







# Sensitization Status of Patients on the Deceased Donor Kidney Transplant Waiting List: A Single-Center Experience

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342

## ABSTRACT

**Objectives:** This study aimed to analyze the features of patients on the deceased donor kidney transplant waiting list and risk factors associated with sensitization that affect panel reactive antibody status in our center.

**Methods:** Patients' data were collected retrospectively. Panel reactive antibody screening and definition tests were studied for class I (A, B, and C) and class II (DR, DP, DQ) antigens with Luminex every 6 months. Patients with panel reactive antibody >5% and antibody strength >1000 median fluorescence intensity were considered panel reactive antibody-positive. Based on the panel reactive antibody status, the patients were divided into 2 groups: the panel reactive antibody-positive group and -negative group.

**Results:** A total of 338 patients (60% male, mean age: 52.6 ± 14.6 years) were included in the analysis. Panel reactive antibody positivity was detected in 117 (34.6) patients on the waiting list. Compared with the panel reactive antibody-negative patient group, the panel reactive antibody-positive patient group had higher rate of women and lower age ( $P < .001$  and  $P < .001$ , respectively). The patients in the panel reactive antibody-positive group also had longer dialysis vintage ( $P = .027$ ), higher rate of blood transfusion history ( $P < .001$ ), organ transplant ( $P < .001$ ), and higher number of blood transfusion ( $P < .001$ ). Female gender (odds ratio:4.094, 95% CI:2.275-7.368,  $P < .001$ ), history of blood transfusion (odds ratio:2.027, 95% CI:1.131-3.633,  $P = .018$ ), and organ transplant (odds ratio:16.894, 95% CI:7.212-39.578,  $P < .001$ ) were independent risk factors associated with panel reactive antibody positivity.

**Conclusion:** Updates of the organ allocation system to consider sensitized patients and new strategies to expand the donor pool and donation rates are needed in Türkiye.

**Keywords:** Blood transfusion, deceased donor kidney transplant waiting list, kidney transplant, organ allocation system, sensitized patients

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## INTRODUCTION

Sensitization is caused by previous exposure to foreign human leukocyte antigens (HLA), such as organ transplants, blood transfusions, and pregnancies and is an important barrier to a successful kidney transplant.<sup>1</sup> There are a growing number of patients who are added to the waiting lists every year due to substantial lack of deceased donor kidney transplants (DDKT) in our

country.<sup>2</sup> Sensitized patients have more difficulty in finding a suitable donor and have a higher risk of mortality associated with longer waiting times for dialysis.<sup>3-5</sup>

According to the 2019 registry report of the Turkish Society of Nephrology, only 20% of kidney transplant patients were deceased donor source.<sup>6</sup> Compared to developed countries, considering the scarcity of DDKT in



our country,<sup>7</sup> efforts and new strategies for the expansion of the donor pool are needed. Pre-transplant assessment of the candidate's clinical and sensitization status is important to decide on appropriate kidney replacement treatment. The aim of this study was to identify the characteristics of patients on the DDKT waiting list and risk factors associated with sensitization that affect panel reactive antibody (PRA) status in our center.

## METHODS

A total of 338 patients with end-stage kidney disease (ESKD) who were on the DDKT waiting list by January 2020 were included in this retrospective cohort study. Data regarding patients' demographics, clinical features, sensitization events, and immunologic properties were collected. Panel reactive antibody screening and identification tests were performed using Luminex assay kits (Luminex Corporation, Austin, Tex, USA) according to the manufacturer's guidelines. A screening test was performed on all candidates, by collecting sera every 6 months while on the waiting list. Panel reactive antibody positivity was defined as >5%, with the identification of only immunoglobulin (Ig)G anti-HLA isotype-positive cases after dithiothreitol treatment. Then, screened positive samples were further tested for the identification of class I (A, B, C) and class II (DR, DP, DQ) antibodies. For single antigen assays, the cut-off level was defined as a raw median fluorescence intensity of  $\geq 1000$  and considered PRA-positive. Sensitization status of patients on the waiting list was evaluated with actual PRA. In the last actual PRA assessment and face-to-face interviews, questions about risk factors associated with sensitization, such as blood transfusion, pregnancy (including normal pregnancy, miscarriage, and abortion), and history of organ transplant to patients on the waiting list were administered and recorded. Finally, risk factors associated with sensitization that affect PRA positivity were analyzed accordingly.

