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HLA-DRB1 Alleles Distribution in Patients With Rheumatoid Arthritis in A Tertiary Center in the Southeastern Anatolia Region of Turkey

Türkiye'nin Güneydoğu Anadolu Bölgesinde Tersiyer Bir Merkezdeki Romatoid Artritli Hastalarda HLA-DRB1 Alel Dağılımı

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Summary

Objective: The HLA-DRB1 alleles play an important role in the genetic predisposition to rheumatoid arthritis (RA). The relationship between HLA-DRB1 and rheumatoid arthritis show differences according to various ethnic groups and geographical distributions. The aim of this study was to determine the distribution of HLA-DRB1 among the Turkish RA patient population in the Southeastern Anatolia Region.

Materials and Methods: 96 patients diagnosed with RA and a control group consisting of 84 healthy individuals were enrolled in the study. The HLA-DRB1 type and subtypes were specified using the polymerase chain reaction with sequence specific primers (PCR-SSP) method. The between-group HLA-DRB1 type and certain subtype frequencies were compared.

Results: The DRB1*10 alleles were found to be statistically significantly higher in patients with RA compared to the control group (p=0.001). However, the DRB1*07 and DRB1*11 alleles were statistically significantly lower in patients with RA (p<0.001 and p=0.02, respectively). In the RA patient group, the DRB1*0401, DRB1*0408 and DRB1*1001 subtypes were found to be statistically significantly higher (p=0.04, p=0.01, p=0.005, respectively), while the DRB1*0402, DRB1*0403 and DRB1*0701 subtypes were statistically significantly lower (p=0.01, p=0.02, p<0.001, respectively).

Conclusion: This study has revealed the HLA-DRB1 distribution in the Southeastern Anatolia Region of Turkey. DRB1*10 type and DRB1*0401, DRB1*0408, DRB1*1001 subtypes were observed to be related with RA. DRB1*07, BRB1*11 types and DRB1 *0402, DRB1*0403, DRB1*0701 subtypes were accepted as protective alleles and subtypes. *Turk J Phys Med Rehab 2013;59:123-7.*

Key Words: Rheumatoid arthritis, HLA-DRB1, subtype

Özet

Amaç: Romatoid Artrit (RA)'in genetik yatkınlığında HLA-DRB1 alelleri önemli bir yere sahiptir. Değişik etnik gruplarda ve coğrafik dağılımlarda HLA-DRB1 ile RA ilişkisi farklılık göstermektedir. Bu çalışmanın amacı Güneydoğu Anadolu Bölgesi Türk popülasyonundaki RA'lı hastalarda, HLA-DRB1 alel dağılımının belirlenmesidir.

Gereç ve Yöntem: RA tanısı almış 96 hasta ve kontrol grubu olarak 84 sağlıklı kişi çalışmaya dahil edildi. HLA-DRB1 tip ve subtipleri 'polymerase chain reaction with sequence specific primers' (PCR-SSP) metodu kullanılarak belirlendi. Gruplar arası HLA-DRB1 tip ve bazı subtip frekansları karşılaştırıldı.

Bulgular: RA'lı hastalarda, kontrol grubu ile kıyaslandığında, DRB1*10 aleli istatistiksel olarak anlamlı yüksek bulundu (p=0,001). RA'lı hastalarda DRB1*07 ve DRB1*11 alelleri ise istatistiksel olarak anlamlı düşük bulundu (p<0,001 ve p=0,02, sırasıyla). RA'lı hasta grubunda DRB1*0401, DRB1*0408 ve DRB1*1001 alt tipleri istatistiksel olarak anlamlı yüksek (p=0,04, p=0,01, p=0,005, sırasıyla); DRB1*0402, DRB1*0403 ve DRB1*0701 alt tipleri ise istatistiksel olarak anlamlı düşük bulundu (p=0,01, p=0,02, p<0,001, sırasıyla).

Sonuç: Bu çalışma Türkiye'nin Güneydoğu Anadolu Bölgesinde HLA-DRB1 dağılımını ortaya koymaktadır. DRB1*10 tip ve DRB1*0401, DRB1*0408, DRB1*1001 subtipleri RA ile ilişkili bulunmuştur. DRB1*07, BRB1*11 tipleri ve DRB1 *0402, DRB1*0403, DRB1*0701 subtipleri koruyucu alel ve alt tipleri olarak kabul edilmiştir. *Türk Fiz Tıp Rehab Derg 2013;59:123-7.*

Anahtar Kelimeler: Romatoid artrit, HLA-DRB1, subtip

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Introduction

Rheumatoid arthritis (RA) is a multifactorial disease that occurs due to genetic and environmental factors (1). The genetic predisposition to RA has been supported through the evidence obtained from studies conducted on siblings and twins (2,3). The relationship between HLA class II genes and the genetic predisposition to RA has been clearly defined. The main determinant among the genes causing a predisposition to RA is the HLA-DRB1 alleles (4). This relationship between HLA-DRB1 and RA varies according to different populations. The association between the HLA-DRB1 alleles and RA has been shown in many populations, such as Italian, Caucasian, Finn, Korean and Latin American populations (4-8).

