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LABORATORY STUDY

Association of HLA phenotypes of end-stage renal disease patients preparing for first transplantation with anti-HLA antibody status

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ABSTRACT

Patients with pre-transplantation high levels of panel reactive antibody (PRA) have an increased risk of graft failure, and renal transplantation in sensitized patients remains a highly significant challenge worldwide. The influence of anti-human leukocyte antigen (HLA) antibodies on the development of rejection episodes depends on patient-specific clinical factors and differs from patient to patient. The HLA typing of the recipient might influence the development of anti-HLA antibodies. Some HLA antigens appear to be more immunogenic than others. The aim of this study is to demonstrate the distribution of HLA phenotypes in PRA-positive and PRA-negative end-stage renal disease (ESRD) patients on the basis of having sensitizing events or not. Our study included 642 (mean age: 41.54; female/male: 310/332) ESRD patients preparing for the first transplantation and who are on the cadaveric kidney transplantation waiting list of Istanbul Medical Faculty in 2008–2009. Class I HLA-A,B typing was performed by complement-dependent cytotoxicity (CDC) method, whereas class II HLA-DRB1 typing was performed by low-resolution polymerase chain reaction (PCR)-sequence-specific primer (SSP). All serum samples were screened for the presence of IgG type of anti-HLA class I- and II-specific antibodies by enzyme-linked-immunosorbent assay (ELISA). PRA-negative group consisted of 558 (86.9%) and PRA-positive group included 84 (13.1%) patients. We have found statistically significant frequency of HLA-A3 ($p = 0.018$), HLA-A66 ($p = 0.04$), and HLA-B18 ($p = 0.006$) antigens in PRA-positive patients and DRB1*07 ($p = 0.02$) having the highest frequency in patients with sensitizing event history but no anti-HLA development suggesting that DRB1*07 might be associated with low risk of anti-HLA antibody formation.

Keywords: HLA phenotype; panel reactive antibody; end-stage renal disease; renal transplantation; anti-HLA antibody

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INTRODUCTION

It is clearly evident that renal transplantation is the best treatment option for patients with end-stage renal disease (ESRD).[1] The presence of anti-human leukocyte antigen (HLA) antibodies in recipient sera before transplantation is an important risk factor.[2] Patients with high levels of panel reactive antibody (PRA) pretransplantation have an increased risk of graft failure, and renal transplantation in sensitized patients remains a highly significant challenge worldwide.[3–5] Furthermore, *de novo* synthesis of these antibodies posttransplantation is proven to be detrimental for the graft.[6] The influence of anti-HLA antibodies on the development of rejection episodes depends on patient-specific clinical factors and differs from patient to patient. It is now clearly evident that anti-HLA antibodies present pretransplantation destroy the graft eventually by adhering to the endothelium of the graft.[6,7]

Sensitization occurs through exposure to HLA antigens through blood transfusions, previous grafts, or pregnancies. However, although rare, presence of anti-HLA antibodies in a couple of nonimmunized cases has also been reported suggesting that these antibodies are cross-reactive microbial determinants.[8–12]

The permanency of anti-HLA antibodies in sensitized recipient sera might differ from person to person, either disappears after some time or remain at high

levels for a long time. On the other side, the reason why some people do not produce antibodies despite foreign HLA exposure remains unclear.[13] The HLA typing of the recipient might influence the development of anti-HLA antibodies. Some HLA antigens appear to be more immunogenic than others.[14]

The aim of this study is to demonstrate the distribution of HLA phenotypes in PRA-positive- and PRA-negative ESRD patients on the basis of having sensitizing events or not.

MATERIALS AND METHODS

Patient group

Our study included 642 (mean age: 41.54, female/male: 310/332) ESRD patients preparing for the first transplantation and who are on the cadaveric kidney transplantation waiting list of Istanbul Medical Faculty in 2008–2009. Information on the demographic characteristics of the patients (n = 642) was retrieved from the records of the Department of Medical Biology and the Transplantation Unit.