According to PRA status, the patients were divided into 2 groups: the PRA-positive patient group and -negative group. Patient characteristics and the risk factors associated with PRA positivity were compared among 2 groups. The study was approved by the Ethical Review Committee of the Institute. All

of the protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

## Statistical Analyses

Data were analyzed with Statistical Package of Social Science version 16.0 (IBM Inc, Chicago, IL, USA). Data were expressed as mean  $\pm$  standard deviation for continuous variables and as number of patients or percentages for categorical variables. The differences between the 2 groups were determined by Student's *t* test or Mann-Whitney *U* test. Categorical variables were examined using the chi-square test or Fisher's exact test. Logistic regression analysis was performed to determine risk factors associated with PRA positivity. The value of *P* <.05 was considered statistically significant.

## RESULTS

### Patients Characteristics

A total of 338 patients on the DDKT waiting list were analyzed. The mean age of the study population was  $52.6 \pm 14.6$  years and most of them were male (60%). The mean body mass index of patients was  $25.3 \pm 4.4$ . Blood types A and O were more common in patients on the waiting list (40% and 35%, respectively). Prevalence of the most common comorbidities, that is, hypertension, diabetes mellitus, and cardiovascular disease was 82%, 28%, and 25.5%, respectively. The cause of ESKD was diabetic kidney disease in 21%, glomerulonephritis in 14%, hypertensive kidney disease in 8%, and in 36.5% of patients on the waiting list, the ESKD cause was unknown. Prevalence of hepatitis B (2%) and C (2%) was very low. In wait-listed patients, the most preferred modality was hemodialysis (95%), and primary arteriovenous fistula was used for hemodialysis access in 84% of patients. Mean dialysis vintage was 61 months. Mean time on the waiting list was  $33.6 \pm 23.9$  months. Totally, 157 patients (46.5%) had a history of blood transfusion, 59 (17.5%) patients had a history of organ transplant (all transplanted organs were kidneys), and of the women, 102 (75.5%) patients had a history of pregnancy. The characteristics of study patients are summarized in Table 1.

### Sensitization Events and Comparison of 2 Groups

Class I and/or class II anti-HLA antibody positivity were detected in 117 (34.6%) patients. Class I antibody positivity was detected in 5% of the patients, for class II, detected in 9%, and for class I and class II, 20.6%. The PRA-positive patient group had significantly higher rate of history of blood transfusion (68% vs. 35%, *P* <.001), organ transplant (41% vs. 5%, *P* <.001), and mean number of blood transfusion ( $2.77 \pm 2.82$  vs.  $1.22 \pm 2.53$ , *P* <.001) than the PRA-negative patient group.

The mean age of the PRA-positive patient group was lower than the PRA-negative patient group ( $48.1 \pm 15.2$  vs.  $55.1 \pm 13.7$ , *P* <.001). In the PRA-positive patient group, rate of women was higher than in the PRA-negative patient group (55.6% vs. 31.7%, *P* <.001). There were no statistically significant differences

## MAIN POINTS

- Sensitization is caused by previous organ transplants, blood transfusions, and pregnancies and is a major barrier to successful kidney transplants.
- There are a growing number of patients who are sensitized and an increasing number of patients are added to the waiting lists each year.
- Sensitization is an important issue to solve in patients on the wait list in our country, and therefore, besides the updates to the organ allocation system, we need new strategies to increase the number of the deceased donor kidney transplants.

