# Association Between HLA Antibodies and Different Sensitization Events in Renal Transplant Candidates

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## ABSTRACT

**Background.** Human leukocyte antigen (HLA) allo-immunization is caused by various events such as blood transfusions, pregnancies, or organ transplantations, which can lead to sensitization. In this retrospective study, we evaluated different sensitization models and their effects on panel-reactive antibody (PRA) profiles of renal transplantation candidates.

**Methods.** Anti-HLA class I/II antibody screening tests were performed in 906 renal transplantation candidates with the use of a microbead-based assay (Luminex).

**Results.** Two hundred ninety-seven (32.8%) of the patients were determined as positive in terms of PRA, and 609 (67.2%) were negative. Sensitized and non-sensitized patients were compared separately in terms of each sensitization type. The anti-HLA class I, II, and I+II positivity rates in patients sensitized only by blood transfusion were 13.1%, 6.3%, and 14.1%, the rates with pregnancy sensitization were 35.5%, 29%, and 45.2%, and rates with previous transplantation sensitization were 15.6%, 34.4%, and 38.9%, respectively. Prevalence of PRA positivity was significantly higher in patients with previous pregnancy than with transplantation and transfusion (odds ratio, 1.003; 95% confidence interval, 0.441–2.281; $P = .031$). The risk of developing HLA class I antibodies was higher in pregnancies ($P < .001$), and the risk of developing anti-HLA class II antibodies was higher in patients who had undergone a previous transplantation ($P < .001$). The rate of developing HLA-B antibodies in patients sensitized by pregnancy were significantly higher compared with sensitization after transfusion ($P = .015$), as was the rate of developing HLA-DQ antibodies in patients sensitized by previous transplantation compared with sensitization through pregnancy ($P = .042$).

**Conclusions.** In patients who are waiting for kidney transplantation, sensitization by pregnancy and transplantation have a significant impact on development of HLA class I and class II antibodies.

RENAL transplantation has long been recognized as the best treatment for end-stage renal disease. Despite reliable pre-transplant screening methods and improvement of immunosuppression therapy, failure of renal allografts still occurs because of cellular- or humoral-mediated rejections. Allo-immunization remains a critical factor that affects the success of renal transplantation. Several recent studies evaluated the prevalence of human leukocyte antigen (HLA)-specific antibodies and the clinical importance of these antibodies in acute allograft rejection [1–3]. The occurrence of allo-antibodies against HLA can be induced by previous transplantation, transfusions, or pregnancies.

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0041-1345/17
http://dx.doi.org/10.1016/j.transproceed.2017.02.004

**Table 1. Demographic and Clinical Charecteristics**

| | |
|---|---|
| All Patients (n) | 906 |
| Age (years), ±SD | 44.25 ± 15.69 |
| Sex, female/male, n (%) | 422 (46.6%)/484 (53.4%) |
| No sensitization event, n (%) | 183/906 (20.3%) |
| Patient with pregnancy only | 62 (6.8%) |
| Patient with transfusion only | 191 (21.1%) |
| Patient with transplantation only | 90 (9.9%) |
| PRA (−), n (%) | 609 (67.2%) |
| PRA (+), n (%) | 297 (32.8%) |
| Total class I (+), n (%) | 193 (21.3%) |
| Total class II (+), n (%) | 221 (24.4%) |
| Class I–positive and class II–negative, n (%) | 76 (8.4%) |
| Class I–negative and class II–positive, n (%) | 104 (11.5%) |
| Class I– and class II–positive, n (%) | 117 (12.9%) |
| Anti–HLA antibody–positive patients with pregnancy only | 28/62 (45.2%) |
| Anti–HLA antibody–positive patients with transfusion only | 27/191 (14.1%) |
| Anti–HLA antibody–positive patients with transplantation only | 35/90 (38.9%) |