Demographics were listed by classifying the patient group as PRA-negative and PRA-positive as shown in Table 1. To compare the HLA phenotypes, the study group was divided into three groups as follows:

Group 1: patients who had sensitizing events and negative PRA (n = 370).
Group 2: patients who had sensitizing events and positive PRA (n = 84).
Group 3: patients who did not have sensitizing events and had negative PRA (n = 188).

Methods

HLA tissue typings were performed in European Federation for Immunogenetics (EFI)-accredited HLA laboratories of Department of Medical Biology. Class I HLA-A,-B typing was performed by complement-dependent cytotoxicity (CDC) method, whereas class II HLA-DRB1 typing was performed by low-resolution polymerase chain reaction (PCR)-sequence-specific primer (SSP), as has been described elsewhere.[15,16] In case of an ambiguity in class I typing, PCR-SSP was performed as well. All serum samples were screened for the presence of IgG type of anti-HLA class I- and II-specific antibodies by using commercially produced enzyme-linked-immunosorbent assay (ELISA) kits according to the manufacturer's instructions (LATM20×5, One Lambda, Canoga Park, California, USA). Microtiter trays were read at 630 nm using an ELISA reader (Bio-Tek ELX 800; Bio-Tek Instruments Inc., Winooski, Vermont, USA) and analyzed by One Lambda software. The positive threshold (cutoff) was calculated as 0.2 times the average positive serum control minus the blank.

TABLE 1. Demographic characteristics of the study group.

	PRA-negative	PRA-positive	
Number of patients	558 (86.9%)	84 (13.1%)	
Gender			
Females	240 (43%)	70 (83.3%)	p = 0.000
Males	318 (57%)	14 (16.7%)	
Mean age	40.44 ± 13.65	42.65 ± 10.31	
Potential risk factors			
Dialysis duration (years)	4.3 ± 3.9	6.2 ± 4.8	p = 0.004
Number of transfusions (unit)	1.87 ± 3.44	4.48 ± 7.6	p = 0.000
Pregnancies (number of females)	240 (54.6%)	70 (78.6)	p = 0.000
Blood type			
A	263 (47.1%)	34 (40.5%)	
B	63 (11.3%)	12 (14.3%)	
O	198 (35.5%)	32 (38.1%)	
AB	34 (6.1%)	6 (7.1%)	
Rh factor			
(–)	68 (12.2%)	12 (14.3%)	
(+)	490 (87.8%)	72 (85.7%)	
Underlying original disease			
Diabetic nephropathy	39 (7%)	3 (3.6%)	
Hypertensive nephrosclerosis	105 (18.8%)	21 (25%)	
Chronic glomerulonephritis	112 (20.1%)	12 (14.3%)	
Chronic pyelonephritis	35 (6.3%)	8 (9.5%)	
Vesicoureteral nephropathy	24 (4.3%)	3 (3.6%)	
Amyloidosis	15 (2.7%)	2 (2.4%)	
Polycystic kidney diseases	49 (8.8%)	7 (8.3%)	
Urologic abnormalities	12 (2.2%)	1 (1.1%)	
Other	11 (2%)	3 (3.6%)	
Etiology unknown	156 (28%)	24 (28.6%)	

Statistical analyses

Statistical analyses were performed by using SPSS 10.0 software. Frequencies and percentage (%) rates of HLAs together with demographic characteristics of the patients were calculated. Chi-square and Fisher's exact tests were used for the comparison

of percentage rates between groups. Comparison of continuous variables was performed by Student's *t*-test. The level of significance was considered as $p < 0.05$.

RESULTS

Demographic characteristics of the patients according to PRA status are shown in Table 1.

PRA-negative group consisted of 558 (86.9%) patients and PRA-positive group included 84 (13.1%) patients. PRA-positivity was significantly associated with female gender ($p = 0.000$) and pregnancy ($p = 0.012$), long dialysis duration ($p = 0.004$), and blood transfusions ($p = 0.000$) (Table 1).

Of 84 PRA-positive patients, 40 (47.6%) were class I (+) and class II (–); 14 (16.7%) were class I (–) and class II (+); and 30 (35.7%) were both class I and class II (+). In the entire study group, 70 patients (10.9%) had anti-HLA class I antibodies whereas 44 (6.8%) had anti-HLA class II antibodies.

