

Assessment of donor specific IgG Anti-HLA subclasses before and after kidney transplantation in Upper Egypt: A Single Center Study

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Abstract

Donor specific antibodies (DSAs) are known as the leading cause of antibody mediated rejection (AMR), graft loss in kidney transplant (KT) recipients. DSAs characteristics, as immunoglobulin (Ig) classes, subclasses, and strength, are important to assess the immunological risk, early prediction of AMR, and therefor proper management. This longitudinal, case control study included 32 KT recipients at Assiut University Urology Hospital and 10 age and sex matched normal subjects as the control group. Total IgG, its subclasses and anti-human leukocyte antigen (anti-HLA) panel reactive antibody (PRA) were detected pre-transplantation (pre-TX), at 6-12- and 24-36-months post-TX. Rejection occurred in 4 recipients, 3 of them had high total IgG, IgG1 and/or IgG3. IgG2 and IgG4 were normal in all recipients. There were preformed anti-DSAs antibodies in 3/32 recipients (9.4%). Of these, two recipients became negative with no rejection occurred. The third recipient had high post-TX mean fluorescence intensity (MFI) and AMR occurred. The pre-TX PRA was negative in 29/32 recipients (90.6%). The PRA was negative in 8/29 recipients (27.6%) and the remaining 21/29 recipients (72.4%) developed de novo DSAs post-TX (MFI <3000->10000). Rejection occurred with both low and high MFI. In 11 recipients, anti-HLA class I and II were not different between pre-TX, 3-6- and 24-36 months post-TX with no rejection occurred. The frequency and median levels of total IgG, IgG1 and IgG3 were increased in all recipients 24-36 months post-TX when compared with their levels pre-TX and 6-12 months post-TX in the 11 recipients and with the control group. The graft survival time significantly decreased in recipients with positive post-TX class I PRA. In conclusion, preformed DSAs may persist post-TX or turn negative. De novo DSAs developed post-TX even in non-sensitized recipients. Serum total IgG, IgG1 and IgG3 frequency increase 2-3 years post-TX.

Keywords kidney transplantation, DSAs, PRA, MFI, IgG subclasses.

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Introduction

Epidemiological data from the past decade suggest that the global burden of patients with renal failure who receive renal replacement therapy exceeds 1.4 million and that figure is growing by about 8% a year.^{1,2} In Egypt, Mansoura University, Cairo University among other universities, military, general and private hospitals (39 centers) entered the field of kidney transplantation (KT). By the end of 2018, the overall experience of KT reached 19,079 cases, and during 2019, 1,338 KT were performed. The 10-year patient and graft survival rates of the Mansoura experience were 77.8% and 65.5% respectively.³

KT is the gold standard treatment for many patients with end stage renal disease (ESRD) and it is associated with prolonged survival, improved quality of life, reduced morbidity, and lower health care costs compared with dialysis. In addition to the medical and surgical challenges in KT, the major biological barrier is immunological, and this barrier may lead to graft rejection and loss.^{1,4}

Antibody mediated rejection is defined as allograft rejection caused by recipient's antibodies directed against donor human leukocyte antigen (HLA) molecules (Class I and class II), called donor specific antibodies (DSAs) and blood group antigens.⁵ Pre-existing antibodies or the development of de novo antibodies post-transplantation (post-TX) has become a biomarker for antibody mediated rejection (AMR) graft loss.⁵ HLA antibodies are risk factors for hyperacute, acute, and chronic allograft rejections.⁷ However, there are also "benign" DSAs that may not be clinically relevant, because they are not associated with antibody mediated rejection or graft failure.⁸ Recently, studies demonstrated that AMR is not usually associated with long term graft failure.^{9,10}

The destructive power of DSAs vary depending on the level of immunoglobulin classes, subclasses, complement binding capacity, strength, target antigen, and the type of organ transplanted.⁸ In kidney transplant, DSAs interact with the endothelial cells of the kidneys, mainly with HLA antigens, resulting in

activation of endothelial cells, activation of classical pathway of the complement, recruitment of inflammatory cells into glomerular and peri-tubular capillaries of the graft leading to graft dysfunction and AMR. DSAs pathogenicity modulated according to IgG subclasses.¹¹ The most abundant subclass in serum is IgG1 and considered the mirror of total IgG level. IgG1 and IgG3 are the most efficient classical complement cascade activators, but IgG3 is more efficient. Although IgG2 may bind and activate complement weakly (in high antibody titer or high antigen density), the IgG4 cannot at all, but both bind Fc receptor and recruit the effector cells. The biomarker of advanced stage of rejection is IgG4 because it is the most terminal subclass produced due to chronic antigen stimulation.^{12,13} Several researchers investigated the DSA IgG subclasses profile and if they predict graft dysfunction, AMR, and graft loss or not. Some of them reported that presence of IgG3 is associated with poor graft outcome.^{14,15} In the past decades, the short-term graft survival in KT recipients was improved, with no noticeable improvement in long-term graft survival.^{16,17}

The present work aimed to analyze the distribution of anti-HLA class I and class II PRA, IgG, and its subclasses in the pre and post KT period and their association with acute AMR and graft survival. Also, to determine the potential benefit of implementing IgG subclasses assessment to the current transplant medicine practice.

Subjects and Methods

Study setting and design

This longitudinal, case control study included 32 patients who underwent KT after they were diagnosed as they had ESRD at Assiut University Urology Hospital during the period from September 2018 to September 2021. In addition, 10 age and sex matched apparently healthy subjects were included in the study as a control group.

The study protocol was ethically reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Assiut University,

Assiut, Egypt (approval dated: November 2016). The importance of the study was explained to all participants and informed consents were taken from them before enrollment in this study.

Selection criteria

Any patient with age between 18 and 70 years who received renal transplant secondary to ESRD, with a duration of more than 6 months' post-transplant, was recruited in the study. Exclusion criteria included patients with extreme age (<18 or >70 years), duration of <6 months' post-transplant or patient's refusal.