Genetic research has revealed that the HLA-DRB1 alleles code a conserved sequence, which is characterized by the RAA pattern at position 72-74 of the third hypervariable region of different HLA-DR chains. This region in the third hypervariable region of the DRB1 molecule is defined as the 'shared epitope (SE)' and the SE is connected to the occurrence and severity of arthritis (2,3,7,9). Due to the differences in the geographical distribution of the HLA genes, it is yet to be clarified which HLA-DRB1 alleles are protective (10).

Although the relationship between RA and HLA has been evaluated in many populations, there are rather few studies conducted on the Turkish population (11,12). In the present study, our aim was to determine the genotyping of the HLA-DRB1 alleles and certain HLA-DRB1 subgroups (HLA-DRB1*01, *04, *07, *08, *10) among the population in the Southeastern Anatolia Region of Turkey.

Materials and Methods

This study was approved by the Ethics Committee of the Dicle University School of Medicine. Ninety-six patients (78 female, 18 male) who have applied to the physical medicine and rehabilitation outpatient clinic at Dicle University School of Medicine, and were diagnosed with RA according to the 1987 American College of Rheumatology (ACR) classification criteria were enrolled in the study together with 84 healthy individuals (48 female, 36 male) from the same region as the control group. The control group consisted of individuals who applied to the molecular hematology laboratory at Dicle University School of Medicine as donors. Written informed consent was obtained from all the subjects. Data on age and sex and age at disease onset were recorded. All the patients underwent a detailed physical examination. The RF titres were measured through the nephelometric immunoassay method and values above 20 IU/mL were considered positive. Erythrocyte sedimentation rate was measured by the Westergren method (mm/h), and serum C reactive protein levels were measured by nephelometry (mg/l).

Peripheral blood samples collected from all RA patients and healthy individuals were stored at -30°C for DNA isolation. The DNA isolation was performed using the GenoM-6 model (Qiagen) isolation robot and the EZ1 DNA blood kit (Qiagen). Polymerase chain reaction (PCR) amplification of the DNA was done through the Thermal Cycler (Corbet Research), and the HLA-DRB1 genotyping was performed using the PCR-SSP HLA-DRB1 Low Resolution gene panel manufactured by Olerup. The HLA-DRB1*01, 04, 07, 08, 10 subtypes were detected using the PCR-SSP HLA-DRB1 High Resolution genotyping panel also manufactured by Olerup.

Computerized statistical analyses were performed using SPSS 15.0 for Windows and MedCalc Version 10.1.6.0 Biostatistics package. The standard distribution, frequency and percentages of the variables were calculated. Fisher's exact Chi-Square test was used for the comparison of proportional variables and the relationships were calculated in odds ratio (OR) and 95% CI (confidence interval). A p value of less than 0.05 was considered statistically significant.

Results

The demographic data and the disease features of the 96 patients diagnosed with RA are presented in Table 1. Extraarticular involvements were diabetes mellitus (DM) (4.2%), hypertension (HT) (23.9%), coroner heart disease (4.1%), hypothyroidism (1.1%), and chronic hepatic disease (1.1%).

When the allele type frequencies were compared between RA-patient group and the control group, the DRB1*10 allele was found to be significantly higher in the RA patient group, while the DRB1*07 and DRB1*11 alleles were significantly lower in patients with RA ($p<0.05$). When the patient and control groups were compared, no significant difference was observed in the frequencies of the DRB1*01, DRB1*03, DRB1*04, DRB1*08, DRB1*13, DRB1*14, DRB1*15, and DRB1*16 alleles ($p>0.05$) (Table 2).

