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Prevalence of anti-HLA antibodies in highly sensitized kidney transplant candidates

Farahnooh Farnood, Alireza Mardomi, Sepide Zununi Vahed, Mohammadreza Ardalan*

Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Introduction: Kidney transplantation is the standard gold therapy for the treatment of the majority of end-stage renal diseases (ESRDs). Despite the general success rate of allogeneic transplantation due to immunosuppressive therapy, it is difficult to find an appropriate donor for some sensitized patients.

Objectives: This study aimed to estimate the prevalence and titers of anti-HLA-class I and anti-HLA-class II antibodies in sensitized patients in a kidney transplantation center. The history of the risk factors of sensitization was studied.

Patients and Methods: Twenty highly sensitized ESRD patients with a calculated panel-reactive antibody (CPRA) ≥50% were selected, and anti-HLA-I and anti-HLA-II antibodies were assessed in their sera using a single antigen bead (SAB) Luminex assay.

Results: The previous history of kidney transplantation was the most critical sensitization risk factor. The results indicated that HLA A*24:02 and DQA1*02:01/DQB1*06:02 were the most frequent antibodies in class I and class II, respectively. Moreover, the mean fluorescence intensity (MFI) levels of anti-HLA class II antibodies were significantly higher than the MFI levels of anti-HLA class I antibodies.

Conclusion: According to the findings of this study, matching HLA alleles, particularly class II molecules, can reduce sensitization in the first kidney transplant. A better understanding of the sensitization status of transplant candidates could be gained by examining CPRA values.

Implication for health policy/practice/research/medical education:
The presence of pre-existing donor-specific anti-HLA antibody (DSA) increases the risk of hyperacute allograft rejection. The present study evaluated the anti-HLA-class I and anti-HLA-class II antibodies and defined the common causes of sensitization among renal transplantation candidates. The results indicated that the previous history of kidney transplantation was the most prevalent cause of sensitization. Besides, the proper matching of some HLA loci, HLA DRB1, and HLA DQBI was more important in the prophylaxis against the formation of a broad spectrum of anti-HLA antibodies and the establishment of sensitization.

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Introduction

Kidney transplantation is linked with better long-term survival and life quality in patients suffering from end-stage renal disease (ESRD) (1). Although recent advancements in cross-matching and immunosuppressive therapy have increased the survival of renal allografts, some risk factors may still have major effects on the overall success of organ transplantation. The sensitization of recipients is a key factor that strongly impacts the allograft function. Sensitization is a situation in which the recipient contains antibodies against a broad spectrum of HLA haplotypes. This event is defined by a high panel reactive antibody (PRA) score that makes the recipient prone to developing hyperacute rejection. The diversity and cross-reactivity of HLA molecules are the main drivers of sensitization. Previous history of transplantation, blood transfusion, and pregnancy account for the most cases of sensitization in clinical cases (2). Moreover, the sensitization and presence of preformed donor-specific antibodies (DSAs) are the major factors contributing to antibody-mediated rejection (ABMR) and chronic graft failure (3). It is now well understood that ABMR is responsible for most of the phenomena related to allograft loss and one of the current challenges in transplant immunology. Furthermore, the

***Corresponding authors**: Prof. Mohammadreza Ardalan, Email: ardalan34@yahoo.com, ardalanm@tbzmed.ac.ir

detection of preformed DSA is a critical procedure in the pre-transplant evaluations (4).

In recent years, facilitated DSA detection methods such as flow cytometry and single antigen bead (SAB) Luminex assays have been combined with the virtual cross-match approaches to yield better comprehension of the potential sensitization of recipients, defined as calculated panel-reactive antibody (CPRA). These methods can provide useful clues on the sensitization score based on the DSA information (5). Bead-based and virtual approaches are capable of gathering more sensitive information on the existence of anti-HLA antibodies, even though there are old PRA approaches, such as the complement-dependent cytotoxicity test. Together, the aforementioned assays may facilitate the introduction of candidate antibodies for appropriate desensitization therapy (6).

From the global perspective, the population of chronic kidney disease patients awaiting a second renal transplant is ever-increasing (7). According to the Eurotransplant data, the percentage of patients waiting for a kidney transplant with a PRA ≥85% increased from 2.0% to 5.6% from 2011 to 2019 (8). A considerable population of patients is not placed in acceptable mismatch programs for finding suitable donors due to their high sensitization scores (9). Full HLA matching of these patients will not be possible and alternative options, including desensitization and monitoring of DSA are the rescue options (9).

