DOI: 10.14744/cm.2023.82084
Comprehensive Medicine 2023;15(2):120-124

RESEARCH ARTICLE

Immunological Risk Monitoring in Patients with High Immunologic Risk and Its Effects on Clinical Outcomes

Zuhal Atan Uçar[1], Ayşe Sinangil[2], Mustafa Emre Özçilsal[3], Yener Koç[4], Alaattin Yıldız[5], Emin Barış Akın[6]

[1]Division of Nephrology, Department of Internal Medicine, Liv Hospital Vadistanbul, İstanbul, Türkiye
[2]Division of Nephrology, Department of Internal Medicine, T.C. Demiroğlu Bilim University Faculty of Medicine, İstanbul, Türkiye
[3]Department of Internal Medicine, T.C. Demiroğlu Bilim University Faculty of Medicine, İstanbul, Türkiye
[4]Division of Nephrology, Department of Internal Medicine, Cumhuriyet University Faculty of Medicine, Sivas, Türkiye
[5]Division of Nephrology, Department of Internal Medicine, İstanbul University Faculty of Medicine, Istanbul, Türkiye
[6]Department of General Surgery, Unit of Renal Transplantation, Demiroğlu Bilim University, Florence Nightingale Hospital, İstanbul, Türkiye

ABSTRACT

Objective: In this study, it was aimed to follow up the immunological risk of patients with high immunological risk and to determine the effect of desensitization treatment in these patients.

Materials and Methods: Living donor transplantation patients with panel reactive antibody (PRA), donor specific antibody (DSA), and/or single antigen bead test positivity and retransplantation patients were included in the study. PRA and/or DSA levels of pre-transplant and post-transplant period were evaluated in all patients. We compared follow-up of immunological data and clinical outcomes of patients who had desensitization (Group 1) versus who did not (Group 2).

Results: Totally 117 patients were included in this study. Thirty-four patients had desensitization treatment. There was no statistically difference between the groups based on age, hepatitis serology, history of blood transfusion, pregnancy, history of dialysis, and acute rejection episodes ($p>0.05$). Female gender was higher in Group 1 patients ($p<0.05$). HLA-MM, PRA Class 2, DSA Class 2 levels were higher in Group 1 in pre-transplant period ($p<0.05$). During the follow-up period, it was determined that the patients in Group 1 had significantly lower PRA Class 2 values at the 1st month and DSA Class 2 values at the 1st and 3rd months compared to the pre-transplant period ($p<0.05$).

Conclusion: Immunological risk decreases with desensitization therapy in the patients with high immunological risk. This decrease is more distinctive in the first 3 months of post-transplant period in which acute rejection attacks are more common.

Keywords: Desensitization treatment, DSA, immunological risk, PRA, single BEAD

How to cite this article: Atan Uçar Z, Sinangil A, Özçilsal ME, Koç Y, Yıldız A, Akın EB. Immunological Risk Monitoring in Patients with High Immunologic Risk and Its Effects on Clinical Outcomes. CM 2023;15(2):120-124

INTRODUCTION

Renal transplantation (RT) is the best treatment for the end stage renal disease. Renal transplant recipients (RTRs) have an improved survival and quality of life compared to hemodialysis patients. Exposure to non-selfhuman leukocyte antigens (HLA) such as pregnancy, blood transfusion, and previous transplantation, can result in the formation of anti-HLA antibodies. This condition is called sensitization. In sensitized RTRs candidates, the risk of graft loss due to rejection may be high after RT. Therefore, the waiting times of these patients are usually long.[1] Various desensitization protocols are applied to prevent rejection and increase renal graft survival in high sensitized RT candidates.[2,3] In this study, immunological follow-up of living donor transplantation patients with high immunological risk and to determine the effect of this treatment in patients who were treated with desensitization were aimed.