Sensitization is one of the most important barriers against transplantation. Antibodies against HLA that developed after blood transfusions, pregnancies, and graft rejections were generally described as PRA. The development of anti-HLA antibodies is likely after exposure to allo-antigens in pregnant women. Leukocytes in blood cells are assumed to be responsible for transfusion-associated HLA sensitization. After sensitization occurs, antibodies against both HLA class I and HLA class II are produced. Many studies reported the relevance of various incidences of allo-antibodies detected after transplantation [4–8]. After sensitization, antibodies appear against both HLA class I and HLA class II, and these antibodies activate different cells, initiate the immune response, and contribute to rejection. HLA antibodies that develop in the early term of transplantation damage allografts more than antibodies that develop 1 year after transplantation [9]. Higher panel-reactive antibody (PRA) levels are also related to an increased risk of graft failure. The PRA assay is commonly accepted as a routine method for determining sensitization to HLAs in transplant candidates. Many studies reported the relevance of various incidences of allo-antibodies detected after transplantation [10]. This variability can be attributed to the use of different antibody detection techniques. Recently, the sensitive solid-phase assays such as enzyme-linked immunosorbent assay (ELISA) and microbead-based assay were introduced, which can detect low levels of anti-HLA antibodies. The microbead-based assay is known as the most sensitive among them. We evaluated how different sensitization events affected PRA status through the use of a microbead-based assay (screening and specificity anti-HLA antibodies) in solid-organ transplantation candidates [11].

## METHODS

A total of 906 patients (484 men, 422 women; mean age, 44.25 ± 15.69 years) who were renal transplant candidates between 2012 and 2016 at Istanbul Faculty of Medicine Hospital were included in this retrospective, observational study.

### Antibody Detection

Microbead-based PRA assays were performed with the use of LABScreen Mixed kits (One Lambda Inc, Canoga Park, Calif, United States) for antibody screening. An additional test was performed to identify HLA specificities with the use of class I and class II Lambda antigen tray kits (LS1PRA and LS2PRA commercial) (One Lambda Inc). Microbead-based PRA screening and identification assays were used in accordance with the protocol recommended by the manufacturer (median MFI values >1000 were taken as positive).

### HLA Typing

All patients were tested for HLA. HLA typing (HLA-A, -B, -DR, and -DQ) was performed with the use of a polymerase chain reaction sequence-specific oligonucleotide (PCR-SSO) assay combined with microbead-based assay technology with an SSO-LABType commercial kit (One Lambda, Inc). The manufacturer's instructions were followed during the procedure.

### Statistical Analysis

Continuous data are described as mean (standard deviation [SD]), and categorical data are expressed as n (%). Negative and positive PRA values were tested for significance between patients by use of the Student *t* test. Data were compared by use of the Pearson $\chi^2$ or Fisher exact test for categorical data. Binary logistical regression tests with univariate and multivariate analyses were performed to determine the relationships to positivity of PRA for the single significant parameter and the multiple significant parameters, respectively. Multiple logistic regression was performed to determine HLA alleles associated with anti-HLA antibodies. A value of $P < .05$ was considered statistically significant. Statistical calculations were performed with the use of SPSS for Mac, version 21.0 (SPSS, Chicago, Ill, United States).

## RESULTS

Nine hundred six patients presented to our laboratory for PRA testing between 2012 and 2016. Demographic and clinical features of the study patients are shown in Table 1.

**Table 2. Comparison of Anti-HLA Development Between Sensitization Event Only and No Event**

| | No Sensitizing Event (n = 183) | Transfusion Only (n = 191) | *P* Value* | Pregnancy Only (n = 62) | *P* Value* | Transplantation Only (n = 90) | *P* Value* |
|---|---|---|---|---|---|---|---|
| Class I (+) | 6 (3.3%) | 25 (13.1%) | .001* | 22 (35.5%) | <.001* | 14 (15.6%) | .001* |
| Class II (+) | 10 (5.5%) | 12 (6.3%) | .827 | 18 (29.0%) | <.001* | 31 (34.4%) | <.001* |
| Class I and/or II (+) | 14 (7.7%) | 27 (14.1%) | .048* | 28 (45.2%) | <.001* | 35 (38.9%) | <.001* |

*Comparison of no sensitizing and sensitizing event (pregnancy, transfusion, and transplantation).