Participants

A total of 32 patients with KT were enrolled in the study. All transplants required a negative flowcytometric crossmatch for IgG T cell and B cell, and ABO blood group compatibility. Serum samples were collected from the 32 recipients within 24-36 months post-TX. Out of the 32 study recipients, serum samples could be collected from 11 patients pre-TX, within 6-12 months and 24-36 months post-TX to evaluate the effect of timing on studied tests.

In this study, recipients received induction immunosuppressive therapy. These included Basiliximab (20 milligrams were given at day zero and day+4 post-TX), Methylprednisolone (250 milligrams given at day -1 pre-TX, 250 mg intra-operative and another 250 mg at day zero then tapered gradually till 5 milligrams daily within 1-2 months. Other maintenance immunosuppressive included Mycophenolate mofetil (was given up to 2 gram/day) and Tacrolimus (given 1 milligram/kilogram/day) and adjusted according to its trough level.

Blood samples:

Venous blood samples (10 ml) were collected from each study participant into plain tube, left to clot for 30 min at 37°C and centrifuged at approximately 1000 xg, for ten minutes. Sera were separated, divided into aliquots and kept frozen in - 20°C until use.

Methodology

1. Pretransplant anti-HLA class I (loci; A, B and C) and II (loci; DR, DP and DQ) PRA were

collected from recipient's medical records. These antibodies were detected in patients' sera using a commercial solid phase immunoassay test kit (Lot No. 018, LABScreen™ PRA Class I, and LABScreen™ PRA Class II,) supplied by ONELAMBDA, using an automated multiplex immunoassay system which detects fluorescent emission from fluorescent dye-conjugates (LABScan3D, Luminex® FLEXMAP 3D®, ONELAMBDA, USA), according to the manufacturer's instructions.

2. Detection of total IgG, IgG1, IgG2, IgG3 and IgG4 in pretransplant serum samples of the eleven recipients. These antibodies were determined using commercial kits: N Antiserum to human IgG (Lot No. 153076M, NAS/IgG), N AS IgG1 (Lot No. 090197, NAS/IgG1), N AS IgG2 (Lot No. 090208E, N AS/IgG2), N Latex IgG3 (Lot No. 086199, N IgG3), and N Latex IgG4 (Lot No. 086002, N IgG4), supplied by Siemens Healthineers, using a fully automated immunonephelometric analyzer (BN ProSpec® System, Siemens Healthineers, Germany), according to the manufacturer's instructions.

3. Posttransplant investigations for recipients and the control group:

a. Urea and creatinine tests were performed using an automatic chemistry analyzer (ADVIA 1800 chemistry Auto-Analyzers, Siemens Healthineers, Germany).

b. Detection of IgG antibodies to HLA class I and class II PRA in patients' serum samples using the LABScreen™, as mentioned above.

c. Detection of total serum IgG, IgG1, IgG2, IgG3 and IgG4, as mentioned above.

Statistical analysis

Data analysis was performed using statistical package for the social science (IBM-SPSS) version 26.0 software. Categorical data were presented in form of frequencies and percentages. All numerical variables were tested before evaluation to determine the normality of data by Shapiro–Wilk test and median (range) was used to express data. After testing data normality, non-parametric tests were performed. Mann Whitney U test was used to compare difference between two

groups of dependent continuous variables, Friedman test was used to compare median IgG and its subclasses over time (pre-TX, 6-12- and 24-36 months post-TX). Chi square and Fisher Exact tests were used to compare proportion between two groups. Related-Samples Cochran's Q Test was used to compare proportions over time: pre-TX, 6-12- and 24-36 months post-TX. Survival analysis. Log rank test was used, and graft survival time was calculated using Kaplan–Meier curve. The level of significance was considered at p value < 0.05 .

Results

Demographic data of the recipients and control groups

There was no difference between the mean age, sex, and blood urea nitrogen levels of the recipients and control groups (Table 1). However, serum creatinine was significantly higher in recipients (median level of 97.5 $\mu\text{mol/L}$, mean \pm SD 135.5 \pm 106.89 $\mu\text{mol/L}$) when compared with the control group ($p < 0.001$).

Table 1. Demographic features of KT recipients and the control group.

Variable	KT recipients (n=32)	Control group (n=10)	p -value*
Age (years)			
Mean \pm SD.	29.72 \pm 8.22	38 \pm 16.53	
Median	28.0	38.5	NS
range	20.0-58.0	15.0-70.0	
Gender			
Male	29 (90.6%)	9 (90.0%)	NS
Female	3 (9.4%)	1 (10.0%)	
Urea (mmol/L)			
Mean \pm SD	7.01 \pm 3.17	5.62 \pm 1.74	
Median	6.70	5.25	NS
range	(2.2-16.9)	2.9-8.2	
Creatinine ($\mu\text{mol/L}$)			
Mean \pm SD	135.50 \pm 106.89	72.90 \pm 13.94	
Median	97.50	71.00	0.001
range	(37.0-613.0)	(44.2-96.0)	

Data expressed as median (range) or frequency (%), mean (SD). $P > 0.05$ is not significant (NS). KT: kidney transplant.

Class I and class II PRA mean fluorescence intensity (MFI) in recipients (24-36 months post-TX) and control group

The study groups were classified into MFI < 3000 and > 3000 . Positive class I alone with MFI > 3000 was detected in 1 recipient, positive class II alone with MFI > 3000 was detected in 4

recipients and positive both class I & II with MFI > 3000 was detected in 1 recipient. No difference was detected between recipients and the control group regarding class I (loci; A, B and C) and Class II (loci; DR, DP and DQ) PRA MFI (Tables 2 and 3).

Table 2. Class I PRA MFI in recipients (24-36 months post-TX) and the control group.

