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Impact of Anti-HLA-DR51/52/53 Antibody Positivity on Predicting Flow Cytometry Crossmatch Results in Kidney Transplant Candidates

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Abstract

Objective: Genetic differences between the patient and the donor and the immune system cells response to these differences are among the causes of allograft rejection. Human leukocyte antigen (HLA)-DR, DP, DQ antigens have been shown to be expressed in renal epithelial cells, and in addition, a marked increase in HLA class-II expression has been reported in rejected renal allografts. Human leukocyte antigen-DR contributes to the rejection process due to its role in the activation of $CD4^+$ T cells. Polymorphism in the HLA-DRB3 (DR52), DRB4 (DR53), and DRB5 (DR51) loci is weak, and they are not encoded by the same loci as HLA-DRB1. In addition, these loci are inherited together with the alleles of the DRB1 locus encoding HLA antigens. In our study, we aimed to investigate the correlation between anti-HLA DR51/52/53 antibody positivity detected alone or in combination in the sera of patients on the kidney transplant waiting list and flow cytometry crossmatch (FCXM) positivity.

Materials and Methods: In our study, the panel reactive antibody (PRA) identification and FCXM test results of 200 patients who tested positive for PRA screening between 2019 and 2023 at the Tissue Typing Laboratory of İstanbul Faculty of Medicine were retrospectively analysed.

Results: Of the patients included in the study, PRA screening tests were positive (n=200), and antibodies against at least one antigen belonging to the anti-HLA-DR51/52/53 subgroups were detected in 55.5% (n=111) of these patients in the PRA identification test. All alleles in the DR51, DR52, DR53 subgroups were found to be associated with FCXM-B positivity. In addition, DR16 (p=0.017) correlated with FCXM-T positivity both alone and in combination with DR15 (p=0.019), while in the DR52 subgroup, the simultaneous positivity of DR13, DR14, DR17, and DR18 (p=0.027) was significantly correlated with FCXM-T positivity.

Conclusion: The findings obtained in our study suggest that HLA-DR51/52/53 antibodies may be effective in predicting FCXM positivity.

Keywords: Donor specific antibody, flow cytometry crossmatch, HLA-DRB3, HLA-DRB4, HLA-DRB5

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INTRODUCTION

Human leukocyte antigen (HLA) -DR is one of the class II HLA proteins and consists of heterodimeric α and β chains. The HLA-DR alpha chain is encoded by *HLA-DRA*. There are four different genes that can encode the β chain, the most common of which is *HLA-DRB1*. Other genes that can encode the β chain are *HLA-DRB3*, *HLA-DRB4*, or *HLA-DRB5*. These genes are generally expressed at lower levels than *HLA-DRB1*, but they may be present in a specific haplotype depending on the associated *HLA-DRB1* gene (1-3). The *HLA-DRB3/4/5* genes are closely related to the *HLA-DRB1* locus (4). It has been reported that they show a strong linkage disequilibrium (5). Although the expression levels of the *HLA-DRB3*, *HLA-DRB4*, and *HLA-DRB5* loci are lower than those of *HLA-DRB1*, they can be detected by serological methods and are therefore referred to as the HLA-DR52, -DR53, and -DR51 antigens, respectively (6).

HLA-DRB3 (i.e., DR52) is linked with the DR11, DR12, DR13, DR14, DR17(3), and DR18(3) antigens, while *HLA-DRB4* (i.e., DR53) is linked with the DR4, DR7, and DR9 antigens. DRB5 (i.e., DR54) is linked with the DR15(2) and DR16(2) antigens. (7,8) The genes linked to *HLA-DRB1* and *HLA-DRB3/4/5* are shown in Table 1 (9).

HLA-DR plays a critical role in the rejection process, particularly due to its effect on the activation of $CD4^+$ T cells (9). Our hypothesis is that anti-HLA-DR 51/52/53 antibody positivity may be effective in predicting flow cytometry crossmatch (FCXM) outcome. In our study, we aimed to investigate the effect of anti-HLA DR51/52/53 antibody positivity on flow FCXM results.

MATERIALS AND METHODS

Between 2019 and 2023, 200 patients on the kidney transplant waiting list who tested positive for panel reactive antibody (PRA) screening were included in the study. The PRA identification and FCXM test data of the patients, which were studied simultaneously, were analyzed retrospectively.

