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Atatürk Üniversitesi Böbrek Nakli Bekleme Listesindeki Hastalarda Panel Reaktif Antikor Profillerinin Belirlenmesi

Panel Reactive Antibody Profiles of Patients on the Kidney Transplant Waiting List of Atatürk University

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ÖZET

Amaç: İnsan lökosit antijenlerinde (HLA) duyarlılaşma çeşitli kan transfüzyonu, gebelik veya organ transplantasyonları ile oluşur. Nakil adaylarının panel reaktif antikor (PRA) taraması ve PRA pozitif olan hasta grubunun anti-HLA profillerinin belirlenmesi amaçlandı.
Materyal ve Metod: Kronik böbrek yetmezliği tanısı ile laboratuvarımıza başvuran ve panel reaktif antikor tarama testi sonuçları pozitif olan 40 hastanın serum örnekleri antikor tiplerinin tanımlanması için kullanıldı. Kırk hastanın antikor profilleri mikroboncuk tabanlı yöntem (Lumınex) ile tanımlandı. Tek antijenli boncuk (single antigen bead [SAB]) testi yapıldı ve çalışmada uygulanacak prosedür kitin prospektüsünde belirtildiği şekilde yapıldı. Çalışma için yerel etik kurul onayı alındı.
Bulgular: En fazla tespit edilen sınıf I anti-HLA antikorları B7 (%8.7), A2 (%8), A24 (%8), sınıf II anti-HLA antikorları ise DR01 (%11.67), DR11 (%11.67) ve DQ3 (%16.70) olarak belirlendi.
Sonuç: Elde edilen sonuçlarımız, toplumdaki antijen frekanslarıyla ilgili yapılan diğer çalışmalarla benzer sonuçlar elde edildi. Fakat çalışmamızda bazı antikorların (A23, A68, B15, B40, DR04, DR07, DR09, DR16) yüksek oranda pozitifliği tesbit edildi.

Anahtar Kelimeler: Anti-HLA, Sınıf I, Sınıf II, PRA, tek antijenli boncuk testi

ABSTRACT

Objective: Human leukocyte antigen (HLA) sensitization can be caused by various events such as blood transfusions, pregnancy, or organ transplantations. The aim of this retrospective study was to screen panel reactive antibodies (PRA) in transplant candidates and determine the anti-HLA profiles of PRA-positive patients.
Materials and Methods: Serum samples from 40 patients who were diagnosed with chronic renal failure and had positive PRA screening test results were used to identify antibody types. Antibody profiles of 40 patients were evaluated using a microbead-based method (Luminex). Single antigen bead (SAB) assay was performed using the manufacturer-recommended procedure. Local ethics committee approval was obtained.
Results: The most frequently detected class I anti-HLA antibodies were B7 (8.7%), A2 (8%), and A24 (8%), and the most common class II anti-HLA antibodies were DR01 (11.67%), DR11 (11.67%), and DQ3 (16.70%).
Conclusion: Our results are similar to those of other studies on antigen frequencies in the population. However, we observed that certain antibodies (A23, A68, B15, B40, DR04, DR07, DR09, and DR16) were produced at high levels.

Key Words: Anti-HLA, Class I, Class II, PRA, single antigen bead assay

Introduction

Kidney transplantation is the preferred treatment for end-stage renal disease because it provides better quality of life and longer life expectancy compared to dialysis. (1,2) As patients with high panel reactive antibody (PRA) levels represent a growing group on transplantation waiting lists, determining PRA sensitization is important. Sensitization is the result of a series of events that occur after the immune system encounters antigenic proteins, resulting in permanent B lymphocyte memory and a strong humoral response upon repeat encounter. In order to develop anti-HLA antibodies, an individual must be introduced to foreign HLA antigens. This encounter may occur during the course of a previous transplantation, full blood transfusion, or

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Table 1. Demographic features

Number of patients	N:40
Mean Age	51
Number of patients entering dialysis	40
Hemodialysis	38
Peritoneal dialysis	2
Female	34
Male	6
Transplantation Story	5
Living Transplant	2
Cadaver Transplant	3

multiple births. (3,4) To determine the HLA antigen sensitivity of patients waiting for kidney transplantation from living or cadaveric donors, antibodies in the recipients' serum against a panel of various HLA class I and II antigens are assayed, which is referred to as the PRA screening test. The PRA screening test can determine sensitization level and antibody titer. High PRA levels are known to be associated with lower transplantation success rates. For patients with positive PRA screening, PRA identification tests are used to determine the specificity of the anti-HLA antibodies. The content of the cell panel is important in PRA identification tests. HLA alleles found in the population should be included in the panel. In patients showing high sensitivity, beads coated with a single HLA antigen are used to determine anti-HLA antibody profile. Therefore, this assay is called the single antigen bead (SAB) assay. (3-5,6) In the present study, we aimed to determine the antibody profiles of patients with chronic kidney disease who were on the active transplantation waiting list and tested positive for anti-HLA antibodies in HLA class I and II screening.