Variables	Recipients (24-36 months post-TX) (n=32)	Control group (n=10)	p-value*
Class I A.			
MFI <3000			
Negative	19 (59.4%)	7 (70.0%)	NS
Positive	13 (40.6%)	3 (30.0%)	
MFI >3000			
Negative	30 (93.8%)	10 (100.0%)	NS
Positive	2 (6.3%)	0 (0.0%)	
Class I B			
MFI <3000			
Negative	21 (65.6%)	6 (60.0%)	NS
Positive	11 (34.4%)	4 (40.0%)	
MFI >3000			
Negative	31 (96.9%)	10 (100.0%)	NS
Positive	1 (3.1%)	0 (0.0%)	
Class I C			
MFI <3000			
Negative	30 (93.8%)	10 (100.0%)	NS
Positive	2 (6.3%)	0 (0.0%)	
MFI >3000			
Negative	32 (100.0%)	10 (100.0%)	
Positive	0 (0.0%)	0 (0.0%)	
Class I PRA			
MFI >3000			
Negative	30 (93.8%)	10 (100.0%)	NS
Positive	2 (6.2%)	0 (0.0%)	

Data expressed as frequency (%). $P > 0.05$ is not significant (NS).

* Fisher Exact test was used to compare proportion between recipients and the control group. TX: Transplantation

Table 3. Class II PRA MFI in recipients (24-36 months post-TX) and the control group.

Variables	Recipients (24-36 months post-TX) (n=32)	Control group (n=10)	p-value*
Class II DR			
MFI <3000			
Negative	26 (81.3%)	10 (100.0%)	NS
Positive	6 (18.8%)	0 (0.0%)	
MFI >3000			
Negative	26 (81.3%)	10 (100.0%)	NS
Positive	6 (18.8%)	0 (0.0%)	

Table 3. Continued.

Variables	Recipients (24-36 months post-TX) (n=32)	Control group (n=10)	p-value*
Class II DP			
MFI <3000			
Negative	30 (93.8%)	10 (100.0%)	NS
Positive	2 (6.3%)	0 (0.0%)	
MFI >3000			
Negative	31 (96.9%)	10 (100.0%)	NS
Positive	1 (3.1%)	0 (0.0%)	
Class II DQ			
MFI <3000			
Negative	24 (75.0%)	10 (100.0%)	NS
Positive	8 (25.0%)	0 (0.0%)	
MFI >3000			
Negative	26 (81.3%)	10 (100.0%)	NS
Positive	6 (18.8%)	0 (0.0%)	
Class II PRA			
MFI >3000			
Negative	26 (81.2%)	10 (100.0%)	NS
Positive	6 (18.8%)	0 (0.0%)	

Data expressed as frequency (%). $p > 0.05$ is not significant (NS). * Fisher Exact test was used to compare proportion between recipients and control group.

Class I and class II PRA MFI in the 11 recipients at different periods (pre-TX, 6-12 months, and 24-36 months post-TX)

There was no difference in class I and class II PRA MFI between the different periods (Tables 4 and 5).

Table 4. Class I PRA MFI in the 11 recipients at different periods (pre-TX, after 6-12 months and 24-36 months post-TX).

Variables	Recipients Pre-TX	Recipients (6-12 months post-TX)	Recipients (24-36 months post-TX)	p-value*
Class I A				
MFI <3000				
Negative	11 (100.0%)	8 (72.7%)	6 (54.5%)	NS
Positive	0 (0.0%)	3 (17.3%)	5 (45.5%)	
MFI >3000				
Negative	11 (100.0%)	11 (100.0%)	10 (90.9%)	NS
Positive	0 (0.0%)	0 (0.0%)	1 (9.1%)	

Table 4. Continued.

Variables	Recipients Pre-TX	Recipients (6-12 months post-TX)	Recipients (24-36 months post-TX)	<i>p</i> -value*
Class I B				
MFI <3000				
Negative	11 (100.0%)	8 (72.7%)	9 (81.8%)	NS
Positive	0 (0.0%)	3 (17.3%)	2 (18.2%)	
MFI >3000				
Negative	11 (100.0%)	11 (100.0%)	11 (100.0%)	---
Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Class I C				
MFI <3000				
Negative	11 (100.0%)	10 (90.9%)	11 (100.0%)	NS
Positive	0 (0.0%)	1 (9.1%)	0 (0.0%)	
MFI >3000				
Negative	11 (100.0%)	11 (100.0%)	11 (100.0%)	----
Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Class I PRA				
MFI > 3000				
Negative	11 (100.0%)	11 (100.0%)	10 (90.9%)	NS
Positive	0 (0.0%)	0 (0.0%)	1 (9.1%)	

Data expressed as frequency (%). $P > 0.05$ is not significant (NS). * Related-Samples Cochran's Q Test compare proportions over time.

Table 5. Class II PRA MFI in the 11 recipients at different periods (pre-TX, after 6-12 months and 24-36 months post-TX).

Variables	Recipients pre-TX	Recipients (6-12 months post-TX)	Recipients (24-36 months Post-TX)	<i>p</i> -value*
Class II DR				
MFI <3000				
Negative	9 (81.8%)	10 (90.9%)	10 (90.9%)	NS
Positive	2 (18.2%)	1 (9.1%)	1 (9.1%)	
MFI >3000				
Negative	11 (100.0%)	11 (100.0%)	10 (90.9%)	NS
Positive	0 (0.0%)	0 (0.0%)	1 (9.1%)	
Class II DP				
MFI <3000				
Negative	11 (100.0%)	11 (100.0%)	11 (100.0%)	-----
Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	
MFI >3000				
Negative	11 (100.0%)	11 (100.0%)	11 (100.0%)	-----
Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	

Table 5. Continued.

