Turk J Immunol 2016;4(1):14-18

Turkish Journal of Immunology - Open Access

www.turkishimmunology.org

Original Article / Özgün Makale

Human Leukocyte Antigen Class I-II Allele Frequencies and Association Between Human Leukocyte Antigen Alleles and ABO Blood Group Antigens

İnsan Lökosit Antijeni Sınıf I-II Alel Frekansları ve İnsan Lökosit Antijeni Alelleri ve ABO Kan Grup Antijenleri Arasındaki İlişki

Mustafa Soyöz,[1] Burcu Akman,[1] İsmail Totur,[1] Sinem Topçu,[1] Aslı Özkızılcık Koçyiğit,[1] Burcu Çerçi Gürbüz,[1] Ceren Yüksel,[1] Toprak Hamdi Güngör,[1] Ayşe Memon,[1] Derya Rasime Güleç,[2] Murat Kılıçoğlu,[2] Tülay Kılıçaslan Ayna,[1] İbrahim Pirim[1]

[1]*Department of Biology and Genetics, Medical Faculty of İzmir Katip Çelebi University, İzmir, Turkey*

[2]*Department of Tissue Typing Laboratory, İzmir Tepecik Training and Research Hospital, İzmir, Turkey*

Correspondence:

Mustafa Soyöz, M.D.
İzmir Katip Çelebi Üniversitesi Tıp Fakültesi Biyoloji ve Genetik Anabilim Dalı, 35040 Bornova, İzmir, Turkey

Tel: +90 232 - 329 35 35

e-mail: mustafa.soyoz@ikc.edu.tr

doi: 10.5606/tji.2016.464

Received: *February 16, 2016*
Accepted: *April 20, 2016*

Objectives: This study aims to investigate human leukocyte antigen (HLA) allele frequencies of healthy individuals and to determine whether there is an association between the HLA alleles and ABO blood groups.

Materials and methods: The HLA-A, HLA-B and HLA-DRB1 allele frequency were evaluated in a sample of 450 unrelated individuals at Izmir Tepecik Education and Research Hospital Tissue Typing Laboratory archives. The HLA genotypes of individuals were analyzed by using single-specific primer-polymerase chain reaction (PCR-SSP) or PCR using sequence-specific oligonucleotide (PCR-SSO) methods. Blood groups were evaluated by using microplate agglutination method.

Results: The most common alleles were HLA-A*02, HLA-B*35, and HLA-DRB1*11 in each locus. A and O blood groups were most common, respectively. A statistically significant association between HLA and ABO blood groups was found in HLA-B and HLA-DRB1 loci.

Conclusion: Our study results confirm the other study findings in the Turkish population and also contribute an additional data for further HLA polymorphisms and anthropological studies. We believe that gaining a better understanding on the HLA allele frequencies would help to constitute national transplantation strategies.

Key words: Allele; blood type; human leukocyte antigen.

Amaç: Bu çalışmada, sağlıklı bireylerde insan lökosit antijen (HLA) alel frekansları tespit edildi ve HLA alelleri ile ABO kan grupları arasında bir ilişki olup olmadığı irdelendi.

Gereç ve yöntemler: İzmir Tepecik Eğitim ve Araştırma Hastanesi Doku Tipleme Laboratuvarı arşivlerinde akrabalık ilişkisi bulunmayan 450 bireyden oluşan HLA-A, HLA-B ve HLA-DRB1 alel frekansları değerlendirildi. Bireylerin HLA genotiplemesi tek spesifik primer-polimeraz zincir reaksiyonu (PCR-SSP) ve diziye özgü oligonükleotid ile PCR (PCR-SSO) yöntemleri kullanılarak analiz edildi. Kan grupları mikroplaka aglütinasyon yöntemi ile değerlendirildi.

Bulgular: Her lokustaki en yaygın aleller HLA-A*02, HLA-B*35 ve HLA-DRB1*11 idi. A ve O kan grupları ise sırası ile en yaygın olanlar idi. HLA-B ve HLA-DRB1 lokuslarında HLA ve ABO kan grupları arasında istatistiksel olarak anlamlı bir ilişki saptandı.