Address for Correspondence: Zuhal Atan Uçar, Division of Nephrology, Department of Internal Medicine, Liv Hospital Vadistanbul, İstanbul, Türkiye
E-mail: zuhal1214@gmail.com **ORCID ID:** 0000-0002-5761-6979

Received date: 04.08.2022
Revised date: 12.01.2023
Accepted date: 23.02.2023
Online date: 25.03.2023

MATERIALS and METHODS

The files of patients who were transplanted at Demiroğlu Bilim University Şişli Florence Nightingale Hospital Kidney Transplant Unit between 2010 and 2018 were evaluated retrospectively. The patients who underwent RT with immunological risk were included in the study. Living donor transplantation patients with panel reactive antibody (PRA), donor specific antibody (DSA), and/or single antigen bead test (single BEAD) positivity and retransplantation patients were accepted as high immunological risk patients. We excluded the patients with deceased donor. Sociodemographic data's such as age, sex, body mass index (BMI), post-transplant follow-up time, hepatitis serology, presence of history blood transfusions and pregnancy, history of dialysis, immunological status such as HLA-miss match (HLA-MM), PRA, DSA and single BEAD levels, immunosuppressive medications, and episodes of acute rejections were recorded. Patients with immunological risk were divided into those who underwent desensitization (Group 1) and those who did not (Group 2). Single BEAD, PRA, and/or DSA levels of pre-transplant and post-transplant period (1st week, 1st, 3rd, 6th, 9th months, and 1st, 2nd years) were evaluated in all patients. The follow-up of immunological data and clinical outcomes of the groups were compared.

In our center, desensitization was applied for sensitized patients in any of the following conditions: (1) Presence of specific PRA Class 1 and/or Class 2 identification, and/or single BEAD; single antibody >MFI 3000 and (2) presence of specific PRA and/or single BEAD multiple antibodies >MFI 2000. The protocol we used to desensitize patients involved only rituximab or a combination of plasmapheresis, intravenous immunoglobulin (IVIG), and rituximab.

Rituximab was administered doses at 375 mg/m^2 at 7th days before transplantation. Plasmapheresis administered at on day 3 and day 1. Total dose of 1–2 g/kg IVIG was given in divided doses. Induction therapy was given to all patients. Immunosuppression comprised rabbit anti-thymocyte globulin according to induction therapy. Methylprednisolone 1000 mg was given intraoperatively followed by sequential tapering to daily oral prednisone 5 mg on the 12th week. All patients used the same immunosuppressive therapy consisting of prednisolone (5 mg/day), tacrolimus adjusted according to blood levels and mycophenolate mofetil (1000 mg bid), or mycophenolate sodium (720 mg bid).

Statistical Analysis

Data were analyzed with SPSS software version 17.0 for Windows (SPSS, Inc., Chicago, Ill, United States). All values are expressed as the mean±standard error of the mean. One-way ANOVA test was used for analyzing biochemical and BMD parameters among groups. Tukey's *post hoc* test was applied to parameters that were determined to present significant differences. Pearson and spearman correlation tests were performed for correlation analyses. Differences were considered statistically significant for $p<0.05$.

RESULTS

Totally 117 patients (45 female/72 male) were included in this study. The mean patient age was 43.9±12.5 years. Thirty-four patients (25 female, mean age 43.7±12.5 years, mean follow-up time 24.3±13.4 months, mean BMI 26.8±6.6 kg/m^2) had desensitization treatment (Group 1). Eleven patients received rituximab for desensitization therapy, rituximab plus plasmapheresis for 19 patients and Rituximab plus plasmapheresis and IVIG for the remaining four patients. Eighty-three patients (20 female, mean age 43.8±12 years, mean follow-up time 18.4±10.2 months, mean BMI 26.8±6.6kg/m^2) had not have desensitization (Group 2). There was no statistical difference between the groups based on age, history of blood transfusion, pregnancy, history of dialysis, and acute rejection episodes ($p<0.05$ for all parameters). Female gender was higher in Group 1 patients ($p<0.001$), and HLA-MM, PRA Class 2, DSA Class 2 levels were higher in Group 1 in pre-transplant period (p:0.02, 0.003 and 0.021, respectively). Demographic data and pre-transplant mean PRA and DSA levels were shown in Table 1. The positivity for DSA/PRA Class 1 was 41.2% and for DSA/PRA Class 2 was 76.4% in Group 1 at pre-transplant period. These values were 5.8% and 32.3% at 7th days, 14.7% and 44.1% 3rd months, only 2.9% and 29.4% at 6th months in post-transplant period, respectively. While the positivity for DSA/PRA Class 1 was 13.1% and for Class 2 was 52.5% in Group 2 in pre-transplant period, these values were 7.1% and 64.3% at 7th days, 7.3% and 34.1% at 3rd months, 6.7% and 53.3% at 6th months in post-transplant period, respectively. DSA/PRA Class 1 and 2 positive patient rates were shown in Table 2 and MFI values of DSA/PRA positive patients were shown in the Figure 1.

