

Human Leukocyte Antigen Frequencies in Highly Sensitized Patients

Yüksek Duyarlılığı Olan Hastalarda İnsan Lökosit Antijeni Sıklıkları

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ABSTRACT

Objectives: This study aims to compare the human leukocyte antigen (HLA) phenotype frequencies between patients with high levels of panel reactive antibodies (PRA) and controls, and analyze the HLA profiles in the study population.

Patients and methods: The study included 144 highly sensitized patients (46 males, 98 females) with positive PRA and end-stage renal failure, and 533 control cadaveric donors. HLA tissue typing and PRA level tests were performed in Akdeniz University Medical Faculty Pediatric Immunology Department, Tissue Typing Laboratory. HLA class I and class II alleles were detected with polymerase chain reaction-sequence-specific oligonucleotide-Luminex, and PRA levels with Luminex.

Results: In the group with positive PRA; HLA-A*24, HLA-B*50, and HLA-DRB1*01 frequencies were statistically significantly different. No significant difference was present between the other HLA A, B, and DR antigens.

Conclusion: While carrying commonly observed HLA antigens seems to be associated with lower PRA rates, patients carrying rarely observed HLA antigens should be given priority in organ transplant waiting lists.

Keywords: Highly sensitized patients; human leukocyte antigen; panel reactive antibody; renal transplantation.

ÖZET

Amaç: Bu çalışmada, yüksek düzeylerde panel reaktif antikor (PRA) olan hastalar ile kontroller arasındaki insan lökosit antijeni (HLA) fenotip sıklıkları karşılaştırıldı ve çalışma popülasyonundaki HLA profilleri incelendi.

Hastalar ve yöntemler: Çalışmaya yüksek duyarlılığı olan pozitif PRA'lı ve son dönem böbrek yetmezliği olan 144 hasta (46 erkek, 98 kadın) ve 533 kontrol kadavra donörü alındı. HLA doku tiplendirmesi ve PRA düzeyi testleri Akdeniz Üniversitesi Tıp Fakültesi Pediatrik İmmünoloji Bölümü, Doku Tiplendirme Laboratuvarında yapıldı. HLA sınıf I ve sınıf II allelleri polimeraz zincir reaksiyon-sekans-spesifik oligonükleotid-Luminex ile PRA düzeyleri Luminex ile saptandı.

Bulgular: Pozitif PRA'lı grupta HLA-A*24, HLA-B*50 ve HLA-DRB1*01 sıklıkları istatistiksel olarak anlamlı şekilde farklıydı. Diğer HLA A, B ve DR antijenleri arasında anlamlı farklılık yoktu.

Sonuç: Yaygın görülen HLA antijenlerini taşıyor olmak düşük PRA oranları ile ilişkili görünürken, nadir görülen HLA antijenlerini taşıyan bireylere organ nakli bekleme listelerinde öncelik verilmelidir.

Anahtar sözcükler: Yüksek sensitize hasta; insan lökosit antijeni; panel reaktif antikor; böbrek nakli.

The human leukocyte antigen (HLA) is the most polymorphic system in the body.^[1] In addition, the major histocompatibility complex (MHC) system and its gene products play an important role in the immune response, and polymorphic HLA antigens are the main target in graft or organ rejection because HLA antigen mismatches between the donor and recipient are the main obstacle to a successful transplantation. Furthermore, MHC genes

determine which peptide epitopes are presented to CD8+ and/or CD4+ T cells. Moreover, some patients waiting for transplants develop anti-HLA antibodies because of blood transfusions, pregnancy, or previously unsuccessful grafts.^[2,3] Finding suitable donors for these highly sensitized patients awaiting transplantation is problematic for transplant centers. It is also not totally clear why some patients develop a higher rate of anti-HLA

antibodies, namely the highly positive panel reactive antibody (PRA), whereas others do not.^[4] Hence, finding a marker to identify or predict which patient will be highly positive for PRA would be helpful for national or international organ sharing centers when they decide on organ allocation.

In this study, we investigated the HLA phenotype frequencies in highly positive (>70%) PRA patients and compared them with the data derived from a control group composed of cadaveric donors that were chosen because they were a non-sensitized population. We also compared the HLA data from our control group with that of other transplantation centers in order to establish our HLA profile.

PATIENTS AND METHODS

This retrospective study was comprised of 144 end-stage renal disease (ESRD) patients who were highly positive for PRA (>70% for least two determinations) (46 males, 98 females) and were considered to be highly sensitized because they had been waiting for a renal transplant for less than three years. We then compared the HLA antigen profiles of these patients with the HLA data from 533 cadaveric donors. Retransplantation patients and multiparous women were excluded from the study because we could not access the information regarding the number of transfusions from their medical files because some of them were being followed up at other hospitals. We perform PRA determination using the Luminex (Austin, TX, USA) every three months for every patients awaiting renal transplants at our center.