Numerous studies showed that known sensitized patients with DSA undergoing a desensitization program have a significant survival benefit before live donor kidney transplantation compared to the similar patients who still undergo dialysis therapy (2). On the other hand, candidates may be classified into low-, intermediate-, and high-risk groups during the pre-transplant period based on sensitization ratings. This classification could help the clinicians personalize each recipient's treatment and properly manage them in the post-transplant period (10).

Objectives

The proper HLA matching in the first transplantation could prohibit the development of sensitization. Not only can the detailed evaluation of the previously sensitized patients help avoid hyperacute rejection but it can reinforce the proper selection of desensitization strategies (11,12). This study aimed to evaluate the prevalence, titers, and types of anti-HLA antibodies in highly sensitized patients (CPRA ≥50%) who wait for kidney transplantation in our center.

Patients and Methods

Study design

This cross-sectional study was performed in Imam Reza general hospital of Tabriz university of medical sciences, Tabriz, Iran. To incorporate the highly sensitized candidate recipients in this study, ESRD patients in the age range of 18-70 with a body mass index (BMI) <35 kg/m^2 were included from February 2020 to 2021. Serum samples of patients were screened by a bead-based flow cytometric PRA assay (Flow-PRA; One-Lambda, CA, USA) using a FACS Calibur instrument (BD Biosciences, USA). Patients with a flow PRA>5% were selected for further evaluations. Patients' comprehensive medical histories were also recorded, including prior transplantation, blood transfusion, and pregnancy. The included ESRD patients (n = 100) were under dialysis. To select the highly sensitized patients out of the sensitized population, the CPRA values were calculated for each patient using the UNOS CPRA calculator on the UNOS website (https://optn.transplant.hrsa.gov/resources/allocation-calculators/cpra-calculator). For this purpose, the serum samples of sensitized patients were further tested for specific anti-HLA antibodies using the SAB LABScreen Luminex assay (One-Lambda, CA, USA). Figure 1 illustrates the flowchart of the selection strategy of the studied patients. The procedure was conducted according to the

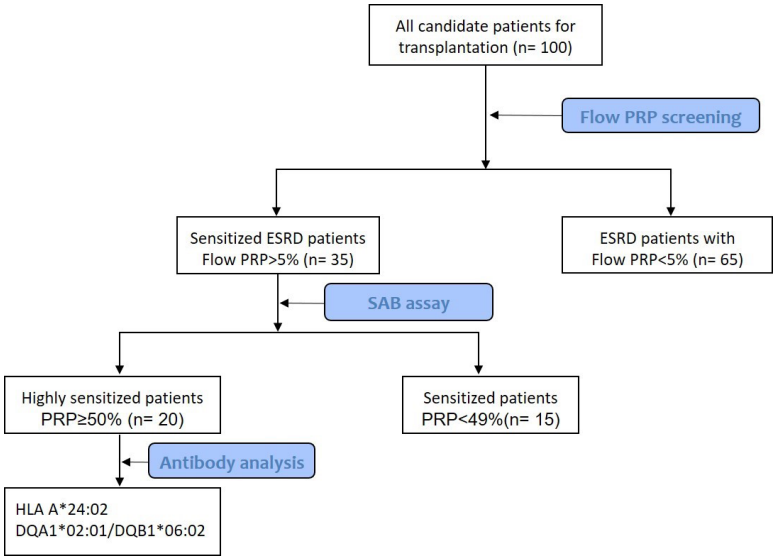

Figure 1. Flowchart of the selection of highly sensitized candidates.

manufacturer's instructions. In brief, serum samples were incubated with Luminex beads, and after washing with phosphate-buffered saline (PBS), phycoerythrin (PE)-conjugated anti-IgG secondary antibody was added. After incubation, the beads were further washed with PBS and analyzed by LAB Scan™ 100 analyzer (One-Lambda, CA, USA). Data were analyzed using Luminex 100 IS version 2.3 software (Luminex Corporation, USA). Patients with CPRA ≥50% (n = 20) were considered highly sensitized and the results of their SAB Luminex anti-classes I and II antibodies were investigated in terms of prevalence and mean fluorescence intensity (MFI).

Data analysis

All quantitative data were presented as mean ± standard deviation or median (minimum-maximum) based on the data normality calculated by the Shapiro-Wilk test. Qualitative data were presented as numbers (percentage). *P* values below 0.05 were considered statistically significant. SPSS version 21.0 was used for the data analysis.

Results

Causes of sensitization

The highly sensitized candidates had a mean age of 36.6, with a 55:45 male to female ratio. The obtained results showed that 70% of the sensitized patients had a history of transplantation (n = 14), 25% of them had a history of blood transfusion (n = 5), and 15% were documented with a history of pregnancy (n = 3). One patient (5%) had no risk factor for sensitization. As a result, the most often cited cause of sensitization in the examined cohort was prior kidney transplantation (Table 1).