**Table 1.** Demographic and Clinical Data of Patients on the Deceased Donor Kidney Transplant Waiting List

Parameters	All Patients (n = 338)
Age (years) (mean ± SD)	52.6 ± 14.6
Gender	
Male, n (%)	203 (60)
Female, n (%)	135 (40)
Body mass index (kg/m <sup>2</sup> ) (mean ± SD)	25.3 ± 4.4
Blood types	
A, n (%)	135 (40)
B, n (%)	62 (18)
AB, n (%)	23 (7)
O, n (%)	118 (35)
Comorbidity	
Diabetes mellitus, n (%)	94 (28)
Hypertension, n (%)	277 (82)
Hyperlipidemia, n (%)	64 (7)
Cardiovascular disease, n (%)	83 (25.5)
Cerebrovascular disease, n (%)	12 (3.5)
Peripheral vascular disease, n (%)	8 (2.5)
Primary kidney disease	
Diabetic kidney disease, n (%)	70 (21)
Hypertensive kidney disease, n (%)	26 (8)
Glomerulonephritis, n (%)	47 (14)
Polycystic kidney disease, n (%)	21 (6)
Amyloidosis, n (%)	7 (2)
Tubulointerstitial nephritis, n (%)	5 (1.5)
Vesicoureteral reflux/pyelonephritis, n (%)	17 (5)
Urinary stone disease/obstructive uropathy, n (%)	21 (6)
Unknown, n (%)	124 (36.5)
Hepatitis B+, n (%)	7 (2)
Hepatitis C+, n (%)	7 (2)
Dialysis modality	
Hemodialysis, n (%)	320 (95)
Peritoneal dialysis, n (%)	18 (5)
Type of hemodialysis vascular access	
Arteriovenous fistula, n (%)	269 (84)
Central venous catheter, n (%)	51 (16)
Dialysis vintage (months) (mean ± SD)	61 ± 55
Time on the waiting list (months) (mean ± SD)	33.6 ± 23.9

(Continued)

**Table 1.** Demographic and Clinical Data of Patients on the Deceased Donor Kidney Transplant Waiting List (Continued)

Parameters	All Patients (n = 338)
History of blood transfusion	
Yes, n (%)	157 (46.5)
No, n (%)	181 (53.5)
Number of blood transfusion (mean ± SD)	1.76 ± 2.72
History of organ transplant	
Yes, n (%)	59 (17.5)
No, n (%)	279 (82.5)
History of pregnancy	
Yes, n (%)	102 (75.5)
No, n (%)	33 (24.5)
Number of pregnancy (mean ± SD)	2.95 ± 2.38
SD, standard deviation.	

between 2 groups in most clinical parameters such as body mass index, blood types, time on the waiting list, history of pregnancy, and mean number of pregnancy. The PRA-positive patient group had longer mean dialysis vintage than that of PRA-negative patient group (70.6 ± 59 vs. 56.3 ± 53.3, *P* = .027). The characteristics and comparisons of 2 groups are presented in Table 2.

On univariate analysis, age, gender (female), dialysis vintage, history of blood transfusion, number of blood transfusion, and history of organ transplant were found to be associated with PRA positivity. On multivariate analysis, gender (female) (odd ratio [OR]:4.094, 95% CI:2.275-7.368, *P* < .001), history of blood transfusion (OR:2.027, 95% CI:1.131-3.633, *P* = .018), and previous transplant (OR:16.894, 95% CI:7.212-39.578, *P* < .001) were found to be independent risk factors for PRA positivity. Risk factors for positive PRA antibody status are presented in Table 3.

**DISCUSSION**

In the present study, we described the clinical characteristics and immunologic profile of patients on the DDKT waiting list and investigated the effects of different sensitization events on HLA alloimmunization. We found that the PRA-positive patient group had lower age and a higher rate of women. Moreover, patients in the group also had longer dialysis vintage, higher history of blood transfusion, number of blood transfusions, and history of organ transplants compared to the PRA-negative patient group. Although age, female gender, longer dialysis vintage, history of blood transfusion, transplant, and higher number of blood transfusion were associated with anti-HLA antibody positivity, female sex, history of blood transfusion, and transplant were independent risk factors.

**Table 2.** Demographic and Clinical Data of Patients on the Deceased Donor Kidney Transplant Waiting List, According to Panel Reactive Antibody Status

Parameters	PRA-Positive Patient Group	PRA-Negative Patient Group	P
	(n = 117, 34.6%)	(n = 221, 65.4%)	
Age (years) (mean ± SD)	48.1 ± 15.2	55.1 ± 13.7	<b>&lt;.001</b>
Gender			
Male, n (%)	52 (44.4)	151 (68.3)	<b>&lt;.001</b>
Female, n (%)	65 (55.6)	70 (31.7)	
Body mass index (kg/m <sup>2</sup> ) (mean ± SD)	25 ± 5.1	25.4 ± 4.1	.537
Blood types			
A, n (%)	46 (39)	89 (40)	.994
B, n (%)	21 (18)	41 (18.5)	
AB, n (%)	8 (7)	15 (7)	
O, n (%)	42 (36)	76 (34.5)	
Dialysis vintage (months) (mean ± SD)	70.6 ± 59	56.3 ± 53.3	<b>.027</b>
Time on the waiting list (months) (mean ± SD)	35.3 ± 24.8	33 ± 23.4	.438
History of blood transfusion			
Yes, n (%)	80 (68)	77 (35)	<b>&lt;.001</b>
No, n (%)	37 (32)	144 (65)	
Number of blood transfusion (mean ± SD)	2.77 ± 2.82	1.22 ± 2.53	<b>&lt;.001</b>
History of organ transplant			
Yes, n (%)	48 (41)	11 (5)	<b>&lt;.001</b>
No, n (%)	69 (59)	210 (95)	
History of pregnancy			
Yes, n (%)	45 (69)	57 (81.5)	.136
No, n (%)	20 (31)	13 (18.5)	
Number of pregnancy (mean ± SD)	2.77 ± 2.40	3.17 ± 2.34	.283