The most frequently observed DRB1*04 subtypes were the DRB1*0405, DRB1*0408, DRB1*0403, DRB1*0401, DRB1*0402 and DRB1*0404 in order of frequency (17.5 %, 9.3 %, 8.2 %, 7.2 %, 6.2 %, 6.2 %, respectively). There was no relationship between accompanying systemic disease and DRB1 alleles ($p>0.05$). When the allele subtype frequencies in the patient group and the control group were compared, the DRB1*0401, DRB1*0408 and DRB1*1001 subtypes were found to be significantly higher ($p<0.05$) in the RA patient group, while the DRB1*0402, DRB1*0403 and DRB1*0701 subtypes were found to be significantly lower ($p<0.05$) (Table 3). No significant

Table 1. Demographic and clinical characteristics in RA patients.

Characteristics	n=96
Age (years)	46.97±13.44
Sex (Female%)	81.2%
Accompanying systemic disease (%)	32.3%
Morning stiffness±SD (minutes)	83.71±53.70
Median disease duration±SD (years)	7.52±6.86
ESR mean±SD (mm/h)	28.55±21.38
CRP mean±SD (mg/dl)	3.20±6.17
RF mean±SD (IU/mL)	123.10±18.44

ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; RF: Rheumatoid Factor; SD: Standart Deviation

Table 2. The distribution of HLA DRB1 alleles in RA and control groups.

HLA-DRB1	RA patients		Control		OR	95% CI	p
	n%	AF	n%	AF			
DRB1*01	13	6.8%	4	2.4%	2.98	0.95-9.32	0.08
DRB1*03	17	8.9%	17	10.1%	0.86	0.43-1.75	0.72
DRB1*04	53	27.6%	33	19.6%	1.56	0.95-2.56	0.08
DRB1*07	8	4.2%	25	14.9%	0.25	0.11-0.57	0.001
DRB1*08	6	3.1%	1	0.6%	5.39	0.64-45.21	0.12
DRB1*10	17	8.9%	2	1.2%	8.06	1.83-35.44	0.006
DRB1*11	28	14.6%	41	24.4%	0.53	0.31-0.90	0.02
DRB1*13	14	7.3%	16	9.5%	0.75	0.35-1.58	0.45
DRB1*14	6	3.1%	5	3%	1.05	0.32-3.51	0.93
DRB1*15	26	13.5%	19	11.3%	1.23	0.65-2.31	0.63
DRB1*16	4	2.1%	5	3%	0.69	0.18-2.63	0.59
Total	192	100%	168	100%			

RA: Rheumatoid Arthritis, AF: Allele Frequency

Table 3. The distribution of HLA DRB1 subtypes in RA and control groups.

HLADRB1	RA patients		Control		OR	95% CI	p
	n%	AF	n%	AF%			
DRB1*0101	13	13.4%	4	6.2%	2.43	0.76-7.86	0.13
DRB1*0401	7	7.2%	0	0%	10.69	0.6-190.55	0.04
DRB1*0402	6	6.2%	13	20.3%	0.26	0.09-0.75	0.01
DRB1*0403	8	8.2%	14	21.9%	0.32	0.13-0.86	0.02
DRB1*0404	6	6.2%	1	1.6%	4.15	0.49-35.35	0.25
DRB1*0405	17	17.5%	5	7.8%	2.51	0.88-7.18	0.10
DRB1*0408	9	9.3%	0	0%	14.50	0.79-242.26	0.01
DRB1*0701	8	8.2%	24	37.5%	0.15	0.06-0.36	<0.001
DRB1*0801	6	6.2%	0	0%	9.16	0.51-165.57	0.08
DRB1*0804	0	0%	1	1.6%	0.22	0.01-5.41	0.4
DRB1*1001	13	13.4%	2	3.1%	6.59	1.47-29.59	0.05
Total	93	100%	64	100%			

RA: Rheumatoid Arthritis, AF: Allele Frequency

difference in the frequencies of the DRB1*0101, DRB1*0404, DRB1*0405, DRB1*0801 and DRB1*0804 subtypes were observed between the patient and control groups (p>0.05). There was no relationship between RF seropositivity and DRB1 alleles (p>0.05).

Discussion

In the present study, the distribution of the HLA-DRB1 alleles among patients diagnosed with RA in the Southeastern Anatolia Region of Turkey was investigated. Although the DRB1*04 allele was more frequently observed among the patients with RA compared to controls, the result was not statistically significant.

The association between HLA-DR and RA is attributable to the susceptibility alleles that encode a homologous amino acid sequence in the third hypervariable region of the first domain of the HLA-DR beta chain. This sequence was determined as the ''shared epitope'' (SE) and this epitope is considered to explain the main role of the HLA-DRB1 in susceptibility leading to RA (1-2). Variations among the subtypes related to RA mainly originate from the different distributions of the HLA-DRB1 alleles among the ethnic groups (1).