Initially, all patients underwent PRA screening tests. Panel reactive antibody identification tests were performed on 200 patients who tested positive for PRA screening tests to detect donor specific antibody (DSAs) using the Lifecodes® kit (Immucor, Norcross, GA, USA) according to the manufacturer's instructions. The PRA tests were performed using the Luminex® 200 system (Luminex Corp., Austin, TX, USA). In addition, FCXM tests were performed on all patients and donors. Panel reactive antibody identification and FCXM tests were conducted simultaneously at İstanbul University İstanbul Faculty of Medicine Department of Medical Biology, Tissue Typing Laboratory. In PRA screening and identification tests, anti-HLA antibodies with a mean fluorescence intensity (MFI) ≥1000 were considered positive (10).

Crossmatching tests were performed using the FCXM method. Lymphocyte cells to be used in the FCXM test were isolated from peripheral blood samples obtained from patients and donors via density gradient centrifugation method. Two-color flow cytometry was performed. CD3-PC5 monoclonal antibodies were used to label T cells, and CD19-PE monoclonal antibodies were used to label B cells. Immunoglobulin (IgG) antibodies were labeled with IgG-FITC secondary antibody (11).

Statistical Analysis

All statistical analyses were performed using SPSS Statistics for Windows, version 21.0 (IBM Corp.; Armonk, NY, USA). Results for continuous variables are calculated as mean (SD). The $\chi 2$ test was used to compare categorical data. Gender ratios are expressed as percentages. The p-value <0.05 is considered statistically significant.

RESULTS

The mean age of the 200 patients included in the study was 52.7 ± 7.6 years, whereas the mean age of their donors was 45.6 ± 8.8 years. The female-to-male ratio among patients was 54.5% (n=109) to 45.5% (n=91). In the PRA screening, one or more antigens belonging to the anti-HLA-DR51/52/53 subgroups were detected in the sera of 55.5% (n=111) of the 200 patients who tested positive. Among the DR51 subgroup antibodies, DR15 (p=0.007) and DR16 (p=0.011) were independently correlated with B cell FCXM positivity, while the combination of these antibodies was also correlated with B cell FCXM positivi-

Table 1. Linked genes between *HLA-DRB1* and *HLA-DRB3/4/5 (9).*

Gene	Coding protein	Linked HLA-DR antigen
DRB3	Beta chain of DR52	DR11, DR12, DR13, DR14, DR17(3), DR18(3)
DRB4	Beta chain of DR53	DR4, DR7, DR9
DRB5	Beta chain of DR51	DR15(2), DR16(2)
None	None	DR1, DR8, DR10

HLA: Human leukocyte antigen.

ty (p=0.006). Additionally, DR16 was found to be correlated with FCXM-T positivity both alone (p=0.017) and in combination with DR15 (p=0.019) (Table 2).

Table 2. Correlation of DRB5 (DR51) antibody and FCXM positivity.

Antigen	FCXM-T cell positivity	FCXM-B cell positivity
DR15	p=0.123	p=0.007
DR16	p=0.017	p=0.011
DR15 + DR16	p=0.019	p=0.006

HLA: Human leukocyte antigen; **FCXM:** Flow cytometry crossmatch.

Table 3. Correlation of DRB3 (DR52) antibody and FCXM positivity.

Antigen	FCXM-T cell positivity	FCXM-B cell positivity
DR11	p=0.050	p<0.001
DR12	p=0.015	p=0.038
DR14	p=0.068	p=0.003
DR17	p=0.103	p=0.016
DR18	p=0.103	p=0.016
DR13 + DR14 + DR17 + DR18	p=0.027	p=0.263

HLA: Human leukocyte antigen; **FCXM:** Flow cytometry crossmatch.

Table 4. Correlation of HLA-DRB4 (DR53) antibody and FCXM positivity.

Antigen	FCXM-T cell positivity	FCXM-B cell positivity
DR4	p=0.048	p=0.064
DR7	p=0.266	p=0.026
DR9	p=0.218	p=0.001
DR4 + DR9	p=0.667	p=0.024

HLA: Human leukocyte antigen; **FCXM:** Flow cytometry crossmatch.

In the DR52 subgroup, DR12 alone was correlated with FCXM-T positivity (p=0.015), while DR11 (p<0.001), DR12 (p=0.038), DR14 (p=0.003), DR17 (p=0.016) and DR18 (p=0.016) were individually correlated with FCXM-B positivity. In addition, the association of DR13, DR14, DR17 and DR18 was correlated with FCXM-T positivity (p=0.027) (Table 3).