Sonuç: Çalışmamızın sonuçları, Türkiye nüfusunda yapılan diğer çalışma bulgularını desteklemekle birlikte, HLA çeşitliliği ve antropolojik çalışmalar için ilave katkı sağlayacaktır. İnsan lökosit antijen alel frekanslarının daha iyi anlaşılmasının ulusal nakil stratejilerinin oluşturulmasına yardımcı olacağı kanısındayız.

Anahtar sözcükler: Alel; kan grubu; insan lökosit antijenleri.

Major histocompatibility complex (MHC) is located at 6p21.3 region of chromosome 6 and plays a key role in the humoral and cell-mediated immune response via intercellular recognition and self/non-self discrimination.[1] In humans, MHC molecules are also called human leukocyte antigens (HLA).[2] Human leukocyte antigen system consisting numerous loci and highly polymorphic comprises of Class I HLA and Class II HLA molecules. Classical Class I HLA molecules contain HLA-A, -B, and -C loci, while classical Class II molecules contain HLA-DR, -DQ, and -DP loci.[3]

Human leukocyte antigen molecules are expressed on the cell surface of nearly all nucleated cells. Human leukocyte antigen molecules play a defining role in presenting pathogen derived peptides and modified proteins of host mediate to immune system response to infectious disease. The graft survival period is associated with the HLA matching between host and donor. Also, it is known that some HLA alleles are related with susceptibility to diseases.[4]

In HLA region where genetic recombination occurs infrequently codominant HLA alleles are inherited as haplotypes. The main factors contributing the distribution of HLA alleles and haplotypes are isolation, migration, origin and mixture of populations.[5] Human leukocyte antigen alleles and haplotype frequencies of populations exhibit wide range variations. Regional differences can be observed in a single country.[6] 10,574 alleles in HLA Class I region and 3,658 alleles in HLA Class II region were identified up to now..[7]

Blood groups are antigenic determinants on the cell surfaces of red blood cells, granulocytes and platelets.[8] However, the blood group term is mostly used for red blood cell antigens. There are 33 different blood group systems accepted by the International Society of Blood Transfusion (ISBT).[9] ABO system is the most important one in transfusions and transplantations.[8,10] ABO blood group locus is located on chromosome 9. Rhesus system (D) locating on chromosome 1 is the second important blood system.[9]

In this study, we aimed to identify HLA allele and haplotype frequencies of individuals who were tested in Tepecik Education and Research Hospital Tissue Typing Laboratory between 2011 and 2015 and to assess the HLA genotyping results of healthy individuals.

MATERIALS AND METHODS

This study was approved by local ethics committee and a written informed consent was obtained from each subject. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The HLA genotyping results of 450 unrelated healthy individuals, who were tested in Tepecik Education and Research Hospital Tissue Typing Laboratory between 2011 and 2015, were screened. These individuals were donor applicants of bone marrow transplantation and kidney failure patients.

Genomic DNA was isolated from the whole blood by using EZ-1 isolation kit (QIAGEN GmbH, Hilden, Germany) and low resolution genotyping was performed by using PCR-SSP (Olerup, SSP AB, Stockholm, Sweden) and Luminex-SSO (Lifecodes, Immucor, Norcross, GA, USA) methods. Exon 2 and exon 3 of HLA-A, -B were amplified and only exon 2 of HLA-DRB1 was amplified by SSP and SSO methods. Amplicons of PCR-SSP were visualized by 2% agarose gel electrophoresis. Bands were analyzed under ultraviolet light and the results were obtained from the SCORE Software Program. Amplicons of PCR-SSO were denatured and hybridized to oligonucleotide probes on fluorescent beads, and subsequently, they were labeled with phycoerythrin-conjugated streptavidin. The results were analyzed using the MatchIT Software Program (version 1.1.15).

