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Frequency of HLA-Class I Allel in Patients with Spondyloarthropathy

Spondiloartropatili Hastalarda HLA Sınıf I Allel Sıklığı

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ABSTRACT

Spondyloarthropathy (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. Brevverton 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 lymphotoxicity 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, Spondyloarthropaty, HLA-B27

ÖZ

Spondiloartropati (SpA), spinal inflamasyon ve periferal artrit ile daha az oranda da eklem dışı tutulumla karakterize, patogenezi henüz tam olarak bilinmeyen multisistemik bir grup hastalıktır. Genetik faktörlerin hastalığın gelişiminde önemli rolü vardır. 1973 yılında Brevverton ve Schlosstein, HLA-B27 ile hastalık ilişkisini ortaya çıkarmıştır. HLA-B27 ile birlikte diğer HLA moleküllerinin (DR1, DR4, DR8, DR15, A24, B39 ve B60)hastalıkla ilişkisi yapılan çalışmalarda gösterilmiştir. Bu bilgilerden yola çıkarak ve etnik farklılığın da hastalıkla ilişkili olduğunu düşünerek SpA'lı Türk hastalarda HLA-A ve –B allellerinin rolünü araştırmayı amaçladık. Hastalardan (n=784) heparinli kan örneği alındı. Lenfosit izolasyonunu takiben komplemana bağımlı lenfositotoksisite yöntemi ile ticari kitler (Biotest-HLA-ABC tipleme plağı, 144X2, USA) kullanılarak tipleme 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 ile B27 negatif kontroller 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 anlamlılık elde edilemedi (pc>0.05). B27 pozitif hastalarla kontrolleri karşılaştırdığımızda 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:0.04, CI:0.002-0.7) kontrol grubunda, HLA-B27 (p<0.0001, pc<0.003, OR:52, CI:36.2-74.7) ise hasta grubunda anlamlı olarak yüksek bulundu. Patogenezi tam olarak bilinmeyen SpA'ların gelişiminde genetik faktörlerin rolü büyüktür. HLA-B27 allelinin hastalığın gelişimi ile ilişkili olduğu bilinmektedir. Etnik farklılıklarla birlikte diğer HLA moleküllerinin de hastalığa karşı yatkınlık ve koruyuculukta etkili olabileceği yapılan çalışmalarla gösterilmiştir.

Anahtar Kelimeler: HLA, Spondiloartropati, HLA-B27

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INTRODUCTION

Spondyloarthropathy (SpA) is a heterogeneous multisystemic disease which is characterized by spinal inflamation, 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; 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, OR:0.04, 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 Betcet' 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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REFERENCES

1. Kahn MA. HLA-B27 subtypes in world populations. Curr Opin Rheumatol 1995;7:263.
2. Chopra A, Raghunath D, Singh A. Spectrum of seronegative arthropathies (SSA) with special reference to HLA profiles. J Assoc Phys India 1990;38:351-5.
3. Lau CS, Burgos-Vargas R, Louthrenoo W, Mok MY, Wordsworth P,Zheng QY. Features of spondyloarthropathies around the world. Rheum Dis Clin North Am 1998;24:753-70.
4. Lopez de Castro JA. HLA-B27 and the pathogenesis of spondyloarthrapathies. Immunol Lett 2006;15:27-33.
5. Reveille JD, Arnett FC. Spondyloarthritis: update on pathogenesis and management. Am J Med 2005;118:592-603.
6. Brewerton DA, Caffrey M, Hart FD et al. Ankylosing spondylitis and HLA-B27. Lancet 1973;1:904-7.
7. Schlostein L, Terasaki PI, Bluestone R, Pearson CM. High association of an HLA antigen, BW27, with ankylosing spondylitis. N Engl J Med 1973;288:704-6.
8. Gonzales-Roces S, Alvarez MV, Gonzales S et al. HLA-B27 polymorphism and worldwide susceptibility to ankylosing spondylitis. Tissue Antigens 1997;49:116-2.
9. Nasution AR, Mardjuadi A, Kunmartini S, Suryadhana NG et al. HLA-B27 subtypes positively and negatively associated with spondyloarthropathy. J Rheumatol 1997;24:1111-4.
10. Oguz FS, Ocal L, Diler AS, Ozkul H, Asicioglu F, Kasapoglu E, Bozkurt G, Konice M, Carin M. HLA B-27 subtypes in Turkish patients with spondyloarthropathy and healthy controls. Dis Markers. 2004;20(6):309-12.
11. Abualrous ET, Fritzsche S, Hein Z, Al-Balushi MS, Reinink P, Boyle LH, Wellbrock U, Antoniou AN, Springer S. F pocket flexibility influences the tapasin dependence of two differentially disease-associated MHC Class I proteins. Eur J Immunol. 2015 Jan 23. doi: 10.1002/eji.201445307.
12. Gunal EK, Sarvan FO, Kamali S, Gul A, Inanc M, Carin M, Konice M, Aral O, Ocal L. Low frequency of HLA-B27 in ankylosing spondylitis patients from Turkey. Joint Bone Spine. 2008 May;75(3):299-302.
13. Toussirot E, Wendling D. Immunogenetic of ankylosing spondylitis. Rev Med Interne 2006;27:762-71.
14. Brown MA, Kennedy GL, Darke C, Gibson K, Pile KD, Shatford JL, et al. The effect of HLA-DR genes on susceptibility to and severity of ankylosing spondylitis. Arthritis Rheum 1998;41:460–5.
15. Miehle W, Schattenkirchner M, Albert D, Bunge M. HLA-DR4 in ankylosing spondylitis with different patterns of joint involvement. Ann Rheum Dis 1985;44:39–44.
16. Robinson WP, Van Der Linden SM, Khan MA, Rentsch HU, Cats A, Russell A, et al. HLA-Bw60 increases susceptibility to ankylosing spondylitis in HLA-B27 + patients. Arthritis Rheum 1989;32:1135–41.
17. De Juan MD, Reta A, Cancio J, Belzunegui J, Cuadrado E. HLA-A*9, a probable secondary susceptibility marker to ankylosing spondylitis in Basque patients. Tissue Antigens 1999;53:161–6.
18. Islam SM, Numaga J, Fujino Y, Masuda K, Ohda H, Hirata R, et al. HLA-DR8 and acute anterior uveitis in ankylosing spondylitis. Arthritis Rheum 1995;38:547–50.