The HLA tissue typing was performed at the Akdeniz University Medical Faculty Pediatric Immunology Department in our specialized laboratory, and the HLA class I (A and B) and class II (DRB1) alleles were identified using a Luminex polymerase chain reaction-sequence-specific oligonucleotide (PCR-SSO) technique. All serum samples were also screened for the presence of the immunoglobulin G (IgG) type of class I and class II anti-HLA antibodies using the Luminex assay.

Statistical analyses

All statistical analyses were performed using the PASW version 18.0 for Windows software program (SPSS, Inc., Chicago, IL, USA), and the allele frequencies were calculated using the following formula: whole of the individual allele/2N in which N represented the number of participants. We compared the genotype frequencies among the two groups by employing Fisher's exact test and chi-square test with one degree of freedom (df), with the level of significance being established at $p < 0.05$ (two-sided).

RESULTS

When the HLA profiles of our highly sensitized patients were compared with the control group, the HLA-A*24 ($p=0.029$), HLA-B*50 ($p=0.042$), HLA-DRB1*01 ($p=0.031$) frequencies were statistically significantly different in the PRA-positive group. However, no significant differences were found in the other HLA A, B, and DR antigens. The frequencies of HLA-A*24, B*50 and HLA-DRB1*01 in the PRA and control groups were 26%, 7%, 10% and 36%, 3.5%, 18%, respectively. We noted that HLA-A*24 and HLA-DRB1*01 were associated with a lower risk for high PRA positivity, whereas HLA-B*50-positive patients seemed to have a higher risk. Furthermore, our data showed that patients with both the HLA A24 and DRB1*01 alleles seemed to be associated with a lower risk for PRA positivity. In addition, 0.69% of these patients had high PRA positivity, and 2.06% of the control group had these two alleles. The HLA-A, HLA-B, and HLA-DRB1 allele frequency in our study population is summarized in Tables 1-3.

The most frequent HLA-A alleles were A*02 (af:0.212), A*24 (af:0.181), and A*03 (af:0.138) while on the HLA-B locus, B*35 (af:0.195), B*51 (af:0.128), and B*44 (af:0.073) were the most frequent. On the HLA-DRB1 locus, DRB1*11 (af:0.187) occurs the most followed by DRB1*04 (af:0.165) and DRB1*15 (af:0.102).

DISCUSSION

The long waiting period for highly sensitized patients to receive a transplant is one of the primary problems faced by transplantation centers because the presence of anti-HLA IgG antibodies significantly decreases the chance of finding suitable donors.

TABLE 1

Distribution of human leukocyte antigen-A allele frequency in Turkish population

HLA-A	Number of allele	%	AF
A*01	109	0.20	0.102
A*02	226	0.42	0.212
A*03	148	0.27	0.138
A*11	86	0.16	0.080
A*23	28	0.052	0.026
A*24	194	0.36	0.181
A*25	3	0.005	0.002
A*26	51	0.09	0.047
A*29	20	0.03	0.018
A*30	50	0.09	0.046
A*31	9	0.016	0.008
A*32	38	0.07	0.035
A*33	14	0.026	0.013

HLA-A: Human leukocyte antigen-A; AF: Allele frequency.

TABLE 2

Distribution of human leukocyte antigen-B allele frequency in Turkish population

HLA-B	Number of allele	%	AF
B*07	45	0.084	0.042
B*08	62	0.11	0.058
B*13	37	0.06	0.034
B*14	12	0.02	0.011
B*18	57	0.10	0.053
B*27	30	0.05	0.028
B*35	208	0.39	0.195
B*37	10	0.01	0.009
B*38	65	0.121	0.060
B*39	11	0.02	0.010
B*40	28	0.05	0.026
B*41	13	0.02	0.012
B*44	78	0.146	0.073
B*45	2	0.004	0.001
B*46	3	0.006	0.002
B*48	4	0.008	0.003
B*49	60	0.112	0.056
B*50	19	0.035	0.017
B*51	137	0.257	0.128
B*52	17	0.031	0.015
B*55	20	0.037	0.018
B*56	8	0.015	0.007
B*57	21	0.039	0.019
B*63	24	0.04	0.022

HLA-B: Human leukocyte antigen-B; AF: Allele frequency.