The Hardy-Weinberg Equilibrium (HWE) for each locus was evaluated by Arlequin Software Program (version 3.5.2.2). The allele and haplotype frequencies were calculated by using IBM SPSS for Windows version 20.0 (IBM Corporation, Armonk, NY, USA) software program, and relationship between ABO blood group and HLA alleles were assessed by using Pearson chi-square test in SPSS.

RESULTS

The results of the Hardy-Weinberg equilibrium test are shown in Table 1. According to the test results, the null hypothesis of HWE is rejected in HLA-A and HLA-DRB1 loci ($p>0.05$), but not in HLA-B locus ($p<0.05$).

TABLE 1

Hardy-Weinberg Equilibrium for the three human leukocyte antigen loci

Locus	HeObs	HeExp	*p*	SD
Human leukocyte antigen-A allele	0.85202	0.87782	0.05273	0.00013
Human leukocyte antigen-B	0.87982	0.92052	0.00189	0.00004
Human leukocyte antigen-DRB1	0.84247	0.87707	0.09320	0.00024

HeObs: Observed heterozygosity; HeExp: Expected heterozygosity; p: Significance value; SD: Standard deviation.

TABLE 2

Human leukocyte antigen-A allele frequencies

Human leukocyte antigen-A allele	Allele frequency
A*02	0.225
A*24	0.173
A*03	0.113
A*01	0.109
A*11	0.089
A*26	0.057
A*32	0.043
A*68	0.043
A*23	0.031
A*30	0.025
A*33	0.024
A*31	0.020
A*25	0.016
A*29	0.016
A*66	0.011
A*69	0.003
A*74	0.002
A*34	0.001

In this study, allele frequencies of 450 individuals in HLA-A, HLA-B, and HLA-DRB1 loci were analyzed and 18 different HLA-A alleles were determined. HLA-A*02 (22.5%), HLA-A*24 (17.3%) and HLA-A*03 (11.3%) were found as the most frequent alleles (Table 2).

In HLA-B locus, 28 different alleles were detected. The most frequent alleles were HLA-B*35 (18.8%), HLA-B*51 (12.7%), HLA-B*18 (7.1%) and HLA-B*44 (7%), respectively (Table 3).

In the case of HLA-DRB1, 15 different alleles were observed in the locus. HLA-DRB1*11 (21.7%), HLA-DRB1*04 (15.7%) and HLA-DRB1*15 (12.9%) were determined as the most frequent alleles (Table 4).

In addition, ABO blood groups of 450 individuals were analyzed. Table 5 lists the frequencies of ABO blood groups and ABO-D blood groups.

We also investigated the association between HLA alleles and ABO blood group antigens by using the Pearson chi-square test. There were statistically significant associations between HLA and ABO blood groups in HLA-B and HLA-DRB1 loci ($p<0.05$). The most frequent HLA alleles in ABO blood groups are also shown in Table 6.

DISCUSSION

A and B types became the basis of the first genetic variation study in humans. Another substantial inherited variability is based on the transplantation observation. [11] The HLA complex is important for transplantation outcome. Due to the incompatible HLA genotypes,

TABLE 3

Human leukocyte antigen-B allele frequencies

Human leukocyte antigen-B allele	Allele frequency
B*35	0.188
B*51	0.127
B*18	0.071
B*44	0.070
B*07	0.051
B*40	0.047
B*52	0.047
B*49	0.046
B*08	0.043
B*38	0.038
B*15	0.036
B*50	0.033
B*13	0.028
B*41	0.028
B*27	0.027
B*55	0.027
B*14	0.019
B*58	0.018
B*57	0.015
B*37	0.014
B*39	0.009
B*56	0.005
B*45	0.003
B*53	0.003
B*46	0.002
B*54	0.001
B*73	0.001
B*78	0.001

recipient's immune system recognizes the donor tissue as a foreign tissue. These differences are able to be identified by the agglutination of white blood cells rather than red blood cells. Thus, we assessed the relationship between HLA genotypes of 450 healthy voluntary individuals who