When the HLA phenotype frequencies of the patients were compared in PRA-negative ($n = 558$) and PRA-positive ($n = 84$) groups, HLA-A3 ($p = 0.018$), HLA-A66 ($p = 0.04$), and HLA-B18 ($p = 0.006$) were significantly high in PRA-positive group. In addition, the frequency of HLA-B49 ($p = 0.05$) in PRA-positive group was high but at the limit of statistical significance.

When the HLA phenotype frequencies of the study group were evaluated in Group 1 (patients who had sensitizing events and negative PRA; $n = 370$) and Group 2 (patients who had sensitizing events and positive PRA; $n = 84$), HLA-A3 ($p = 0.04$) and HLA-B18 ($p = 0.006$) were significantly high in Group 2, and although HLA-A66 ($p = 0.08$) and HLA-B49 ($p = 0.07$) in Group 2 were statistically insignificant, their frequency was high.

In Group 1 patients who did not develop any anti-HLA antibodies, although they have been exposed to foreign HLA, the frequency of HLA-DRB1*07 ($p = 0.02$) was significantly high.

There were no patients in our study group who had anti-HLA antibodies although not having any sensitizing event history.

DISCUSSION

Anti-HLA antibody production occurs as a result of sensitizing events although there are individual differences. Our findings confirm the significant correlation of long waiting time and blood transfusions with PRA positivity.[17] Furthermore, female gender and pregnancy were also statistically significant in PRA-positive patients because of paternal HLA exposure.[18]

Despite sensitizing events, not every patient has the same chance of becoming sensitized after exposure to foreign HLA antigens. The reason for that might be the immunogenicity of the product or immune response genes in the patient that activate the antibody formation against HLA antigens.[17] Although immune response gene functions are known to be accomplished by the genes within major histocompatibility class II locus, immune response gene control of anti-HLA antibody formation is not entirely clarified. Furthermore, data presented by Papassavas et al. suggested that HLA class II allele and the type of the bound allopeptide might influence the humoral and cellular response.[19] In a study of Kreisler et al. in which CDC was the typing method, DR2 was found to be associated with immune response against class I antigens.[20] Heise et al. could not confirm the results of Kreisler with their results of single HLA antigen analyses in 19,440 renal transplant patients, but DR2 in combination with B44, B53, and A2 was found to be correlated with high PRA response. Univariate analysis of the whole cohort concluded that nine HLA allelotypes (DR1,4,7; B8,12,40; A1,A2,A11) were associated with reduced risk of sensitization while five allelotypes (B42,53; A10,19,36) were associated with elevated risk of sensitization.[13] Fuller and Fuller demonstrated that 79% of anti-HLA-Bw4 antibody-producing patients expressed either DRB1*01 or DRB1*03 and concluded that these alleles might confer a high risk for both humoral allosensitization and renal allograft failure in case of HLA-Bw4 incompatibility.[21] On the other hand, another study showed that the presence of DR1 in the recipient was correlated with low immune response.[22] Although Heise et al. concluded that DR1 and DR4 phenotypes were associated with low PRA and good graft survival, and DR3 with high PRA and poor graft survival, they drew attention to the point that it would be incorrect to infer DR1 phenotypes as poor responders to all HLA epitopes.[13]

The results of this study do not fully confirm the results of the above-mentioned studies as we have found statistically significant frequency of HLA-A3 ($p = 0.018$), HLA-A66 ($p = 0.04$), and HLA-B18 ($p = 0.006$) antigens in PRA-positive patients and DRB1*07 ($p = 0.02$) having the highest frequency in patients with sensitizing event history but no anti-HLA development suggesting that DRB1*07 might be associated with low risk of anti-HLA antibody formation.

To our knowledge, this study is the first study investigating the relationship of anti-HLA antibody response with HLA phenotypes in Turkish population. Although an analysis in terms of anti-HLA antibody specificities and related HLA haplotypes could not be

established in this study, we believe that we could provide information on possible immunogenic and protective HLA antigens taking roles in anti-HLA antibody response specific to Turkish ESRD patients.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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