SD, standard deviation.

Kidney transplant is the best therapeutic modality for the majority of patients with ESKD.<sup>8</sup> Pre-transplant sensitization remains one of the important issues in kidney transplants. The presence of anti-HLA antibodies is a known risk factor for antibody-mediated rejection, long-term graft failure, and death.<sup>9,10</sup> The prevalence of PRA positivity was 34.6% in our wait-listed patients. In the literature, PRA positivity rates are variable, ranging between 36% and 49.4%.<sup>11-13</sup> In studies reported from

**Table 3.** Logistic Regression Model. Risk Factors for Positive Panel Reactive Antibody in Patients

Parameters	Univariate	P	Multivariate	P
	OR (95% CI)		OR (95% CI)	
Age	0.968 (0.952-0.983)	<.001	-	NS
Gender (female)	2.696 (1.699-4.279)	<.001	4.094 (2.275-7.368)	<b>&lt;.001</b>
Dialysis vintage	1.004 (1-1.009)	.032	1.004 (0.999-1.009)	.096
Time on the waiting list	1.004 (0.995-1.013)	.408	-	-
History of blood transfusion	3.985 (2.448-6.487)	<.001	2.027 (1.131-3.633)	<b>.018</b>
Number of blood transfusion	1.242 (1.129-1.367)	<.001	-	-
History of organ transplant	14.242 (6.815-29.764)	<.001	16.894 (7.212-39.578)	<b>&lt;.001</b>
History of pregnancy	1.865 (0.817-4.254)	.139	-	-
Number of pregnancy	0.930 (0.803-1.077)	.334	-	-

OR, odds ratio.

2 different Turkish kidney transplant centers, the prevalence of PRA positivity was found to be 32.8% and 50.5%.<sup>14,15</sup> Currently, 40% of patients on the waiting list in the United States are sensitized with PRA level >1% and about 15% of patients are classed as highly sensitized with PRA level >80%.<sup>16</sup> Each sensitization event on class I and/or class II anti-HLA antibody positivity through blood transfusion, pregnancy, and history of transplant has been evaluated in different studies.<sup>11-15</sup> Hyun et al<sup>13</sup> reported that the PRA (class I and/or class II) positivity rates were significantly higher in patients with transfusion (33%), pregnancy (71.4%), or transplant (76.9%). Another study reported by Lopes et al<sup>11</sup> showed higher prevalence of class I and class II anti-HLA antibody positivity in patients with these sensitization events (class I: transfusion, 18.9%; pregnancy, 38.3%; transplant, 75% and class II: transfusion, 11%; pregnancy, 39.5%; transplant, 71.2%). Similar to previous studies, we found that anti-HLA class I, II, and I and II positivity rates were 55.5%, 70%, and 66% in patients sensitized only by transfusion, 60%, 50%, and 70% in patients sensitized by pregnancy, and 33%, 53%, and 37% in patients with previous transplant sensitization, respectively.

Akgul et al<sup>14</sup> were able to identify the relationship between PRA positivity and higher rate of women, pregnancy, increased number of pregnancy, and transfusion. They also showed that pregnancy has independent significant association with a positive

PRA.<sup>14</sup> These data were partially compatible with our study, and we did not determine pregnancy and number of pregnancy associations. This may be related to relatively small sample size and numerically inadequate pregnancy. We found that female sex, history of transfusion, and transplant were the strongest risk factors for positive PRA. Lopes et al<sup>11</sup> demonstrated that anti-HLA antibodies against class I or II were significantly higher in patients with transplantation than with transfusion. Similar to that, a study conducted by Sahin et al<sup>15</sup> showed that transplantation, followed by transfusion, had the highest immunization effect against HLA antigens.