Variables	Recipients pre-TX	Recipients (6-12 months post-TX)	Recipients (24-36 months Post-TX)	p-value*
Class II DQ				
MFI <3000				
Negative	10 (90.9%)	10 (90.9%)	9 (81.8%)	NS
Positive	1 (9.1%)	1 (9.1%)	2 (18.2%)	
MFI >3000				
Negative	11 (100.0%)	11 (100.0%)	10 (90.9%)	NS
Positive	0 (0.0%)	0 (0.0%)	1 (9.1%)	
Class II PRA				
MFI > 3000				
Negative	11 (100.0%)	11 (100.0%)	10 (90.9%)	NS
Positive	0 (0.0%)	0 (0.0%)	1 (9.1%)	

Data expressed as frequency (%). $P > 0.05$ is not significant (NS). * Related-Samples Cochran's Q Test compare proportions over time.

Comparison of total IgG and its subclasses in recipients at 24-36 months post-TX and in the control group

The frequency and median level of total IgG showed no difference between recipients and the control group (Table 6). There was a statistically significant increase in the frequency and median levels of IgG1 in recipients when compared with the control group ($p < 0.001$). The frequency of IgG3 was significantly increased in recipients when compared with the control

group. However, there was no difference in the IgG3 median level between the recipients and the control group. Levels of both IgG1 and IgG3 were high in 21/32 recipients (65.6%), but within normal level in 2/32 recipients (6.3%). The level of IgG1 was high in 8/32 recipients (25%), and IgG3 high in 1/32 recipient (3.1%). Levels of both IgG2 and IgG4 were normal in all recipients with no difference between recipients and the control group (Table 6).

Table 6. Total IgG and its subclasses in recipients (24-36 months post-TX) and the control group.

Variables	Recipients (24-36 months post-TX) (n=32)	Control group (n=10)	p-value*
Total IgG (g/L)			
High	18 (56.2%)	5 (50.0%)	NS
Normal	14 (43.8%)	5 (50.0%)	
Median total IgG level (range)	16.60 (6.790-27.100)	14.55 (9.65-20.50)	NS
IgG1 (g/L)			
High	29 (90.6%)	1 (10.0%)	<0.001
Normal	3 (9.4%)	9 (90.0%)	
Median IgG1 level (range)	13.60 (8.42-23.50)	7.55 (5.50-11.00)	<0.001
IgG2 (g/L)			
High	-----	-----	NS
Normal	32 (100.0%)	10 (100.0%)	
Median IgG2 level (range)	4.40 (1.27-6.68)	3.97 (3.76-6.28)	NS

Table 6. Continued.

Variables	Recipients (24-36 months post-TX) (n=32)	Control group (n=10)	p-value*
IgG3 (g/L)			
High	22 (68.8%)	3 (30.0%)	0.041
Normal	10 (31.2%)	7 (70.0%)	
Median IgG3 level (range)	1.01 (0.50-2.55)	0.75 (0.28-6.46)	NS
IgG4 (g/L)			
High	0 (0.0%)	1 (10.0%)	NS
Normal	32 (100.0%)	9 (90.0%)	
Median IgG4 level (range)	0.58 (0.04-1.57)	0.64 (0.20 -3.14)	NS

Data expressed as median (range) or frequency (%). $P > 0.05$ is not significant (NS).

* Mann-Whitney U test was used to compare the median between recipients and control group, Fisher Exact test was used to compare proportion between recipients and the control group.

Total IgG and its subclasses in the 11 recipients at different periods (pre-TX, 6-12 months, and 24-36 months post-TX)

Table 7 shows the total IgG and its subclasses in the 11 recipients at the different study periods. Because of heterogenous IgG subclass response, variable settings of previous studies that detected IgG subclasses (pre-TX and post-TX at different periods) using different assay protocol,

it is hard to get firm conclusion about the IgG subclasses pattern and their effect on graft outcome. We found that the frequency and median level of total IgG, IgG1 and IgG3 increased in recipients at 24-36 months post-TX when compared with pre-TX and 6-12 months post-TX. IgG2 and IgG4 were normal in all 11 recipients (100%) at the 3 periods with normal median level (Table 7).

Table 7. Total IgG and its subclasses in the 11 KT recipients at different periods (pre-TX, after 6-12 months and 24-36 months post-TX).

Variables	Recipients pre-TX	Recipients (6-12 months post-TX)	Recipients (24-36 months Post-TX)	p- value*	p- value1	p- value2	p- value3
Total IgG (RR 7-16 g/L)							
High	2 (18.2%)	0 (0.0%)	6 (54.5%)	0.009	NS	0.003	0.046
Normal	9 (81.8%)	10 (100.0%)	5 (45.5%)				
Median level (range)	11.10 (6.34-20.70)	7.99 (4.63-9.38)	19.20 (11.90- 27.10)	0.001	NS	<0.001	NS
IgG1 (RR 4.1-10.1 g/L)							
High	0 (0.0%)	0 (0.0%)	11 (100.0%)	<0.001	NS	<0.001	<0.001
Normal	11 (100.0%)	11 (100.0%)	0 (0.0%)				
Median level (range)	4.15 (0.95-7.90)	4.18 (2.77-6.09)	14.28 (10.40- 23.30)	<0.001	NS	0.001	0.002

Table 7. Continued.

Variables	Recipients pre-TX	Recipients (6-12 months post-TX)	Recipients (24-36 months Post-TX)	p-value*	p-value1	p-value2	p-value3
IgG2 (RR 1.7- 7.9 g/L)							
High	0 (0.0%)	0 (0.0%)	0 (0.0%)	-----	-----	-----	-----
Normal	11 (100.0%)	11 (100.0%)	11 (100.0%)				
Median level (range)	1.22 (0.34-3.84)	1.85 (1.27-2.57)	5.36 (3.44-6.68)	<0.001	NS	0.003	0.001
IgG3 (RR 0.11- 0.83 g/L)							
High	1 (9.1%)	0 (0.0%)	9 (81.8%)	<0.001	NS	0.001	0.003
Normal	10 (90.9%)	11 (100.0%)	2 (18.2%)				
Median level (range)	0.66 (0.31-0.88)	0.43 (0.19-0.64)	1.11 (0.50-1.77)	<0.001	NS	0.001	0.041
IgG4 (RR 0.03- 2.0 g/L)							
High	0 (0.0%)	0 (0.0%)	0 (0.0%)	-----	-----	-----	-----
Normal	11 (100.0%)	11 (100.0%)	11 (100.0%)				
Median level (range)	0.55 (0.09-1.12)	0.30 (0.01-0.56)	0.67 (0.13-1.06)	<0.001	0.032	<0.001	NS