Materials and Methods

Demographic Characteristics of the Patient Group: The study group comprised 40 patients who were assessed in the Atatürk University Medical Biology Tissue Typing Laboratory due to chronic kidney disease and had positive PRA (class I and/or class II PRA) screening test between 2012 and 2017. These patients were evaluated using SAB assay. The patients' demographic data are shown in (Table 1)

Anti-HLA Antibody (PRA) Assay: PRA screening is performed routinely in our laboratory. Blood samples (6 cc) obtained from the patients were centrifuged at 3,000 rpm for 15 minutes at +4 °C and the separated sera were stored at -80 °C. The serum samples were brought to room temperature prior to analysis. The sera were prepared for the assay by centrifuging at 15,000 rpm for 30 minutes. Each bead in the class I/class II bead mixture is coated with an HLA antigen (LMX LIFECODES LSA CLASS I LOT:08256N, LMX LIFECODES LSA CLASS II LOT:09156A). The beads were washed three times after incubation with the patients' serum samples. The beads were incubated with phycoerythrin (PE)-conjugated antihuman immunoglobulin IgG for 30 minutes. The excess unbound antibody was removed by washing the microbeads in the wells. After incubating the plates in the dark, the results were analyzed using a Luminex 100 Fluoroanalyzer (USA) and LIFECODES MATCH IT! Antibody Software.

Results

The patient sera that were positive in PRA class I and II screening were used to identify anti-HLA antibodies. Anti-HLA antibodies were detected in a total of 40 patients. Class I and/or class II PRA sensitivity rates and class I/II antibodies were determined in these patients. The detected antibodies are shown in (Table 2) and (Figures 1 and 2). The most frequent class I anti-HLA antibodies were B07 (8.7%), A02 (8%), and A24 (8%), while the most frequent class II anti-HLA antibodies were DR11 (11.6%), DR01 (11.6%), and DQ03 (16.7%).

Data Analysis: Categorical data were expressed as n (%). PRA positivity rates were analyzed by frequency analysis. Statistical analysis of the obtained data was performed using SPSS 17.0 SPSS,

Discussion

Anti-HLA antibodies can develop following pregnancy, blood transfusion, or transplantation. The presence of antibodies against donor HLA antigens prior to transplantation is an important factor in graft survival in organ transplantation.

Table 2. Class I and Class II anti-HLA antibodies of the identified patients

Patient No	HLA –Class I Ab	HLA –Class II Ab	CI SAB	CII SAB
1	Positive (+)	Negative (-)	A26,B07, B40	
2	Positive (+)	Negative (-)	A02, A68, B57, B58, B15	
3	Positive (+)	Negative (-)	A02, A68, B07	
4	Positive (+)	Negative (-)	A24, B48,	
5	Positive (+)	Positive (+)	A24, B18	DR01,DQ05
6	Negative (-)	Positive (+)		DR03
7	Positive (+)	Positive (+)	A29, A30	DR01, DR09, DQ09, DQ02
8	Negative (-)	Positive (+)		DR01, DR07, DR09, DR10, DR13, DR16, DR17
9	Positive (+)	Positive (+)	A10, B07, B61	DR07, DQ02
10	Positive (+)	Negative (-)	A10, A31, A33	
11	Positive (+)	Negative (-)	A01, A24, B15, B40	
12	Positive (+)	Positive (+)	A01, A26, B07, B08	DQ03, DR01
13	Positive (+)	Negative (-)	A11, B48, B50, B55	
14	Positive (+)	Positive (+)	A02, A09, A68, A34, B07, B27, B40	DR15, DQ 06
15	Positive (+)	Negative (-)	A02, A28, B55	
16	Positive (+)	Positive (+)	A02, A28, A30, B56, B63, B27, B07, B51, B35	DR08, DR11, DR14, DR15, DR16, DR17
17	Positive (+)	Negative (-)	A11, A24, B12, B47, B48	
18	Positive (+)	Negative (-)	B48, B45	
19	Positive (+)	Negative (-)	A31, B45, B48, B70	
20	Positive (+)	Positive (+)	A03, A23, A24, B17, B40	DR03, DR04, DR11, DR13, DR14
21	Positive (+)	Positive (+)	A02, A29, A30, A26, A68, B13, B14, B15, B27, B37, B44, B45	DR07, DR09, DR10
22	Positive (+)	Negative (-)	A02, A23, B15	
23	Positive (+)	Negative(-)	A02, A35, B51	
24	Positive (-)	Positive (+)		DR07, DR11, DQ2
25	Positive (+)	Positive (+)	B39, B44, B48, B62	DR01, DR04, DR11, DQ03
26	Positive (+)	Positive (+)	A23, A24, A31, A32, B08, B48	DR04, DR11, DR18
27	Positive (+)	Negative (-)	A33,B17	
28	Positive (+)	Positive (+)	A23, A24, B44, B07	DR01, DR16, DQ03
29	Positive (-)	Positive (+)		DR16, DQ03
30	Positive (+)	Negative(-)	A33, B58, B15, B57	
31	Negative (-)	Positive (+)		DQ03
32	Positive (+)	Negative (-)	A68, A69, B08, B17	
33	Positive (+)	Positive (+)	A68, B07, B40	DQ03, DR11
34	Positive (+)	Negative (-)	A24, B07, B15	
35	Positive (+)	Positive (+)	A02,A24, B07	DR07, DR16
36	Positive (+)	Negative (-)	A02,A24, B48	
37	Positive (+)	Positive (+)	A02, A23, B07,B07	DR11, DQ03
38	Negative (-)	Positive (+)		D003
39	Positive (+)	Positive (+)	A24,B07, B40	DR01, DQ03
40	Positive (+)	Negative (-)	A68,B48, A26	