19. Vargas-Alarcón G, García A, Bahena S, Melín-Aldana H, Andrade F, Ibañez-de-Kasep G, et al. HLA-B and complotypes in Mexican patients with seronegative spondyloarthropathies. Ann Rheum Dis 1994;53:755–8.

20. Maksymowych WP, Gorodezky C, Olivo A, Alaez C, Wong C, Burgos-Vargas R, et al. HLA-DRB1*08 influences the development of disease in Mexican mestizo with spondyloarthropathy. J Rheumatol 1997;24:904–7.

21. Parasannanavar DJ, Rajadhyaksha A, Ghosh K. Role of HLA-B Alleles and Clinical Presentation of B27 Negative Spondyloarthritis Patients from Mumbai, Western India. Autoimmune Dis. 2014;2014:327315. doi: 10.1155/2014/327315.

22. Koehler L, Kuipers JG, Zeidler H. Managing seronegative spondyloarthritis. Rheumatology 2000;39:360-8.

23. Prete M, Guerriero S, Dammacco R, Fatone MC, Vacca A, Dammacco F, Racanelli V. Autoimmune uveitis: a retrospective analysis of 104 patients from a tertiary reference center. J Ophthalmic Inflamm Infect. 2014 Jul 24;4:17.

24. Radouane A, Oudghiri M, Chakib A, Naya A, Belhouari A, El Malki A, Bennani S. HLA-B*27 allele associated to Behc¸ et's disease and to anterior uveitis in Moroccan patients. Ann Biol Clin (Paris). 2011 Jul-Aug;69(4):419-24.

25. Gustincich S, Manfiolett G, Del Sal G, Schneider C, Carninci P. A fast method for high-quality genomic DNA extraction from whole human blood. Bio Techniques 1991;11:298-302.

26. Olerup O, Zetterquist H. HLA-DRB1*01 subtyping by allele-specific PCR amplification: A sensitive, specific and rapid technique. Tissue Antigens 1991;37:197-204.

27. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. Nature 1964:204;998-1000.

28. Şendur ÖF, Aydeniz A. Spondiloartropatilerin temel özellikleri ve ayırıcı tanı ve tedavisinin genel kriterleri. ADÜ Tıp Fakültesi Dergisi 2001:2(2);31-5.

29. Vargas-Alarcón G, Londono JD, Hernandez-Pacheco G, Pacheco-Tena C, Castillo E, Cardiel MH, Granados J, Burgos-Vargas R. Effect of HLA-B and HLA-DR genes on susceptibility to and severity of spondyloarthropathies in Mexican patients. Ann Rheum Dis 2002:61;714-7.

30. Madhavan R, Parthiban M, Panchapakesa Rajendran C, Chandrasekaran AN, Zake L, Sanjeevi B. HLA Class I and Class II association with Ankylosing spondylitis in a Southern Indian population. Ann NY Acad Sci 2002:958;403-7.

31. Arnais-Villena A, Carin M, Bendikuze N, Gomez-Casado E, Moscosa J, Oguz FS, Sarper Diler A, De Pacho A, Allende J, Guillen J, Martinez Laso J. HLA alleles and haplotypes in Turkish population: relatedness to Kurds, Armanians and other Mediterraneans. Tissue Antigens 2001:57(4);308-17.

32. Karahan GE, Seyhun Y, Oguz SF, Kekik C, Onal AE, Yazici H, Turkmen A, Aydin AE, Sever MS, Eldegez U, Carin MN. Impact of HLA on the underlying primary diseases in Turkish patients with end-stage renal disease. Renal Failure 2009:31;44-49.

33. Wei JCC, Tsai WC, Lin HS, Tsai CY, Chou CT. HLA-B60 and B61 are strongly associated with ankylosing spondylitis in HLA-B27 negative Taiwan Chinese patients. Rheumatalogy 2004:43;839-42.

34. Mahfoudh N, Siala M, Rihl M, Kammoun A, Frikha F, Fourati H, Younes M, Gdoura R, Gaddour L, Hakim F, Bahloul Z, Baklouti S, Bargaoui N, Sellami S , Hammami A, Makni H. Association and frequency of HLA-A, B and HLA-DR genes in south Tunisian patients with spondyloarthritis (SpA).Clin Rheumatol 2011: DOI 10.1007/s10067-011-1705-6

35. Pimentel-Santos FM, Matos M, Ligeiro D, Moura AF, Ribeiro C, Costa J, Santos H, Barcelos A, Pinto P, Cruz M, Sousa E, Santos RA, Fonseca JE, Trindade H, Guedes-Pinto H, Branco JC, CORPOREA Study Group. HLA alleles and HLA-B27 haplotypes associated with susceptibility and severity of ankylosing spondylitis in a Portuguese population. Tissue Antigens, 2013:82;374–379.