Data expressed as median (range). $P > 0.05$ is not significant (NS). *Friedman test (compare median total IgG and its subclasses over time: pre-TX, 6-12 months, and 24-36 months post-TX). Pairwise comparison: p-value 1: pre-TX Vs 6-12 m, p-value 2: 6-12 m Vs 24-36 m post-TX and p-value 3: pre-TX Vs 24-36 m post-TX. * Related-Samples Cochran's Q Test compare proportions over time: pre-TX, 6-12 months, and 24-36 months post-TX)

Association between rejection occurrence, post-TX IgG subclasses and PRA

The median duration between TX date and the sampling date was 32 months ranged from 24-36 months. Rejection occurred in 4/32 recipients (12.5%), with median duration of 32 month (25 -36 months). Of the four patients with kidney rejection, one recipient had normal total IgG and its subclasses with negative class I and II PRA. His biopsy report revealed acute T cell mediated rejection with chronic changes. The second recipient had high total IgG, IgG1 and IgG3 with class 1 and II PRA MFI <3000. His biopsy report revealed subacute AMR. The third recipient had high total IgG, IgG1 and IgG3 with positive class II PRA only, MFI > 10000. His

biopsy report revealed acute active AMR and acute T cell mediated rejection. The fourth recipient had high total IgG and IgG1 with positive both class I and II PRA, MFI > 10000. His biopsy report revealed acute active AMR and acute T cell mediated rejection.

Graft survival in KT recipients

Recipients with post-TX positive class I PRA showed statistically lower mean survival time than recipients with negative class I PRA ($p=0.025$). However, the gender of the recipients or recipients with high total IgG, IgG1, IgG3 levels and positive class II PRA had no effect on graft survival time (Table 8 and Figure 1).

Table 8. Graft survival in KT recipients.

Variable	Graft survival Time mean (Months), (95% CI)	<i>p</i> -Value*
All patients	35.21 (34.14-36.27)	
Gender		
Male	35.39 (34.27-36.50)	NS
Female	34.0 (30.79-37.20)	
Post transplantation IgG		
IGg		
High	35.33 (34.25-36.42)	NS
Normal	35.00 (33.13-36.86)	
IGg 1		
High	35.56 (34.83-36.30)	NS
Normal	32.33 (26.46-38.20)	
IGg 3		
High	35.62 (34.61-36.63)	NS
Normal	34.42 (32.15-36.68)	
Post transplantation RPA		
Class I		
Positive	34.0 (34.0-34.0)	0.025
Negative	35.32 (34.17-36.46)	
Class II		
Positive	35.50 (34.30-36.70)	NS
Negative	35.15 (34.01-36.30)	

Survival analysis (Log rank test) compare graft survival time. $p > 0.05$ is not significant (NS). CI (confidence interval)

*Log rank test.

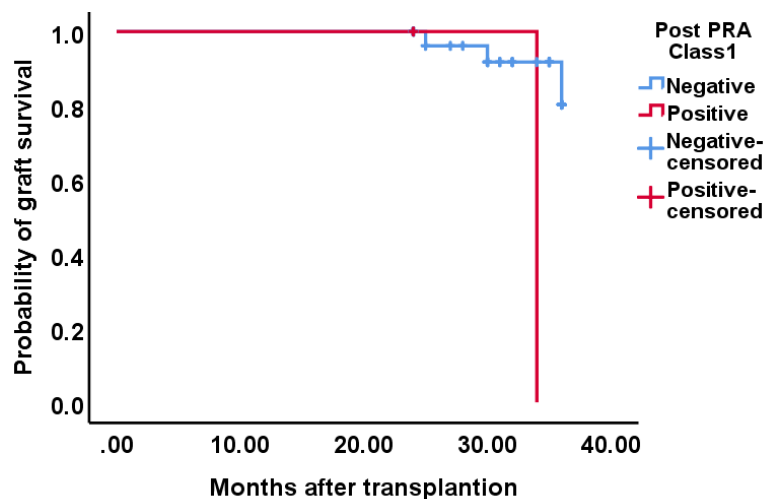


Figure 1. Kaplan–Meier curves for kidney allograft survival according to post PRA class I.

Discussion

The present work aimed to analyze the distribution of anti-HLA class I and class II PRA, IgG, and its subclasses in pre and post KT periods and their association with acute AMR and graft survival. It also aimed to determine the potential benefit of implementing IgG subclasses assessment to the current transplant medicine practice.

In the present study, the median of serum creatinine in KT recipients was slightly statistically significant increased ($p < 0.001$) when compared to the control group. Serum creatinine was increased in 12.5% of recipients during their rejection episode and with rejection management it was returned to its normal level or slightly elevated. This finding agreed with Maraghi et al., 2016, who reported that the serum creatinine is a well-known biomarker for renal function and an important indicator of graft status. The regular measurement of serum creatinine in KT recipients post-TX seems to be one of the most crucial factors to determine the graft dysfunction even before histological diagnosis.¹⁸ Also, Younespour et al., 2016, found that there was strong association between graft dysfunction and elevated serum creatinine levels.¹⁹ However, Josephson, 2011, said that changes in serum creatinine may not be equivalent with the degree of graft injury, and they may change with other renal causes as graft infection, hemodynamic effects of calcineurin inhibitors and prerenal volume depletion.²⁰