In this study, the DRB1*10 type and the DRB1*0401, DRB1*0408, DRB1*1001 subtypes were found to be significantly more frequent in the study group. The relationship of the DRB1*0401 alleles with the predisposition to the disease in our study was in compliance with the studies conducted on Spanish (13), Greek (14) and Caucasian (15) populations, as well as the populations in various regions in Turkey (11,12). Similarly, the relationship of the DRB1*0408 subtype with the predisposition to RA in our study was in line with other studies conducted

on patients from Northern Hungary (16), Finland (6) and the Caucasus (15). However, the DRB1*01, DRB1*04, DRB1*0404 and DRB1*0405 alleles were not observed to have a statistically significant relationship with the disease in our region, although the frequency of these alleles was higher in patients with RA. This may have resulted from the limited number of the patients in our study.

In a meta-analysis on the Asian population, the DRB1*1001 allele was shown to be more frequent in patients with RA compared to controls (17). In the Spanish (13) and Greek populations (14), the DRB1*1001 allele was found to be related with the predisposition to RA. In line with our study, RA patients in Greece were also observed to have the DRB1*10 alleles (14). However, no significant relationship between the DRB1*10 alleles and RA was observed in studies conducted in Morocco (18), Kuwait (19) and Pakistan (13). In our study, the DRB1*10 and DRB1*1001 were found to be statistically significantly associated with the predisposition to RA.

In other studies conducted in Turkey, the DRB1*01 and DRB1*0101 alleles were found to be related with the predisposition to RA (11,12). Similarly, among Ashkenazi Jews (13) and various populations from the Caucasus (5), the DRB1*01 allele has been shown to play a role in the pathogenesis of RA. In our study, the frequency of the DRB1*01 and DRB1*0101 alleles in RA patients were higher than in controls, although the difference was statistically insignificant. In line with our study, another study conducted in Northern Hungary found that the DRB1*01 and its subtypes had no significance in the predisposition to RA (16).

In the present study, the frequency of the DRB1*07, DRB1*11 types and the DRB1 *0402, DRB1*0403, DRB1*0701 subtypes in RA patients were observed to be significantly lower. In a meta-analysis on the Asian population, the DRB1*0403, DRB1*0701, DRB1*1101 and DRB1*1405 subtypes were found as the protective alleles in patients with RA (17). In addition, in another study on RA patients in Korea, DRB1*0701 has been described as the protective subtype (7). In another study conducted by Yelamos et al. in Spain, the DRB1*0402 and DRB1*07 were determined as the negative risk factors for RA (13). These results are also in compliance with those of our study. In a study from Turkey, the DRB1*0402 and DRB1*13 alleles were evaluated as the protective alleles (12). Besides, the DRB1*13 was found as the protective allele against RA in Asians (7,17, 20), Germans (21), and in people from Finland (6) and the Caucasus (22). Also, the DRB1*13 allele was observed more frequently in the control group compared to the patients with RA in our study, the difference was not statistically significant. In contrast with these studies, certain studies conducted in Kuwait and Italy failed to associate any of the subtypes with protectiveness (4,19).

In our study, no significant relationship was observed between seropositivity and the HLA-DRB1 alleles. In a study by Kinikli et al., (11) no association was observed between the HLA-DRB1 alleles and parameters, such as seropositivity, functional status, and radiological erosion indicating the severity of the disease. Similarly, another study conducted in Kuwait failed to point out any relationship between seropositivity and HLA-DRB1. Moreover, the DRB1*04 and the erosive and non-erosive forms of the disease were also found to be unrelated with each other (19). Contrarily, in a study in Morocco, the frequency of RF was observed to be higher in patients carrying the DRB1*04 and it was concluded that the HLA alleles may play an important role in patients with early seropositive RA (18).

In conclusion, the DRB1 *0401, DRB1*0408, DRB1*10 and DRB1*1001 alleles were identified to be related with RA. In contrast, the DRB1*0402 and DRB1*0403, DRB1*07, DRB1*0701 DRB1*11 alleles were observed to be the protective alleles in our region. The results obtained from this study reveal the HLA-DRB1 distribution among RA patients in the Southeastern Anatolia region of Turkey and its relationship with seropositivity. Although the protective alleles we detected in our region differ from the results of the studies conducted in other regions of Turkey (11,12), they still comply with various other studies (13,17).

Further studies conducted on larger patient populations are needed in order to define the relationship between the distribution of the HLA-DRB1 and RA.

Conflict of Interest

Authors reported no conflicts of interest.

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