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The Diagnosis of Human Leukocyte Antigen Class I and Class Ii Allel In Eastern Anatolia Region

Doğuanadolu Bölgesinde İnsan Lökosit Antijen Sınıf I ve Sınıf II Alel Dağılımı

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ABSTRACT

Objective: The aim of our study was to investigate the frequency of HLA class I and class II alleles in Eastern Anatolia.
Materials and Methods: The study included 1050 unrelated bone marrow donors from the archives. HLA class I and II polymorphisms were identified using polymerase chain reactions (PCR) using sequence-specific primers (SSP) and sequence-specific oligonucleotides (SSO). Ethical approval was obtained.
Results: The most common alleles were HLA-A*02 (21.1%), HLA-A*24 (18.4%), and HLA-A*03 (12.3%) in the HLA-A locus; HLA-B*35 (20%), HLA-B*51 (18.3%), and HLA-B*44 (6.3%) in the HLA-B locus; and HLA-DRB1*11 (22.3%), HLA-DRB1*04 (16.2%), and HLA-DRB1*15 (12.4%) in the HLA-DR locus.
Conclusion: Our results are consistent with the HLA antigens identified in previous studies. We believe that our study will contribute to the determination of HLA diversity in our region. Consolidating the data obtained in this study and other national data will facilitate the selection of bone marrow donors.

Key Words: HLA; Class I; Class II

ÖZET

Amaç: Çalışmamızın amacı Doğu Anadolu bölgesindeki HLA sınıf I ve sınıf II alelerinin sıklığının araştırılmasıdır.
Gereç ve Yöntemler: Çalışmaya aralarında herhangi bir akrabalık ilişkisi olmayan 1050 kemik iliği vericisi alındı. HLA sınıf I ve II polimorfizmleri, polimeraz zincir reaksiyonu (PCR) metodu ile sekansa özgü primerler (SSP) ve sekansa spesifik oligonükleotidler (SSO) kullanılarak belirlendi. Etik kurul onayı alınmıştır.
Bulgular: En yaygın aleller, HLA-A lokusunda, HLA-A*02 (21,1%), HLA-A*24 (18,4%), HLA-A*03 (12,3%), HLA-B lokusunda HLA-B*35 (20%), HLA-B*51 (18,3%), HLA-B*44 (6,3), HLA-DR lokusunda HLA-DRB1*11 (22,3%), HLA-DRB1*04 (16,2%), HLA-DRB1*15 (12,4%) olarak belirlendi.
Sonuç: Çalışma sonuçlarımız, daha önce yapılan çalışmalarda tespit edilen HLA antijenleri ile uyumludur. Çalışmamız bölgemizdeki HLA çeşitliliğinin belirlenmesinde katkı sağlayacağını düşünmekteyiz. Çalışmamızda elde ettiğimiz veriler ve diğer ulusal verilerin toplanılması kemik iliği bağışçılarının seçiminde yardımcı olacaktır

Anahtar Kelimeler: HLA; sınıf I; sınıf II

Introduction

The MHC (major histocompatibility complex), which is found in all vertebrates, is a diverse group of genes encoding molecules with and without immune-related functions. The human MHC is called the human leukocyte antigen (HLA) genes. HLAs are glycoproteins expressed by the human MHC region. In humans, the MHC is located on the short arm of chromosome 6. HLA antigens are divided into three groups according to their structural and functional properties: Class I (HLA-A, -B, -C), Class II (HLA-DR, -DQ, -DP), and Class III (properdin factor B [FB], tumor necrosis factor [TNF], C4A, C4B, and C2). The physiological function of HLA molecules is to present peptides derived from protein antigens to antigen-specific T lymphocytes (1).

HLAs have considerable clinical importance. HLA molecules are the main cause of acute rejection after transplantation. Graft survival is associated with HLA compatibility between the recipient and donor.

In addition, it has been determined that some diseases have a higher prevalence among individuals with certain HLA alleles. These include the common conditions ankylosing spondylitis (B27), celiac disease (DR3), and Behçet's disease. There are several hypotheses regarding the role of HLAs in autoimmune disease, such as antigenic mimicry between pathogens and HLA, and antigenic hypo-and hyperresponsiveness regulated

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Table 1. Distribution of human leukocyte antigen (HLA)-A allele frequencies

HLA-A alleles	Allele Frequency (%)
A*02	21.1%
A*24	18.4%
A*03	12.3%
A*11	11.3%
A*01	9.2%
A*26	6%
A*68	4.8%
A*32	3.5%
A*29	3.5%
A*23	3.1%
A*30	2.9%
A*31	1.5%
A*31	1%
A*28	0.5%
A*66	0.4%
A*25	0.3%
A*69	0.1%

by class II genes (2).

There is a broad range of HLA molecules in the population. The frequencies of the HLA alleles show considerable variation between different populations. HLA diversity results in significant immunological differences among individuals. A total of 17,695 HLA alleles have been reported to date, 12,893 in the class I region and 4,802 in the class II region, and this number continues to increase (3).

In the present study, we aimed to determine the frequency of HLA alleles in healthy individuals tested by the Tissue Typing Laboratory of a Health Research and Application Center Hospital between 2007 and 2017, and to assess the results of HLA genotyping (4-6).

Materials and Methods

A total of 1,050 unrelated volunteer bone marrow donors who applied for HLA tissue typing were included. Genomic DNA was isolated from whole blood using EZ-1 isolation kit (QIAGEN GmbH, Hilden, Germany). HLA Class I and II (HLA-A, -B, -DRB1) tissue typing was performed on all samples using low-resolution polymerase chain reaction using sequence-specific primers (PCR-SSP) (One lambda, SSP AB, Stockholm, Sweden) and Luminex-based sequence-specific oligonucleotide primed PCR (PCR-SSO) (Lifecodes, Immucor, Norcross, GA, USA).