In this work, Class I and class II PRA were studied within 24-36 months post-TX in 32 KT recipients, and there was no difference between recipients and the control group. The pre-TX PRA were present in 3 recipients (9.4%) and negative in the other 29 recipients (90.6%). In the 3 recipients (9.4%) with preformed Ab, two recipients of them became negative with no rejection occurred. The third recipient had post-TX class I and II PRA MFI > 10000 and acute active AMR with T cell mediated rejection occurred 2 years post-TX. These agreed with Malheiro et al., 2017, and Callemeyn et al., 2021, who found that either preformed or de novo DSA were indicators of AMR, graft

dysfunction and poor graft survival.^{21,6} Ma et al., 2016, said that sensitized KT recipients with preformed DSA could be against HLA class I, class II or both and target private or public epitopes.²² Also, Caillard et al., 2017, and Senev et al., 2019, reported that most preformed DSA disappeared after KT, but DSA which were persistent after TX with high MFI values caused AMR. Therefore, these researchers disputed that preformed DSAs above certain threshold become deleterious if they persist after TX.^{23,24} Moreover, Wang et al., 2019, demonstrated that high DSA with cumulative MFI values (ranged 1785-14985) in KT recipients undergo acute rejection while recipients with lower DSA MFI values (range, 786-8113) do not undergo acute rejection.²⁵ Also, Phillipott et al., 2022, said that rising DSAs MFI titer is more considerable and of clinical significance than steady or declined titre.²⁶

In the 29 recipients who had negative pre-TX PRA, the PRA was still negative post-TX in 8 recipients (27.6%) and the remaining 21 recipients (72.4%) developed de novo DSAs post-TX. De novo DSAs were class I and/or class II, and MFI was < 3000 up to > 10000 . These supported finding by Chung et al., 2014, Ramon et al., 2017, and Cun et al., 2021, who reported that 13-30% of KT recipients developed de novo DSA although they were non sensitized at TX time or even getting proper pre-TX desensitization program within 5 years post-TX.²⁷⁻²⁹ Also, Song et al., 2012, and Guidicelli et al., 2016, clarified that de novo DSA were mainly directed against donor class II HLA particularly if they were in high titer and this usually occurred during the first year after TX with 20% possibility of occurrence in next 4 years.³⁰⁻³¹

Rejection occurred in only 4 recipients (12.5%) out of the 32 KT recipients with different class I and class II MFI. Of these, the first recipient had negative class I and class II PRA. The second recipient had negative class I and class II PRA, and MFI was < 3000 . The third recipient had negative class I and class II PRA, and MFI was > 10000 . The fourth recipient had positive both class I and class II PRA, MFI was > 10000 . These supported findings by Wiebe et al., 2012, and Yell et al., 2015, demonstrated that there was a very heterogenous graft

outcome after the appearance of de novo DSAs, ranged from no graft injury to rapid graft dysfunction and loss. However, DSAs with same MFI strength did not cause the same outcome.³²⁻³³ This could be explained by findings of Yoo et al., 2014, Tambur et al., 2015, and Lefaucheur et al., 2017, who reported that the binding ability of DSAs to the beads (in Luminex solid phase assay) might not be as the binding ability of DSAs to the HLA antigens. They also reported that there were many limitations of solid phase assays as false positive high DSA titer (due to IgG against denatured HLA antigens or targeting shared epitopes) or false negative low titer (due to inhibitors or prozone phenomena occurred in extremely high DSAs titer).^{34,35,13} However, Arnold et al., 2014, explained that recipients who had post-TX de novo DSA with AMR occurrence were chronic with intense antigen exposure leading to advanced immune response with matured allo-response, such that, it could be used as diagnostic and prognostic indicator of AMR and graft survival.³⁶

In the present work, the total IgG, and its subclasses in the 32 KT recipients were detected by nephelometric method. In these recipients, the median level of total IgG, IgG1 and IgG3 was high, and their frequency was 56.2%, 90.6% and 68.8%, respectively. High levels of both IgG1 and IgG3 was observed in 65.5%, high IgG1 alone in 25% and high IgG3 alone in 3.1%. High IgG1 and IgG3 were significantly increased in recipients 24-36 months post-TX when compared with the control group ($p < 0.001$ and $p < 0.041$, respectively). IgG2 and IgG4 were normal in all recipients. The rejection occurred in 4 recipients (12.5%). Of these, one recipient had normal total IgG and its subclasses. Two recipients had high total IgG, IgG1 and IgG3. The last one had high total IgG and IgG1. These observations were supported by findings of many previous studies which measured DSAs IgG subclasses by using modified Luminex based Single Antigen Bead assay (by replacing SAB-IgG total by secondary antibodies specific for each IgG subclasses). Lefaucheur et al., 2016, found that IgG1 predominate (25%-30%), IgG3 (5%), IgG2 (2%) and IgG4 (2%). They reported that the risk of AMR was higher in recipients with multiple IgG DSA subclasses than recipients with

IgG1 alone, normal IgG subclass or low pan IgG MFI.¹⁴ Also, von Glehn Ponsirenas et al., 2018, said that post-TX DSA were mainly IgG1, followed by IgG3. IgG2 and IgG4 were less frequent, as they disappeared of them due to early antigen clearance. Only one patient with high IgG3 experienced AMR and allograft dysfunction.³⁷ However, Khovanova et al., 2015, Lefaucheur et al., 2016, and Hamdani et al., 2018, when used the modified Luminex method, they found that in 16%-21% of DSAs, particularly those with low MFI, any subclass was detected.^{38,14,39} This may be explained by Lowe et al., 2013, who suggested that this method for subclass detection lacks sensitivity and cross-reactivity between secondary antibodies could be occurred.⁴⁰