Moreover, it is not yet known why some patients rapidly develop these anti-HLA antibodies, but they occur after blood transfusions, pregnancy, and unsuccessful transplantations. At our center, we receive patients with the highest degree of PRA positivity (70%), but others use even higher PRA values as part of their testing platforms. For example, the United Network for Organ Sharing (UNOS) and the Eurotransplant International Federation receive patients with values of 80% and 85%, respectively.^[5,6]

There are a limited number of studies concerning the possible relationship between the effect of the HLA profile and high PRA positivity. Kreisler et al.^[7] found that having the DR2 antigen was associated with increased PRA positivity while Heise et al.^[8] showed that the DR2 positivity in combination with B44, B53, and A2 was correlated with high PRA positivity. Furthermore, Fuller and Fuller^[9] demonstrated that either DRB1*01 or DRB1*03 was correlated with PRA positivity, and Karahan et al.^[10] found that HLA-A*03, HLA-A*66, and HLA-B*18 appeared at significantly higher levels in PRA-positive patients and that there was a significant increase in the frequency of these antigens in these patients versus those who were PRA-negative. Similar to the study by Karahan et al.,^[10]

TABLE 3

Distribution of human leukocyte antigen-DRB1 allele frequency in Turkish population

HLA-DRB1	Number of allele	%	AF
DRB1*01	96	0.18	0.090
DRB1*03	83	0.155	0.077
DRB1*04	176	0.33	0.165
DRB1*07	77	0.14	0.072
DRB1*08	20	0.037	0.018
DRB1*09	9	0.016	0.008
DRB1*10	36	0.06	0.033
DRB1*11	200	0.375	0.187
DRB1*12	15	0.028	0.014
DRB1*13	103	0.193	0.096
DRB1*14	44	0.08	0.041
DRB1*15	109	0.20	0.102
DRB1*16	50	0.09	0.046

HLA-A: Human leukocyte antigen-A; AF: Allele frequency; To determine the allelic frequencies; (whole of the individual allele) / 2N.

ours was also composed of Turkish patients, but our data did not correlate with theirs because we found significant differences between HLA-A*24, HLA-B*50, and HLA-DRB1*01 in the PRA-positive group. It is conceivable that patients with rare HLA alleles might rapidly produce anti-HLA antibodies to common HLA alleles after blood transfusions, and HLA-B*50 was one of the rare alleles in our population (3.5% in the normal population); thus, finding an association between this antigen and high PRA positivity was not a surprise. Furthermore, the other two HLA alleles associated with a lower risk of high PRA positivity occurred relatively often in our population (Tables 1-3). Therefore, our findings suggest that patients who have common HLA alleles have a lower risk of high PRA positivity, but further prospective studies are needed to determine the details of this relationship. If those findings confirm our observation, then patients who have rare or hyperresponsive alleles, such as HLA-B*50, may receive a higher priority in the organ allocation process.

We also investigated the HLA profile of the Turkish patients in our study and determined that the most frequent HLA-A allele was A*02 and the most common HLA-B allele was B*35. This distribution was similar to previous studies conducted in this country as well as our previous report.^[11,12] Furthermore, at the HLA-DRB1 locus, DRB1*11 was the most frequent allele, which correlates with the study by Saruhan-Direskeneli et al.^[13] Tables 1-3 show the allelic frequency of the HLA-A, B, and DRB1 alleles in our Turkish patients, and our findings were comparable to other studies that have focused on this population.^[11-14]

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REFERENCES

1. Terasaki PI. Humoral theory of transplantation. *Am J Transplant* 2003;3:665-73.
2. Cecka JM, Cho L. Sensitization. *Clin Transpl* 1988;365-73.
3. Hariharan S, McBride MA, Bennett LE, Cohen EP. Risk factors for renal allograft survival from older cadaver donors. *Transplantation* 1997;64:1748-54.
4. Paramesh AS, Zhang R, Baber J, Yau CL, Slakey DP, Killackey MT, et al. The effect of HLA mismatch on highly sensitized renal allograft recipients. *Clin Transplant* 2010;24:E247-52.
5. Cecka JM. Calculated PRA (CPRA): the new measure of sensitization for transplant candidates. *Am J Transplant* 2010;10:26-9.
6. Claas FH, Doxiadis II. Human leukocyte antigen antibody detection and kidney allocation within Eurotransplant. *Hum Immunol* 2009;70:636-9.
7. Kreisler JM, Rementeria MC, de Pablo R, Moreno ME. HLA-DR2, a marker for class I antigen sensitization. *Transplantation* 1988;45:1071-4.
8. Heise E, Manning C, Thacker L. HLA phenotypes of ESRD patients are risk factors in the panel-reactive antibody (PRA) response. *Clin Transplant* 2001;15 Suppl 6:22-7.
9. Fuller TC, Fuller A. The humoral immune response against an HLA class I allodeterminant correlates with the HLA-DR phenotype of the responder. *Transplantation* 1999;68:173-82.
10. Karahan GE, Kekik C, Oguz FS, Onal AE, Bakkaloğlu H, Calışkan YK, et al. Association of HLA phenotypes of end-stage renal disease patients preparing for first transplantation with anti-HLA antibody status. *Ren Fail* 2010;32:380-3.
11. 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.
12. Savaş A, İnce S, Coşkun M, Yeğın O. HLA phenotype and haplotype frequencies in Antalya region of Turkey. *Turkish Journal of Immunology* 1996;2:31-40.
13. 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.
14. 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.