During the follow-up period, it was determined that the patients in Group 1 had significantly lower PRA Class 2 values at the 1st month and DSA Class 2 values at the 1st and 3rd months compared to the pre-transplant period (p:0.047, 0.015 and 0.024, respectively).

DISCUSSION

In this study, we evaluated anti-HLA antibody levels in the first 2 years after transplantation in 117 immunologically high-risk patients. We found that anti-HLA antibodies decreased in desensitized patients. PRA and DSA Class II levels

Table 1. Demographic dates and immunological data of the patients

Parameters	Group 1 n=34	Group 2 n=83	p
Age (y)	43.97±12.5	43.8±12	0.05
Sex			
Female	25	20	0.001
Male	9	63	
BMI (kg/m^2)	26.8±6.6	26.8±6.6	0.05
Follow-up time (mo)	24.3±13.4	18.4±10.3	0.05
History of pregnancy (n)	24	20	0.05
HLA-MM	2.9±1.6	2.22±1.14	0.02
PRA-class 1 (MFI) pre-transplant	8562±11523	3558±5100	0.05
PRA-class2 (MFI)	7945±6683	2063±2323	0.003
DSA-class 1 (MFI)	3374±4141	1495±1320	0.05
DSA-class 2 (MFI)	4198±5233	1718±2656	0.021

BMI: Body mass index; HLA-MM: Human leukocyte antigens-miss match; PRA: Panel reactive antibody; MFI: Mean fluorescence intensity; DSA: Donor specific antibody

were significantly lower in the 1st month, and DSA Class II levels were significantly lower in the 3rd month.

Anti-HLA antibodies play a key role in allograft rejection. Approximately 15% of wait-listed candidates have some degree of sensitization.[4] In the highly sensitized recipient, desensitization to lower the levels of antibodies may be the only feasible option for transplantation. Because the rates of acute antibody-mediated rejection (AMR) and allograft loss are higher.

Table 2. DSA/PRA Class 1 and 2 positive patient rates

	Group 1 (n=34)		Group 2 (n=83)	
	Class 1 %	Class 2 %	Class 1 %	Class 2 %
Pre-transplant period	41.2	76.4	13.5	52.5
7th day	5.8	32.3	7.1	64.3
1st month	11.7	47	9.6	46.9
3rd month	14.7	44.1	7.3	34.9
6th month	2.9	29.4	6.7	53.3
9th month	8.8	23.5	9.6	34.9
1st year	8.8	41.1	19.2	33.7

DSA/PRA: Donor specific antibody/Panel reactive antibody

[5] Most series have reported an AMR incidence of 30–40% in the 1st months of post-transplant period with increasing DSA levels at baseline.[6-8] Furthermore, in one study, the incidence of AMR was shown 36.4% in patients with DSA between 3001 and 6000 MFI and 51.3% for >6000 MFI.[9] We detected acute rejection in two patients in both of our study groups, rates were lower than the literature and we did not find any difference in the development of acute rejection in the two groups.

In addition, it was determined that the risk of graft failure was increased by 3.8 in patients with an HLA-DSA MFI value above 3000 compared to patients with an HLA-DSA MFI value below 3000.[10] Increased antibody levels increase the risk of graft failure. Based on the studies, we apply desensitization in the presence of specific PRA Class 1 and/or Class