In the DR53 subgroup, DR4 alone (p=0.048) was found to be correlated with FCXM-T positivity, while DR7 (p=0.026) and DR9 (p=0.001) were individually correlated with FCXM-B positivity. The association of DR4 and DR9 was correlated with FCXM-B positivity (p=0.003) (Table 4).

DISCUSSION

Patients with a history of sensitivity, such as recurrent pregnancies, blood transfusions, and secondary transplants, may develop antibodies against HLA antigens. The presence of antibodies with high MFI levels against donor class I and class II HLA antigens in the patient's serum prior to kidney transplantation is a significant risk factor for antibody-mediated rejection after transplantation (12). Recent studies have shown that donor-specific antibody (DSA) development is associated with antibody-mediated damage and poor graft outcomes, and that HLA class II DSA has a greater effect than class I (13,14). In one study, it was shown that anti-class II DSA may be highly associated with humoral rejection, and it was reported that the determination of anti-class II DSA may be important in the overall assessment of immunological risk in kidney transplant patients (15). In another study, PRA positivity was detected in 20.4% of patients with chronic kidney disease on the waiting list, and the rates of anti-HLA antibodies against HLA class II were reported to be higher (16).

The HLA-DR51 antigen encoded by the *HLA-DRB5* gene, the HLA-DR52 antigen encoded by the *HLA-DRB3* gene, and the HLA-DR53 antigen encoded by the *HLA-DRB4* gene are thought to be weaker than other HLA-DR antigens encoded by *HLA-DRB1* genes (9,17). However, it is known that HLA-DR51, -DR52, and -DR53 antigens are always associated with DR antigens. Anti-HLA-DR53 antibody is a risk factor for acute mediated rejection (AMR) and resistance to elimination (18). Katsuma et al. (19) reported a subclinical AMR case in which the MFI of DR53 increased after intensive immunosuppressive therapy. This case report highlights that high MFI values for HLA-DR53 may be significant in the early diagnosis of subclinical AMR. The study also points out that mismatch in HLA-DR 51, 52, and 53 alleles may be associated with subclinical AMR.

Our study retrospectively evaluated 200 class II PRA-positive patients and found that 55.5% (n=111) were posi-

tive for at least one antigen belonging to the anti-HLA-DR51/52/53 subgroups. Similar to the findings in the literature, the results of our study suggest that HLA-DR51, -DR52, and -DR53 antigens should be included in HLA-DR typing analysis because they are always associated with DR antigens. Additionally, anti-HLA-DR51, -DR52, and -DR53 (-DR51/52/53) antibodies can be evaluated as DSA for antibody analysis. One study showed that mismatches in the HLA-A, -B, and -DRB1 loci, as well as HLA-DR51/52/53 antigen mismatches, also have an effect on HLA allosensitization. In the relevant study, it was determined that the risk of allosensitization is 3, 2; 3, 4; 3,5; and 3,9-fold higher for HLA-A, -B, -DRB1, and -DR51/52/53 mismatches, respectively (9). Another study reported that antibody-mediated rejection occurred more frequently in the group with possible anti-HLA-DR51/52/53 DSA (20). All of this literature data emphasizes the importance of considering these antibodies during HLA-DR51/52/53 typing and DSA evaluation for all donors and recipients.

The FCXM test is a highly sensitive cell-based method that predicts graft rejection. In our study, in addition to the studies in the literature, the correlation between anti-HLA-DR51/52/53 DSA positivity and T cell and B cell FCXM positivity was investigated, and it was found that all DR51/52/53 donor-specific antibodies, either alone or in combination, showed a correlation with B cell FCXM positivity. In addition, it has been found that DR4, DR16, DR11, and DR12 alone, and DR15 and DR16 in combination, may be associated with T cell FCXM positivity. The results of our study suggest that FCXM positivity may predict the presence of HLA-DR52/53/54 antibodies, which are an important risk factor for rejection. Therefore, considering these antibodies during HLA-DR51/52/53 typing and DSA assessment for all donors and recipients may contribute significantly to transplant success and post-transplant immunological follow-up.

However, recent studies have shown that DSA do not always prevent organ transplantation, and therefore, broader studies to understand the subtypes of anti-HLA antibodies and their activity will be very important.

Ethical Approval: This study was approved by the Ethics Committee of İstanbul University on February 3, 2023 (Decision No. 2022/1707).

Informed Consent: N.A.

Peer-review: Externally peer-reviewed

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