In contrary to our findings, Pernin et al., 2020, assessed DSAs IgG subclasses in 69 KT recipients by using mass spectrometry after DSA isolation and that allowing their relative quantification.¹⁵ They reported that all IgG subclasses were present in post-TX de novo DSA as IgG1 was predominant (62.2% of total IgG), IgG2 (23.1%), IgG3 (7.8%) and IgG4 (4.7%). Thus, IgG1, IgG3 and IgG4 fractions were high in alloimmune response than in total IgG and AMR occurrence with poor graft outcome, correlated with high IgG3 DSA percentage.¹⁵ Moreover, Valenzuela and Schaub, 2018, demonstrated that recipients had IgG1 and/or IgG3 only (37% to 48% of isolated IgG subclasses), recipients had IgG1 and/or IgG3 with IgG2 and/or IgG4 (47% to 62%), and recipients had IgG2 and/or IgG4 only (1% to 5%) with rare occurrence of single subclass.⁴¹

In the 11 recipients of this study, class I and class II PRA were not different between pre-TX, 6-12- and 24-36-months post-TX. The frequency and median level of total IgG, IgG1 and IgG3 increased in recipients at 24-36 months post-TX when compared with pre-TX and 6-12 months post-TX. IgG2 and IgG4 were normal in all 11 recipients (100%) at the pre-TX, 6-12- and 24-36-months post-TX with normal median level. No rejection occurred in these 11 recipients. These observations were supported by von Glehn Ponsirenas et al., 2018, said that post-TX DSA subclasses were mainly IgG1, followed by IgG3. IgG2 and IgG4 were less frequent and

disappeared due to early antigen clearance. Because of heterogeneous IgG subclass response, variable settings of previous studies that detected IgG subclasses (pre-TX and post-TX at different periods), using different assay protocol, small recipients' number included and defect in precise histological data of AMR, it is hard to get firm conclusion about the IgG subclasses pattern and their effect on graft outcome.³⁷ Contrary to our findings, Viglietti et al., 2017, studied circulating anti-HLA DSA in 110 KT recipients and reported that the frequency of IgG1, IgG2 and IgG4 was nearly the same at the time of TX, 1- and 2-years post-TX. While the frequency of IgG3 decreased 1- and 2-years post-TX when compared to the time of TX but its presence was associated with AMR occurrence.⁴²

In the present work, the mean graft survival time was not different in recipients who had high total IgG, IgG1 and IgG3 levels or positive post-TX class II PRA. While the mean survival time of the graft decreased in recipients who had positive post-TX class I PRA. This agreed with that of Riella et al., 2014, who reported that despite of the presence of class I or class II DSA with different IgG subclasses distribution, long term renal allograft survival was observed.⁴³ This was explained by von Glehn Ponsirenas et al., 2018, who found that the factors associated with low graft survival time were high MFI, type of IgG subclass and its capability of complement activation.³⁷ In contrary to our findings, Loupy et al., 2013, found that renal allograft survival decreased 5 years in patients with IgG1/IgG3 DSA more than patients with IgG2/IgG4 DSA or no DSA at all. This could be explained by small number of graft rejection, 4 recipients (12.5%), in a relatively narrow follow up period (mean: 2-3 years post-TX) in this study.⁴⁴

In conclusion, preformed class I and class II DSAs may turn to negative post-TX but they become deleterious if they persist after TX in high titer. De novo development of DSAs occurred even when recipients were non-sensitized at TX time. The frequency of post-TX total IgG, IgG1 and IgG3 levels increased mainly 24-36 months post-TX, therefore, recipients should be routinely evaluated for DSA classes

and subclasses at this period but their effect in graft outcome was unclear.

Author Contributions

WTE, SKS and ME contributed to the study conception and design. AD, WTE, SKS, ME, HAA and AAM contributed to material preparation, data collection and analysis. SKA and AR provided clinical support. AD wrote the manuscript draft. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was ethically reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Assiut University, Assiut, Egypt (approval dated: November 2016).

Informed consent

The importance of the study was explained to all participants and informed consents were taken from them before enrollment in this study.

References

1. Joshi S, J., Gaynor, J., Ciancio, G. (2012). Review of ethnic disparities in access to renal transplantation. *Clinical Transplantation*. 26(4): E337-E343.
2. Moeller, S., Gioberge, S., Brown, G. (2002): ESRD patients in 2001: global overview of patients, treatment modalities and development trends. *Nephrology Dialysis Transplantation*. 17(12): 2071-2076.
3. Bakr, M., Elmowafy, A., Abbas, M. (2020). History of renal transplantation in the Arab World. *Arch Hellen Med*. 37: 208-13.
4. Schieppati, A., Remuzzi, G. (2005). Chronic renal diseases as a public health problem: epidemiology, social, and economic implications. *Kidney International*. 68(7): S7-S10.