**Table 3. Comparisons Between Negative and Positive PRA Groups in Patients**

| Patients (n) | Negative PRA (n = 609) | Positive PRA (n = 297) | *P* Value |
|---|---|---|---|
| Age (years), ±SD | 43.95 ± 16.25 | 44.87 ± 14.40 | .410 |
| Sex | | | |
| Female, n (%) | 235 (38.6%) | 185 (62.3%) | <.001 |
| Male, n (%) | 374 (61.4%) | 112 (37.7%) | |
| Only pregnancy, n (%) | 34 (5.6%) | 28 (9.4%) | .031 |
| Mean number of pregnancies, ±SD | 0.62 ± 0.06 | 1.57 ± 0.14 | <.001 |
| Only transfusion number, n (%) | 164 (26.9%) | 27 (9.1%) | <.001 |
| Mean number of transfusions, ±SD | 1.94 ± 0.12 | 3.47 ± 0.29 | <.001 |
| Transplantation, n (%) | 13 (2.1%) | 5 (1.7%) | .802 |

Class I and/or II–positive PRA rates were significantly higher in patients with pregnancy sensitization (45.2%), transplantation events (38.9%), or transfusion events (14.1%) than without sensitization events (7.7%), ($P < .001$, $P < .001$, and $P = .048$, respectively). In the patients with a single sensitizing event, class I–positive rates were significantly higher in patients with previous pregnancy (35.5%), transplantation (15.6%), or transfusion (13.1%), compared with patients without a sensitizing event (3.3%) ($P < .001$, $P = .001$, and $P = .001$, respectively). In patients with a single sensitizing event, class II–positive rates were significantly higher in patients with previous transplantation (34.4%), pregnancy (29.0%), or transfusion (6.3%), compared with patients without a sensitizing event (5.5%) ($P < .001$, $P < .001$, and $P = .827$, respectively). A history of transplantation significantly affected class II anti-HLA antibodies, and pregnancy affected class I antibodies (Table 2).

Previous pregnancy, transfusion, and transplantation were compared between the PRA-positive and PRA-negative groups. In the PRA-positive patient group, the number of women (62.3%) was higher than in the PRA-negative patient group (38.6%) (Table 3). We compared the PRA-positive and PRA-negative groups with regard to other factors such as age, sex, and mean number of pregnancies (Table 3). Univariate logistic regression models were used to show relations between PRA positivity and events such as sensitizing event, age, and sex. Prevalence of anti-HLA antibodies was significantly higher in patients with only pregnancy sensitization than with other sensitizing events. Prevalence of PRA-positive rates in different single-sensitizing event groups is shown Table 4. A multivariate logistic regression model showed that pregnancy has independent significant associations with a positive PRA (Table 5).

The anti-HLA class I antibodies (HLA-B and HLA-A+B loci) ($P = .015$ and $P = .034$) rates were significantly higher in patients with pregnancy sensitization than with other sensitizing events. We found a higher prevalence of anti-HLA antibodies for DQ loci in patients with previous transplantation than pregnancy and transfusion ($P = .042$) (Table 6).

**Table 4. Univariate Logistic Regression Models for Positive PRA Test in Patients**

| | OR | 95% CI | *P* Value |
|---|---|---|---|
| Sex (female vs male) | 8.646 | 0.195–0.574 | .003 |
| Age | 0.679 | 0.010–0.050 | .410 |
| Sensitizing event | | | |
| No | 0.274 | 0.176–0.336 | .600 |
| Transfusion only | 0.076 | 0.105–0.381 | .782 |
| Pregnancy only | 27.727 | 0.413–2.174 | .001 |
| Transplantation only | 0.491 | 0.264–0.377 | .484 |
| Pregnancy/transplantation | 17.537 | 0.365–1.528 | .001 |
| Pregnancy/transfusion | 2.906 | 0.596–1.016 | .088 |
| Transplantation/transfusion | 1.551 | 0.344–0.428 | .213 |

Abbreviations: OR, odds ratio; CI, confidence interval.