Anti-HLA antibodies

The median MFI level of anti-class II antibodies was significantly higher than the MFI levels of class I antibodies in the studied patients – 3400 (500-54934) versus 3008 (100-41160) ($P<0.05$). Besides, the MFI levels of anti-HLA-class I antibodies in sensitized patients with different histories did not show statistically significant differences ($P>0.05$). In the case of class-I antibodies, anti-A*24:02 was the most frequent antibody (n = 9; $P<0.05$), and A*11:01, B*07:02, and B*57:01 were the other prevalent antibodies (n = 8). In the assessment of class-II antibodies, anti-DQA1*02:01 and anti-DQB1*06:02 were the predominant antibodies (n = 12, $P<0.05$). Anti-DRB1*11:01 antibody was the second prevalent class-II antibody (n = 11). The list of anti-class I and class II and their frequencies among the patients are represented in Table 2.

Among the studied patients, 47.4% of anti-HLA-class I antibodies had MFI levels below 3000 (n = 128), 13.7% had MFI levels between 3000 and 6000 (n = 37), and 33.7% had MFI levels above 6000 (n = 91). In the case of class-II antibodies, 46.7% showed MFI levels below 3000 (n = 126), 14.1% between 3000 and 6000 (n = 38), and 39.3% above 6000 (n = 106). Accordingly, it was demonstrated that 39% of anti-HLA-DR and 32.4% of HLA-DQ antibodies had MFI levels above 6000 (n = 71 and n = 23, respectively).

Table 1. Demographic information of candidates for renal transplantation

Demographic information		Values
Age		36.6±12.8
Gender	Male	11 (55%)
	Female	9 (45%)
CPRA	0-50	1 (5%)
	50-80	3 (15%)
	>80	16 (80%)
Only pregnancy		0 (0%)
Only blood transfusion		5 (25%)
Renal transplantation		14 (70%)

CPRA, calculated panel reactive antibody.

Discussion

In this study, highly sensitized patients comprised 20% of all the sensitized population. The most common causative of sensitization was the previous kidney transplantation seen in 70% of the patients. The titers of anti-HLA-class I antibodies were comparatively higher than the titers of anti-HLA-class II antibodies. Anti-A*24:02 and anti-DQA1*02:01/DQB1*06:02 were the predominant antibodies against the class I and class II molecules, respectively.

A recent study in a single-center report from North India in 2021, showed that the prevalence of HLA A*24:02 antibody was 35.4% in kidney transplant candidates (13). High-resolution HLA typing in the Korean population indicated that the HLA A*24:02 allele with a prevalence of 19.5% was the most common HLA genotype in the A allelic group (14). Our outcomes on the higher prevalence of the anti- HLA antibody A*24:02 are in line with the mentioned studies.

Studies showed that the risk of rejection in candidates with DSA MFI levels above 6000 was 100 times higher than the patients with MFI levels below 500 (15). In our study, 33% of anti-HLA-class I had MFI levels above 6000, and 39% of anti-HLA-class II antibodies had MFI levels above 6000. Several studies indicated that anti-HLA-DQ antibodies are the most frequent anti-HLA antibodies being produced following the transplantation and could have a major influence on developing graft glomerulopathy (16). Although the anti-HLA-DQ antibody is the most common de novo antibody resulting in the immunologic rejection and inferior outcomes, there are still a series of centers that neglect the importance of the DQ locus and only consider A, B, and DR loci (17).

The only possible cause of sensitization in the five patients was blood transfusion. Despite advances in leukocyte-free products, blood transfusion remains to be

Table 2. The frequency and median MFI levels of class I and class II antibodies among candidates for renal transplantation