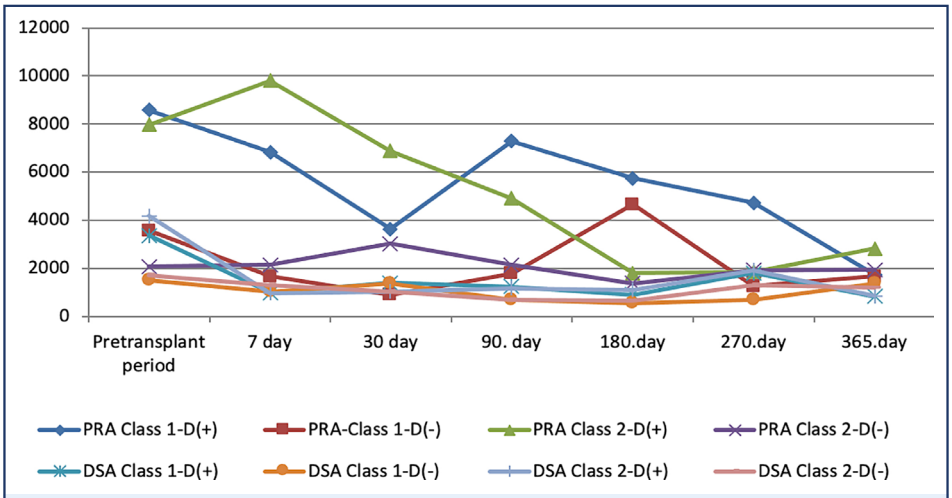

Figure 1. PRA and DSA findings

PRA: Panel reactive antibody; DSA: Donor specific antibody

2 and/or when MFI is over 3000 in a single antibody with single BEAD or in the presence of multiple antibodies with MFI greater than 2000 with specific PRA and/or Single BEAD. In our desensitized group (Group 1), PRA Class 2 and DSA Class 2 levels and HLA-MM ratios were significantly higher in the pre-transplant period. Therefore, desensitization was necessary in these patients.

Alloantibodies that develop after pregnancy are among the factors limiting kidney transplantation from a living donor in female gender. These antibodies also reduce the possibility of kidney transplant from the husband.[11] Anti-HLA antibodies may occur with a single pregnancy or may not be seen in female gender with multiple pregnancies.[12] In one study, Class I anti-HLA antibodies were detected in 18.2% of those who had their first pregnancy, 27.3% of those who had a second pregnancy, and 50% of those who had 3 or more pregnancies. Anti-HLA antibody production seems to increase with the number of pregnancies.[13] In our study, although there was no difference between the groups for pregnancy, we found the female gender ratio to be significantly higher in those who underwent desensitization treatment. This difference may be related to the number of pregnancies of female recipients.

There is no standardized protocol for desensitization. Plasmapheresis or immune-adsorpsion columns are used to decrease the level of DSA and B-cell eliminating and/or modulating agents such as monoclonal antibodies or IVIG preparations are used to prevent increasing post-transplant antibody.[2,3,14-16] We used only rituximab or a combination of plasmapheresis, IVIG, and rituximab. In Group I (desensitization group), 11 patients received rituximab for desensitization therapy, rituximab plus plasmapheresis for 19 patients, and Rituximab plus plasmapheresis and IVIG for the remaining four patients.

Although desensitization protocols permit acceptable graft survival in patients with preexisting DSA, there are few studies regarding the efficacy and long-term durability of DSA removal. In a study presenting 3-year results of 29 highly sensitized patients, there was a 46% reduction in DSA MFI at 1-month post-transplant, and this reduction was sustained over 3-year follow-up for both Class I and II DSAs. Three-year patient and graft survival were 95% and 90%, respectively.[17] In other study, it was found that DSA levels rebounded as early as post-transplant 1–4 weeks post-transplant in two of six recipients who treated with IVIG plus placebo, but DSA levels did not increase in the first 12 months in six patients received IVIG and rituximab.[18]

In our study, PRA Class 2 level which was high in the pre-transplant period in Group 1 decreased significantly at the post-transplant 1st month. Same way, DSA Class 2 values were significantly lower at the 1st and 3rd months compared to those of the pre-transplant period. Although there was a decrease in the mean PRA and DSA Class 1 at 1 month, this decrease was not statistically. The limitation of this study was the small sample size and retrospective design of the study. Further studies with large series are needed to confirm the durability of DSA removal after desensitization.

CONCLUSION

This retrospective study of DSA/PRA monitoring in desensitized living donor kidney transplant recipients shows that immunological risk decreases with desensitization treatment in patients with high immunological risk. This decrease continues more clearly in the first 3 months of post-transplantation period where acute rejection attacks are more common.

Disclosures

Ethics Committee Approval: The study was approved by the Demiroğlu Bilim University Clinical Research Ethics Committee (No: 2022-12-01, Date: 21/06/2022).

Informed Consent: Written informed consent was obtained from all patients.

Peer-review: Externally peer reviewed.

Authorship Contributions: Concept: Z.A.U., A.S.; Design: Z.A.U., A.S. Supervision: A.S., M.E.Ö.; Funding: A.Y., E.B.A.; Materials: E.B.A.; Data Collection or Processing: M.E.Ö., Y.K.; Analysis or Interpretation: Z.A.U., M.E.Ö.; Literature Search: Y.K., A.Y.; Writing: Z.A.U., M.E.Ö.; Critical review: A.Y., E.B.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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