## DISCUSSION

Kidney transplantation is a good option for patients with end-stage renal disease. The role of HLA antibodies in the post-transplant period remains an important issue in renal transplantation. In this study, we investigated the effects of sensitization events such as transfusion, pregnancy, and transplantation on anti-HLA antibody prevalence in patients awaiting kidney transplantation. We compared the class I/II anti-HLA antibody prevalence among patients who were exposed to sensitization events and on nonsensitized patients. LUMINEX PRA screening and identification tests were performed on solid-organ transplant candidates, and the outcomes were compatible with previously reported results [11–13]. In a study with 674 transplant candidates, class I anti–HLA antibody positivity was reported as 23.4%, 47.6%, and 46.2% [11] in patients with a history of transfusion, pregnancy, and transplantation, respectively; and class I anti–HLA antibody positivity was reported as 18.9%, 38.3%, and 75%, respectively [12], in a study with 453 transplantation candidates. Different positivity rates (18%, 86%) of anti-HLA class I antibody were reported in different studies in pregnant patients with sensitization to pregnancy

**Table 5. Multivariate Logistic Regression Model for Positive PRA Test in Patients**

| Sensitizing Event | OR | 95% CI | *P* Value |
|---|---|---|---|
| Transfusion only | 0.204 | 0.093–0.449 | .063 |
| Pregnancy only | 1.003 | 0.441–2.281 | .031 |
| Transplantation only | 0.341 | 0.099–0.377 | .648 |

Abbreviations: OR, odds ratio; CI, confidence interval.

**Table 6. Specific Antibodies Against HLA-A, -B, -C, -DR, and -DQ: Comparison of Previous Pregnancy and Transfusion With Previous Transplantation**

| | Transfusion Only (n = 191) | Pregnancy Only (n = 62) | *P* Value* | Transfusion Only (n = 191) | Transplantation Only (n = 90) | *P* Value† | Pregnancy Only (n = 62) | Transplantation Only (n = 90) | *P* Value‡ |
|---|---|---|---|---|---|---|---|---|---|
| Anti-A | 3 (1.6%) | 2 (3.2%) | .598 | 3 (1.6%) | 3 (3.3%) | .389 | 2 (3.2%) | 3 (3.3%) | .970 |
| Anti-B | 4 (2.1%) | 6 (9.7%) | .015 | 4 (2.1%) | 2 (2.2%) | .944 | 6 (9.7%) | 2 (2.2%) | .063 |
| Anti-A+B | 12 (6.3%) | 10 (16.1%) | .034 | 12 (6.3%) | 8 (8.9%) | .459 | 10 (16.1%) | 8 (8.9%) | .206 |
| Anti-A+B+C | 6 (3.1%) | 4 (6.5%) | .265 | 6 (3.1%) | 1 (1.1%) | .434 | 4 (6.5%) | 1 (1.1%) | .159 |
| Anti-DR | 3 (1.6%) | 8 (12.9%) | .008 | 3 (1.6%) | 3 (3.3%) | .389 | 8 (12.9%) | 3 (3.3%) | .051 |
| Anti-DQ | 0 | 0 | | 0 | 7 (7.8%) | .003 | 0 | 7 (7.8%) | .042 |
| Anti-DR+DQ | 9 (4.7%) | 10 (16.1%) | .009 | 9 (4.7%) | 21 (23.3%) | <.001 | 10 (16.1%) | 21 (23.3%) | .311 |

*Comparison of transfusion and pregnancy.
†Comparison of transfusion and previous transplantation.
‡Comparison of previous pregnancy and previous transplantation.

only [13,14]. In our study, we found anti-HLA class I positivity as 13.1%, 35.5%, and 15.6% in patients sensitive to transfusion, pregnancy, and transplantation, respectively, with the PRA results of 906 patients. The positivity rates related to blood transfusion and pregnancy sensitivity were compatible with other studies; however, class I antibody level was lower in the transplantation group compared with the other groups. The reason could be that the number of patients sensitized to transplantation was small.