PCR-SSO amplification was performed in a Labcycler (SensoQuest GmbH, Germany). Following the oligonucleotide probes and conjugated streptavidin on hybridized fluorescent beads step, the results were analyzed using MatchIT Software (version 3.27). PCR-SSP amplification was performed in a PE 9700 thermal cycler and the resulting PCR products were loaded in an agarose gel, visualized in ultraviolet (UV) light. Positives were documented and analyzed using the Start Scored program.

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee (Atatürk University Faculty of Medicine Ethics Committee no:21). Written informed consent forms were obtained from each volunteer bone marrow donor. The frequencies of HLA-A, -B, and -DR alleles were analyzed using SPSS the study data were analysed using SPSS® version 23.0 (IBM Corp., Armonk, NY, USA) statistical software package. Frequency distributions were used for comparisons.

Results

Seventeen different HLA-A alleles were identified in our group of 1,050 bone marrow donors. The

Table 2. Distribution of human leukocyte antigen (HLA)-B allele frequencies

HLA-B alleles	Allele Frequency (%)
B*35	20.0%
B*51	18.3%
B*44	6.3%
B*49	6.2%
B*52	5.1%
B*18	4.5%
B*38	4.3%
B*55	4.2%
B*07	4.2%
B*50	3.7%
B*08	3.6%
B*41	2.4%
B*40	2.3%
B*27	2.2%
B*15	2.2%
B*13	1.9%
B*57	1.5%
B*58	1.4%
B*14	1.4%
B*39	0.9%
B*53	0.6%
B*37	0.6%
B*45	0.4%
B*47	0.3%
B*56	0.2%
B*60	0.2%

Table 3. Distribution of human leukocyte antigen (HLA)-DR allele frequencies

HLA-DR alleles	Allele Frequency (%)
DRB1*11	22.3%
DRB1*04	16.2%
DRB1*15	12.4%
DRB1*13	11.9%
DRB1*03	9.0%
DRB1*07	6.9%
DRB1*14	6.5%
DRB1*01	5.2%
DRB1*16	3.1%
DRB1*08	2.7%
DRB1*10	2.1%

most common alleles in the HLA-A locus were HLA-A*02 (21.1%), HLA-A*24 (18.4%), and HLA-A*03 (12.3%) (Table 1). Twenty-six different alleles were detected in the HLA-B locus. The most common HLA-B alleles were HLA-B*35 (20%), HLA-B*51 (18.3%), HLA-B*44 (6.3%) (Table 2). In the HLA-DR locus, the most common alleles were HLA-DRB1*11 (22.3%),

HLA-DRB1*04 (16.2%), and HLA-DRB1*15 (12.4%) (Table 3).

Discussion

The most frequently detected HLAs in our study were A*02, B*35, and DRB1*11. Previous studies have identified HLA antigen types in different populations and groups using a variety of methods. Our findings are similar to the most common HLA alleles found in other regional studies conducted in Turkey.

Choukri et al. (7) reported the distribution of HLA-A and -B alleles among 100 healthy, unrelated individuals living in the Casablanca region of Morocco as HLA-A*02 (21%), A*01 (11%), A*03 (10%), B*44 (11.4%), B*50 (9.9%), B*05 (8.5%), and B*35 (6.5%).

In a study by Bardi et al., (8) the most common alleles were HLA-A*02, 24, 01, HLA-B*35, 44, 51, and DRB1*11, 13, 07 among Caucasians; HLA-A*02, 03, 30, HLA-B*35, 15, 44, and DRB1*13, 11, 03 among Afro-Brazilians; and HLA-A*24, 02, 26, HLA-B*40, 51, 52, and DRB1*04, 15, 09 among Asians.

Chen et al. (9) reported the distribution of HLA-A, -B, and -Cw as A*24 (27.2%), B*51 (16.8%), Cw*04 (13.3%), and Cw*070201G1 (13.3%) among 158 unrelated Tibetan Chinese individuals.

In studies conducted in Turkey, Patıroğlu et al. (10) investigated the distribution of HLA antigens in the Kayseri region and identified 185 HLA Class I and 125 HLA Class II molecules using microcytotoxicity assay. They determined the most common alleles in that region to be HLA-A*02, A*09, A*03, HLA-B*05, B*35, B*12, HLA-Cw*04, HLA-DR*05, DR*02, and HLA-DQ*03.

Pala et al. (11) reported that HLA-A*02 (20.5%), HLA-B*35 (22.9%) and HLA-DR*11 (17.6%) were the most common HLAs in a Thracian population.

In a study by Erikoğlu et al. (12) including 362 subjects (57% male, 43% female), the most common HLAs were HLA-A*02 (48%), HLA*B35 (33%), and HLA-DR*B11 (48%).

In another study, Kayhan et al. (13) reported that the most frequent alleles in the HLA-A, -B, and -DRB1 groups were HLA-A*02, 24, 11; HLA-B*35, 51, 44; and HLA-DRB1*11, 04, 13. They also reported the most common haplotypes as HLA-A*02-B*51-DRB1*11, HLA-A*11-B*35-DRB1*11, and A*24-B*35-DRB1*11.

Söyöz et al. (6) determined that the highest frequency alleles among 450 bone marrow donors were HLA-A*02, HLA-B*35, and HLA-DRB1*11.

The results of our study corroborate the findings of other studies conducted in Turkey. We believe our study makes an important contribution to HLA allele frequency data in the Eastern Anatolia region of Turkey. Our next study will investigate HLA haplotype frequency in a larger Turkish population.

Conflict of Interest: None

Source of Funding: None

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