Class I	N	MFI level	Class II	N	MFI level
Total	256	3008 (100-41160)	Total	270	3400 (500-54934)
A*24:02	9	3100 (1000-18000)	DQA1*02:01/DQB1*06:02	12	2014 (1400-23027)
A*11:01	8	1800 (500-21027)	DRB1*11:01	11	1500 (500-40813)
B*07:02	8	800 (500-12100)	DRB1*03:02	8	1600 (500-17415)
B*57:01	8	1700 (800-2100)	DRB1*07:01	8	2250 (500-24069)
B*44:02	7	1000 (500-13200)	DRB1*08:01	8	2945 (600-31568)
B*08:01	6	3000 (100-9000)	DRB1*14:01	8	2529.5 (500-24259)
B*35:01	6	1150 (500-17000)	DRB3*02:02	8	2900 (500-19379)
B*45:01	6	1450 (500-10500)	DRB1*03:01	7	3200 (600-18911)
B*55:01	6	654.5 (500-8000)	DRB1*09:01	7	2900 (1200-24069)
B*15:01	6	1350 (500-4200)	DRB1*12:02	7	2400 (500-54934)
A*03:01	6	1600 (500-10000)	DRB1*13:01	7	2111 (1100-24976)
A*23:01	6	8492 (1304-41160)	DRB1*13:03	7	1625 (500-25628)
A*68:01	6	4200 (1192-23011)	DQA1*03:01/DQB1*04:02	6	750 (500-11700)
A*25:01	5	2300 (500-23118)	DRB1*04:04	6	1900 (500-22029)
A*29:02	5	2700 (500-22097)	DRB1*12:01	6	4010 (1400-27197)
A*33:01	5	500 (500-2000)	DQB1*05:02	5	3840 (1736-24178)
A*01:01	4	5500 (5200-23660)	DRB1*01:01	5	2300 (500-24762)
A*02:01	4	4350 (600-11098)	DRB5*01:01	5	9687 (500-15750)
A*02:02	4	9283 (6000-12566)	DRB1*04:05	5	2300 (500-24431)
A*26:01	4	575 (500-23794)	DRB1*10:01	5	4101 (1150-26400)
A*30:01	4	1225 (500-24292)	DRB1*o4;01	5	1600 (500-25293)
A*32:01	4	8900 (500-23254)	DQA1*01;02/DQB1*05:01	4	10414.5 (500-35000)
A*33:03	4	1559 (900-26362)	DQA1*01:01/DQB1*05:01	4	2350 (1550-3500)
B*14:02	4	750 (500-4900)	DQA1*01:02/DQB1*06:04	4	2826.5 (2518-20854)
B*40:01	4	550 (500-6300)	DQA1*03:02/DQB1*03:03	4	3450 (1700-22400)
B*52:01	4	1000 (650-8700)	DQA1*06:01/DQB1*03:01	4	1850 (500-20284)
			DRB1*01:03	4	2325 (500-9407)
			DRB1*08:02	4	12569 (2753-28335)
			DRB1*11:04	4	21819 (1050-45311)
			DRB1*14:04	4	10121 (3040-20112)
			DRB1*15:02	4	4875 (500-24336)
			DRB4*01:01	4	7468.5 (500-37100)

a risk factor for sensitization in individuals with chronic kidney disease unrelated to the other inflammatory events. Leukocyte-depleted products, such as packed cells contain very low magnitudes of HLA molecules, and the higher volumes and repeated sessions of transfusions make the incidence of sensitization inevitable. Therefore, the blood product infusion which is a treatment in chronic kidney disease patients waiting for a kidney transplant can potentially lead to high CPRA scores (18).

One patient in our study with CPRA ≥50% did not have any history of classical sensitization risk factors. These rare cases of sensitization could occur due to epitope cross-reactions to commensal flora and specific infectious agents in a susceptible genetic background. However, the specific etiology of these sensitizations needs to be fully elucidated.

In spite of the diversity of HLA molecules, adhering to a proper matching program not only can provide a fair long-term outcome, but also can prevent the development of detrimental sensitization. We showed that matching of the class-II DRB1 and DQBI is critically important in avoiding broad sensitization in the first organ transplantation. Novel matching approaches such as virtual cross-matching can reinforce transplantation centers all around the globe to prevent life-threatening and ever-increasing sensitization events. The outcomes of the present study could be regarded as a piece of the puzzle in the development of local and center-based cross-matching databases.

Conclusion

In the kidney allocation system, matching of the HLA antigens between the recipient and donor, especially in HLA DRB1 and HLA DQBI loci seems critical in minimizing the establishment of the sensitization. While recording the history of the sensitization event is demanded for identifying sensitized individuals before transplantation, the measurement of anti-HLA antibodies is required to get meaningful CPRA values utilizing virtual cross-match approaches. The establishment of the center-based cross-matching datasets might be the missing key in the prevention of sensitization in transplant wards, and these kinds of databases are highly recommended.

Limitations of the study

A small sample size due to the limited number of transplantation surgeries during the COVID-19 pandemic was the main limitation of this study.

Authors' contribution

MA conceptualized and managed the project. FF participated in laboratory experiments, analysis, and interpretation of the results. FF, AM and SZV wrote and revised the manuscript. The authors completely observed the ethical issues including data fabrication, falsification, plagiarism, double publication misconduct, or submission and redundancy.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

Informed consent was obtained from all study participants. All obtained information was saved confidential (Ethical code: IR.TBZMED.REC.1398.209). This study was extracted from the postdoctoral thesis of Farahnoosh Farnood (nephrologist) at the Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran (Registration code: 62923).

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