TABLE 4

Human leukocyte antigen-DRB1 allele frequencies

Human leukocyte antigen-DRB1	Allele frequency
DRB1*11	0.217
DRB1*04	0.157
DRB1*15	0.129
DRB1*03	0.103
DRB1*13	0.083
DRB1*07	0.079
DRB1*14	0.066
DRB1*01	0.062
DRB1*16	0.048
DRB1*10	0.022
DRB1*08	0.014
DRB1*12	0.010
DRB1*09	0.008
DRB1*02	0.001
DRB1*17	0.001

TABLE 5

ABO and D blood group frequencies

ABO blood group	Frequency	ABO and D blood group	Frequency
A	0.451	A Rh-	0.040
AB	0.073	A Rh+	0.411
B	0.153	AB Rh-	0.007
O	0.322	AB Rh+	0.067
		B Rh-	0.018
		B Rh+	0.136
		O Rh-	0.024
		O Rh+	0.298

were donor applicants of bone marrow transplantation and kidney failure patients and blood type.

We detected A*02, B*35 and DRB1*11 as the most common HLA antigens. Additionally, A and O blood groups were the most common in the study group and the results were consistent with the other study findings.[12,13] In a few study, the authors found A*02, B*35 and DRB1*11 alleles as the most common HLA alleles in the Turkish populations.[12-16] Our results were also similar to the studies.

Recently, Erikoğlu et al.[12] and Kayhan et al.[13] investigated the relationship between HLA alleles and ABO blood group antigens in the Turkish population. Erikoglu et al.[12] suggested that A*02, A*24, B*35, B*51, DRB1*11 and DRB4 were the most common HLA alleles, A and O blood group antigens were the most in waiting list from cadaver. Kayhan et al.[13] revealed that the same HLA alleles and blood group antigen frequencies in dialysis patients in the East Anatolia However, they did not find any correlation between HLA alleles and ABO group antigens.[12,13] In our study, we found a positive correlation between certain HLA-B, HLA-DRB1 alleles and ABO group antigens. We performed the Pearson chi-square test to analyze the linear relationship and assessed HLA allele/blood group antigen pairs to show significant contributions. We found B*08/A, B*52/A, B*13/B, B*27/AB, B*50/O and DRB1*11/AB, DRB1*13/AB, DRB1*16/AB pairs had a statistically significant association. Phenotype can be affected by multi-genetic influences. Regional differences between studies may cause these distinct results.

In conclusion, our results confirm the other studies about allele frequencies of Turkish population. This study also attributes to the data on HLA polymorphism of our population and anthropological studies. The knowledge of HLA allele frequencies helps to constitute national transplantation strategies. Further analyzes in larger populations can be performed to test the relationship between ABO blood groups and HLA alleles.

Acknowledgements

The authors have no financial conflict of interest. The authors thank the staff of Tissue Typing Laboratory, Department of Internal Medicine Nephrology, and Department of General Surgery of Izmir Tepecik TRH, Izmir Ataturk TRH, Izmir TRH, for their continuous efforts in performing of the study.

TABLE 6

The most frequent human leukocyte antigen alleles in ABO blood groups

ABO blood group/ HLA allele	Frequency	ABO blood group/ HLA allele	Frequency	ABO blood group/ HLA allele	Frequency
A/HLA-A*02	0.214	A/HLA-B*35	0.185	A/HLA-DRB1*11	0.216
A/HLA-A*24	0.173	A/HLA-B*51	0.128	A/HLA-DRB1*15	0.138
A/HLA-A*03	0.121	A/HLA-B*52	0.068	A/HLA-DRB1*04	0.133
A/HLA-A*01	0.106	AB/HLA-B*51	0.242	AB/HLA-DRB1*11	0.333
AB/HLA-A*24	0.258	AB/HLA-B*35	0.152	AB/HLA-DRB1*04	0.167
AB/HLA-A*02	0.258	B/HLA-B*51	0.133	AB/HLA-DRB1*13	0.152
B/HLA-A*02	0.210	B/HLA-B*44	0.074	B/HLA-DRB1*04	0.190
B/HLA-A*24	0.167	B/HLA-B*07	0.074	B/HLA-DRB1*03	0.146
B/HLA-A*03	0.109	O/HLA-B*35	0.210	B/HLA-DRB1*07	0.109
O/HLA-A*02	0.241	O/HLA-B*51	0.098	O/HLA-DRB1*11	0.217
O/HLA-A*24	0.155	O/HLA-B*44	0.066	O/HLA-DRB1*04	0.157
O/HLA-A*01	0.121			O/HLA-DRB1*15	0.129