In one study, however, the rate of PRA positivity was 4.11% in first–kidney transplantation candidates; the rate was considerably high (80.2%) in re-transplant patients [11]. The PRA positivity rate was 13.1% with the use of an ELISA in our previous study in a group of 642 patients who were to receive cadaver-related transplants. The class I/II rates were 47.1%, with 16.7% in PRA-positive patients [15]. PRA positivity was 32.8% with the use of LUMINEX in the present study. The rate was 38.9% in patients on the re-transplantation list.

Rates of PRA positivity were significant in patients with transfusion, pregnancy, or transplant history compared with patients with no sensitization. The anti–HLA antibody prevalence was 3.3% in class I and 5.5% in class II in kidney transplantation candidates with no sensitization in this study. Hyun et al [11] also reported similar results for class I and class II positivity, at 5.6%. Lopes et al [12] reported the anti-HLA antibody prevalence for class I as 10% and 5.2% for class II in kidney transplantation candidates with no sensitization, and the positivity rate was 2% in healthy blood donors with no sensitization. Similarly, Triulzi et al [16] reported the rate as 1.6% in nonsensitized blood donors [16]. Although the rate of sensitization in the patient group with no allo-immunization was higher in our study than the other reported nonsensitized blood donors, these results were similar to those of patients on the solid-organ transplant list. The presence of anti–HLA antibody (5% to 10%) in patients with no identified sensitization might be due to unreported abortion, vaccination, or natural formation of the molecules. Some of these antibodies might have harmful effects in kidney transplantation [17]; the others were related to denatured beads and were proven to be clinically insignificant [18,19].

The effect of blood transfusion on HLA allo-immunization in solid-organ transplant patients was compatible with previous studies [11,12]. We found that transfusion had the smallest effect on HLA allo-immunization compared with pregnancy and transplantation. This could be caused by the use of filters with leukocyte-poor blood products that could decrease the HLA allo-immunization. However, previous studies did not give information about the number of transfusions and PRA positivity because of insufficient data; we identified a correlation between transfusion and positivity as the number of transfusions increased.

Pregnancy is known to cause strong immunization in solid-organ transplant candidates. The effect of pregnancy was investigated in blood donors for HLA allo-immunization, and the PRA positivity rate was 24.4% [16]. The rates vary in different studies as 39.5% [12], 71.4% [11], and 25.5% [13]. PRA positivity related only to pregnancy was found as 45.2% in our study. The researchers reported the pregnancy HLA allo-immunization effect to be intermediate between transplantation and transfusion [12]. In that study, it was shown that prevalence of PRA positivity was significantly higher in patients with previous pregnancy than with transplantation and transfusion.

Hyun et al [11] found that as the number of pregnancies increased, the HLA allo-immunization prevalence also increased. We also found a correlation between an increased number of pregnancies and HLA allo-immunization in our study. Some studies showed that there was a significant association between pregnancy and class I antibodies [12,14]. In our study, class I antibody was 35.5% and class II was 29% in patients with sensitization of pregnancy only. Some publications indicated that development of HLA-A and DQ antibodies with pregnancy sensitization were significantly related [13,20]. We showed the association between the development of HLA-B, HLA-A+B, and HLA-DR antibodies in patients with sensitization by pregnancy only in our study.

In a parallel comparison of the effects of transfusion, pregnancy, and transplantation on HLA allo-immunization, researchers reported that transplantation had a strong effect on allo-immunization in class II HLA antigens in

particular [11]. We found that transplantation had an immunogenic effect on class II antigens, especially for DQ.

In our study, information regarding the type of the pregnancy (childbirth, miscarriage, early abortion), transplantation, and date of last transfusion was missing. We believe that future studies that include these data will be important to confirm our results.

## CONCLUSIONS

We showed that solid-organ transplantation had a stronger HLA allo-immunization effect, particularly for class II HLA antigens, compared with pregnancy and transfusion. Pregnancy had a notable allo-immunization effect for class I HLA antigens. We identified that transplantation and pregnancy had a stronger immunization effect compared with transfusion. The prevalence of class I and class II HLA antibodies was significantly higher in patients exposed to sensitization compared with nonsensitized patients.

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