<0.0001, pc<0.003, OR:0.3, CI:0.2-0.4), HLA-B51 (p<0.0001, pc<0.003, OR:0.3, CI:0.2-0.6) and HLA-B52 (p:0.001, pc:0.03, CI:0.002-0.7) was found significantly high in controls and HLA-B27 (p<0.0001, pc<0.003, OR:52, CI:36.2-74.7) in the patients group.

When we compared B27 negative group II patients and controls, HLA-B55 (p:0.0002, pc:0.006, OR:6.5, CI:2.4-17.5) was found significantly high in patients with FMF. When we compared B27 positive group II patients and controls, HLA-B27 (p<0.0001, pc<0.003) was found significantly high in patients with FMF, Behcet’ disease and uveitis.

The most frequent HLA antigens distribution was like that:
• HLA-A locus: HLA-A2 (n:484, %23), HLA-A24 (n:379, %18) and HLA-A3 (n:277, %13);
• HLA-B locus: HLA-B35 (n:422, %20), HLA-B51 (n:317, %15) and HLA-B44 (n:155, %7)

DISCUSSION

Spondyloarthropathies are characterised by inflammation of the vertebrae, peripheral joints and periarticular tissues. Diseases in this group present with similar clinical features. Ankylosing spondylitis and reactive arthritis are located in this group. Uveitis, FMF and Behcet’ disease are called the non-classified spondyloarthropathies. Since the 1970s, it has been shown that HLA-B27 is remarkably associated with the disease (6,7,28-30). In our study, we found statistically significant HLA-B27 in both group. HLA frequency of the healthy group was concordant with Turkish population’s HLA frequency (31,32).

In studies, an association was shown between SpA and non-classified SpA and HLA alleles. HLA-B60 and -B61 were found associated in HLA-B27 negative patients with SpA of the Taiwan population, HLA-B15 was found associated in the Mexico population with SpA and non-classified SpA in HLA-B27 of negative patients (29,33). In contrast, Deveraj et al. showed the frequency of HLA-B40 was significantly decreased (21). In our study, HLA-A29 was detected significantly high in B27 negative patients with SpA. We think that this allele may be associated with susceptibility to the disease. B60 was found significantly high in HLA-B27 negative patients with SpA, in spite of the significance that remained with the Bonferroni test.

In studies, A3 was found high in HLA-B27 positive patients with SpA of the Tunis population (34). In the India population, HLA-A1 was detected low in patients (30). Pimentel-Santos et al. detected that A36, A69, B42, B52 and B78 were only identified in controls. Furthermore, frequencies of A31 and B8 were increased in AS patients (35). In our study, we showed the frequency of HLA-A3, -B35, -B51 and -B52 as significantly low in patients. HLA-A3, -B35 and -B51 were seen at high frequency, while the frequency of B52 was low in the Turkish population. We think that B52 may be protective against the disease, nonetheless, we should not ignore that B52 and B51 were the cross reactive antigens in serological method.

Genetic factors are important in the development of the non-classified SpAs such as FMF, uveitis and Behcet’s disease. In this study, B55 was found significantly high in B27 negative patients with FMF. But our number of FMF patients is too low, so we think that the number of patients should be increased.

Studies showed that gender differences are important in clinical findings (29,34). Consistent with the literature in this study, there are many more men than women in the B27 positive patients.

In conclusion, the role of genetic factors is important in the development of SpA whose pathogenesis is not exactly known. The HLA-B27
allele is known to be associated with the development of the disease. Other HLA molecules with ethnic differences have been shown in studies to be effective in susceptibility and protection against the disease.

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