The causal relationship between blood transfusion and clinically significant HLA antibody development has been demonstrated. Importantly, the sensitizing effects of transfusion may differentially affect female and male patients, further limiting their access to transplants. These studies emphasize the importance of avoiding unnecessary transfusions for patients on the transplant waiting list.<sup>17,18</sup>

According to the United States and United Kingdom registry reports, the majority of patients on the waiting list were aged 50-64 years old, and men comprised about 60%.<sup>16,19</sup> In studies reported from different countries, the average age of wait-listed patients was between 50 and 52 years, and men comprised 55%-60% of the patients.<sup>11,12,19,20</sup> Consistent with this data, the mean age of our study patients was 52 years, and 60% of the patients were male sex. In a study reported by Akgul et al<sup>14</sup>, mean age of the study patients was 48, and there was no relationship between age and PRA positivity. In our study, age was negatively correlated with PRA positivity. This can be explained by the strength of the immune response that declines with age and a higher rate of transplant history in these patients.

Dialysis vintage in our wait-listed patients was 61 months. Waiting time for dialysis was longer in the PRA-positive patient group (70 months) than in the PRA-negative patient group (56 months). Although longer waiting times for dialysis were associated with PRA positivity in univariate analysis ( $P = .032$ ), it was not reaching statistical significance in multivariate analysis ( $P = .096$ ). Sahin et al<sup>15</sup> reported that dialysis vintage on the wait-listed patients was 70 months, and longer dialysis vintage was associated with anti-HLA antibody positivity. It can be related to the long waiting time for dialysis, the greater possibility that they will encounter antigenic stimulants in blood products. In our country, patients on the DDKT waiting list have longer dialysis vintage. In the United States, 17% of wait-listed patients had been on dialysis for 6 or more years.<sup>16</sup> Longer waiting time for dialysis in our country is associated with the shortage of DDKT.

Mean waiting time of our wait-listed patients was 33.6 months. In a study reported from Türkiye, mean waiting time was approximately 5 years.<sup>15</sup> In Canada, mean waiting time after initial wait-list activation was 2.1 years.<sup>3</sup> In the United States

Kidney Data System 2019 annual report, only a quarter of wait-listed patients received a DDKT within 5 years, and this proportion varies dramatically by donation service area, from 15.5% to 67.8%.<sup>16</sup> Insufficient supply of donor organs leads to extended transplant waiting times in our country.

In 2014, a new kidney allocation system was implemented in the United States for prioritizing highly sensitized patients. Patients with a calculated PRA of 99%-100% represented approximately 8% of the waiting list; historically, they received only 2.5% of kidney transplants, but this was increased to 14.8% in the first 6 months after the kidney allocation system implementation.<sup>21</sup> Similarly, in the Eurotransplant program, highly sensitized patients (calculated PRA equivalent of >85%) received increased donor access through the Acceptable Mismatch Program.<sup>22</sup>

The PRA status has not been considered a part of the standard criteria in the present Turkish deceased donor kidney allocation system. Sensitization is a serious problem to overcome for patients on the DDKT waiting list in our country, and therefore, organ allocation system updates that account for sensitized patients are mandatory to compensate for inequities in access to transplant. We also need new strategies to increase deceased donor organ pool and donation rates due to the low rates of DDKT in Türkiye. Education, community-based workshops, and awareness campaigns about kidney transplant will increase the number of organ donations. Various social, cultural, religious, and traditional concerns about organ donation may hamper its acceptability and cause a lack of willingness to donate. Therefore, these issues need to be addressed and concerns should be alleviated. Considering the global shortage of donor organs, use of expanded criteria donors, donation after cardiac death organs, hepatitis B-infected or hepatitis C-infected donors, old-to-old kidney match programs, ABO-incompatible kidney transplant, and kidney paired donation matches are some of the approaches applied in the world and may help to further expand the kidney donor pool and may improve the problem of kidney transplant in sensitized patients.

## CONCLUSION

Sensitization is an important problem in our wait-listed patients. History of organ transplant had the highest immunization effect against HLA antigens, followed by blood transfusion. National multicenter data are needed to reflect the sensitization status and risk factors in patients on the waiting list in our country. Organ allocation system in our country must be updated to account for sensitized patients, and new strategies should be developed to expand donor pool and donation rates.

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