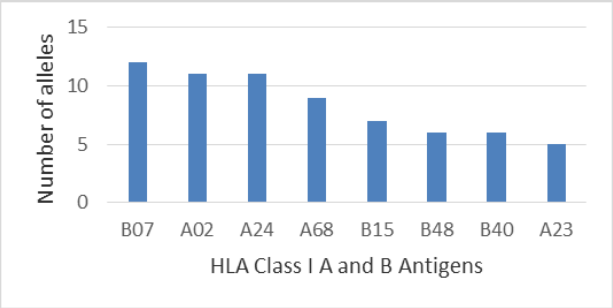

Fig. 1. Identified Class I Anti-HLA antibodies

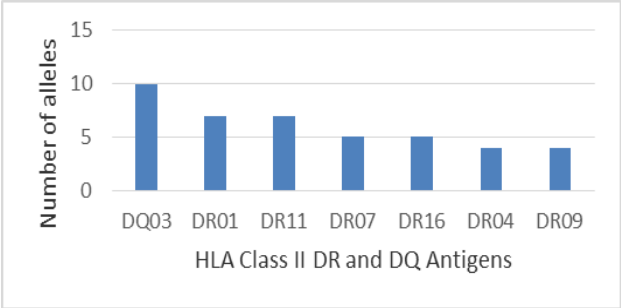

Fig. 2. Identified Class II Anti-HLA antibodies

Pretransplant reactivity against donor HLA antigens is described as a positive crossmatch test and precludes organ transplant. (7-9) To best identify the degree of sensitization, the patient's HLA antibodies should be evaluated based on the frequency of target antigens in a donor population. Knowing the gene frequency of the alleles to which a patient develops anti-HLA antibodies allows calculation of the 'virtual PRA,' which reflects anti-HLA reactivity more accurately than conventional PRA value. Many studies have shown that graft survival is limited to 3-5 years in patients with panel reactive or donor-specific antigens. (10) Karahan et al. reported that A2, A68, Bw4, A23, A66, A69, A33, A24, B27, A26, A32, A25, A29, B57, and B7 were the most common antibodies against class I HLA antigens, while the DQ3, DR52, DR51, DQ5, DR4, DR9, DR11, DR1, DR12, DR53, DR8, and DQ6 antibodies were the most common against class II HLA antigens. Özdemir et al. reported the most frequent antibodies as B56, A2, A34, A1, A23, A24, and B61 for class I and DR11, DR14, DQ7, DR10, DQ5, DR1, and DR7 for class II. In a study by Soyöz et al., A2, A23, A24, A33, A68, B7, and B61 antibodies were most common for class I and DR4, DR7 DR9, DR11, DR10, DR1, DR8, DR17, DQ2, and DQ9 antibodies for class II. (4-10,11) In studies conducted in the Turkish population, Kayhan et al. reported A*2, A*24, B*35, B*52, DRB1*04, DRB1*11 as the most frequent class I and II HLA alleles, while Erikoğlu et al. and Arnaiz-Villena et al. in their 2001 study reported A2, A24, A26, A3, A1, A26, A11, and A23 as the most common HLA class I antigens. B35, B51, B44, B18, B38, B27, and B13 were the most common HLA-B antigens, and DRB1-DRB11, DRB4, DRB13, DRB3, DRB15, DRB7, DQ3, DQ1, and DQ2 were the most common class II antigens. (12-13) Saruhan-Direskeneli et al. also determined that DRB1*1101, DRB1*0301, DRB1*0701, DQB1*0301, DQB1*02, and DQB1*0302 were the most frequent alleles in a class II subtype study. (14) In a allele frequency study by Soyöz et al. including 450 people, the most common HLA antigens were A*02, A*24, A*03, B*18, B*51, B*44, DRB1*11, DRB1*04, and DRB1*15. (15) Pala et al. reported that A*02, A*11, and A*24 were the three most common HLA-A alleles in the Thrace region of Turkey. The most common HLA-B and -DR alleles were B*35, B*51 and B*07, and DR*11, DR*13, DR*15, and DR*04. (16) Karaarslan et al. presented the distribution of HLA antigens determined from 891 blood samples. (17) The present study reports the allele frequencies of A*02, A*24, B*07, DQ*03, DR*11 of the HLA-B and HLADR-DQ locus antigens. We also identified antibodies against A*68, B*15, B*48, DR*01, DR*07, and DR*16 antigens. The results of our study are compatible with previous reports of HLA allele frequencies in the Turkish population. We believe that the discrepancies with other studies are due to differences in antigenicity. In light of all of these studies, we again emphasize the importance of immunological monitoring prior to kidney transplantation to recognize the development of anti-HLA antibodies.

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