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Kindt TJ, Goldsby RA, Osborne BA, Kuby J. Major histocompatibility complex. In: Goldsby RA, Kindt TK, Osborne BA, Kuby J, editors. Immunology. 5th ed. New York: W.H. Freeman and Company; 2007. p. 161-84.
2. Khan AN, Gregorie CJ, Tomasi TB. Histone deacetylase inhibitors induce TAP, LMP, Tapasin genes and MHC class I antigen presentation by melanoma cells. Cancer Immunol Immunother 2008;57:647-54.
3. Mahdi BM. A glow of HLA typing in organ transplantation. Clin Transl Med 2013;2:6.
4. Salvadori Lde C, Santana FC, Marcos EV. Frequency of alleles and haplotypes of the human leukocyte antigen system in Bauru, São Paulo, Brazil. Rev Bras Hematol Hemoter 2014;36:108-14.
5. Andric Z, Popadic D, Jovanovic B, Jaglicic I, Bojic S, Simonovic R. HLA-A, -B, -C, -DRB1 and -DQB1 allele and haplotype frequencies in the Serbian population. Hum Immunol 2014;75:218-26.
6. Tiercy JM, Claas F. Impact of HLA diversity on donor selection in organ and stem cell transplantation. Hum Hered 2013;76:178-86.
7. Available from: http://hla.alleles.org/nomenclature/stats.html [Date last updated: January 13 2016] [Date last accessed: January 27 2016].
8. Daniels G. ABO, Hh and Lewis systems. In Daniels G eds. Human blood groups. 2nd ed. Oxford, UK: Blackwell Science Ltd. 2008. p. 7-67.
9. Mitra R, Mishra N, Rath GP. Blood groups systems. Indian J Anaesth 2014;58:524-8.
10. Dean L. The ABO blood group. In: Dean L, editor. Blood groups and red cell antigens. National Center for Biotechnology Information; 2005.
11. Bodmer W. Genetic characterization of human populations: from ABO to a genetic map of the British people. Genetics 2015;199:267-79.
12. Erikoğlu M, Büyükdoğan M, Cora T. The Relationship Between HLA Antigens and Blood Groups. European Journal of General Medicine. 2011;8:65-8.
13. Kayhan B, Kurtoglu EL, Taskapan H, Piskin T, Sahin I, Otlu G, et al. HLA-A, -B, -DRB1 allele and haplotype frequencies and comparison with blood group antigens in dialysis patients in the East Anatolia region of Turkey. Transplant Proc 2013;45:2123-8.
14. Saruhan-Direskeneli G, Uyar FA, Bakar S, Eraksoy M. Molecular analysis of HLA-DRB1, -DQA1 and -DQB1 polymorphism in Turkey. Tissue Antigens 2000;55:171-4.
15. Arnaiz-Villena A, Karin M, Bendikuze N, Gomez-Casado E, Moscoso J, Silvera C, et al. HLA alleles and haplotypes in the Turkish population: relatedness to Kurds, Armenians and other Mediterraneans. Tissue Antigens 2001;57:308-17.
16. Uyar FA, Dorak MT, Saruhan-Direskeneli G. Human leukocyte antigen-A, -B and -C alleles and human leukocyte antigen haplotypes in Turkey: relationship to other populations. Tissue Antigens 2004;64:180-7.