5. Valenzuela, NM., Reed, EF. (2013). Antibodies in transplantation: the effects of HLA and non-HLA antibody binding and mechanisms of injury. *Transplantation Immunology*. 9(3):41-70.
6. Callemeyn, J., Ameye, H., Lerut, E., et al. (2021): Revisiting the changes in the Banff classification for antibody-mediated rejection after kidney transplantation. *American Journal of Transplantation*. 21(7):2413-23.
7. Maxfield, S., Taylor, C., Kosmoliaptsis, V., et al. (2015). Transfer of HLA-specific allosensitization from a highly sensitized deceased organ donor to the recipients of each kidney. *American Journal of Transplantation*. 15(9):2501-2506.
8. Kumbala, D., Zhang, R. (2013). Essential concept of transplant immunology for clinical practice. *World journal of transplantation*. 3(4):113-119.
9. Lichvar, AB., Tremblay, S., Leino, AD., et al. (2020). Reducing donor-specific antibody during acute rejection diminishes long-term renal allograft loss: comparison of early and late rejection. *Transplantation*. 104(11):2403-14.
10. Krishnan, N., Abimbola, A., Machan, N., et al. (2021). HLA antibody incompatible renal transplantation: long-term outcomes similar to deceased donor transplantation. *Transplantation direct*. 7(8).
11. Stegall, MD., Chedid, MF., Cornell, LD. (2012). The role of complement in antibody-mediated rejection in kidney transplantation. *Nature reviews nephrology*. 8(11):670-8.
12. Sondermann, P., Szymkowski, DE. (2016). Harnessing Fc receptor biology in the design of therapeutic antibodies. *Current opinion in immunology*. 40(6):78-87.
13. Lefaucheur, C., Viglietti, D., Mangiola, M., et al. (2017). From humoral theory to performant risk stratification in kidney transplantation. *Journal of immunology research*. 17(7):46-59.
14. Lefaucheur, C., Viglietti, D., Bentelejewski, C., et al. (2016). IgG donor-specific anti-human HLA antibody subclasses and kidney allograft antibody-mediated injury. *Journal of the American Society of Nephrology*. 27(1):293-304.
15. Pernin, V., Beyze, A., Szwarc, I., et al. (2020). Distribution of de novo donor-specific antibody subclasses quantified by mass spectrometry: high IgG3 proportion is associated with antibody-mediated rejection occurrence and severity. *Frontiers in immunology*. 11:919.
16. Poggio, ED., Augustine, JJ., Arrigain, S., et al. (2021). Long-term kidney transplant graft survival—Making progress when most needed. *American Journal of Transplantation*. 21(8):2824-32.
17. Hariharan, S., Israni, AK., Danovitch, G. (2021): Long-term survival after kidney transplantation. *New England Journal of Medicine*. 385(8):729-43.
18. Maraghi, E., Foroushani, AR., Younespour, S., et al. (2016). Longitudinal assessment of serum creatinine levels on graft survival after renal transplantation: joint modeling approach. *Nephro-Urology Monthly*. 8(4):64-69.
19. Younespour, S., Foroushani, AR., Maraghi, E., et al. (2016). Longitudinal serum creatinine levels in relation to graft loss following renal transplantation: robust joint modeling of longitudinal measurements and survival time data. *Nephro-urology monthly*. 8(5).
20. Josephson, MA. (2011). Monitoring and managing graft health in the kidney transplant recipient. *Clinical Journal of the American Society of Nephrology*. 6(7):1774-80.
21. Malheiro, J., Tafulo, S., Dias, L., et al. (2017). Determining donor-specific antibody C1q-binding ability improves the prediction of antibody-mediated rejection in human leucocyte antigen-incompatible kidney transplantation. *Transplant International*. 30(4):347-359.
22. Ma, J., Patel, A., Tinckam, K. (2016). Donor-specific antibody monitoring: Where is the beef? *Advances in chronic kidney disease*. 23(5):317-325.
23. Caillard, S., Anglicheau, D., Matignon, M., et al. (2020). An initial report from the French SOT COVID Registry suggests high mortality due to COVID-19 in recipients of kidney transplants. *Kidney international*. 98(6):1549-58.
24. Senev, A., Lerut, E., Van Sandt, V., et al. (2019). Specificity, strength, and evolution of pretransplant donor-specific HLA antibodies determine outcome after kidney transplantation. *American Journal of Transplantation*. 2019 Nov;19(11):3100-13.
25. Wang, J., Wang, P., Wang, S., et al. (2019). Donor-specific HLA Antibodies in Solid Organ Transplantation: Clinical Relevance and Debates. *Exploratory Research and Hypothesis in Medicine*. 4(4):76-86.
26. Phillpott, M., Daga, S., Higgins, R., et al. (2022). Dynamic Behaviour of Donor Specific Antibodies in the Early Period Following HLA Incompatible Kidney Transplantation. *Transplant International*. 35:10128.
27. Chung, BH., Choi, BS., Oh, EJ., et al. (2014). Clinical impact of the baseline donor-specific anti-human leukocyte antigen antibody measured by

- L uminex single antigen assay in living donor kidney transplant recipients after desensitization therapy. *Transplant International*. 27(1):49-59.
28. Ramon, DS., Huang, Y., Zhao, L., et al. (2017). Use of complement binding assays to assess the efficacy of antibody mediated rejection therapy and prediction of graft survival in kidney transplantation. *Human immunology*. 78(2):57-63.
29. Cun, H., Hönger, G., Kleiser, M., et al. (2021). Screening strategy for de novo donor-specific HLA antibodies beyond the first year after kidney transplantation: Personalized or "one size fits all"? *Clinical Transplantation*. 35(2):e14170.
30. Song, EY., Lee, Y-J., Hyun, J., et al. (2012). Clinical relevance of pretransplant HLA class II donor-specific antibodies in renal transplantation patients with negative T-cell cytotoxicity crossmatches. *Annals of laboratory medicine*. 32(2):139-144.
31. Guidicelli, G., Guerville, F., Lepreux, S., et al. (2016). Non-complement-binding de novo donor-specific anti-HLA antibodies and kidney allograft survival. *Journal of the American Society of Nephrology*. 27(2):615-625.
32. Wiebe, C., Gibson, I., Blydt-Hansen, T., et al. (2012). Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *American journal of transplantation*. 12(5):1157-1167.
33. Yell, M., Muth, BL., Kaufman, DB., et al. (2015). C1q binding activity of de novo donor-specific HLA antibodies in renal transplant recipients with and without antibody-mediated rejection. *Transplantation*. 99(6):1151-1155.
34. Yoo, PS., Bonnel, A., Kamoun, M., et al. (2014). Clinical outcomes among renal transplant recipients with pre-transplant weakly reactive donor-specific antibodies. *Clinical transplantation*. 28(1):127-133.
35. Tambur, A., Herrera, N., Haarberg, K., et al. (2015). Assessing antibody strength: comparison of MFI, C1q, and titre information. *American journal of transplantation*. 15(9):2421-2430.
36. Arnold, ML., Ntokou, IS., Doxiadis, II., et al. (2014). Donor-specific HLA antibodies: evaluating the risk for graft loss in renal transplant recipients with isotype switch from complement fixing IgG1/IgG3 to noncomplement fixing IgG2/IgG4 anti-HLA alloantibodies. *Transplant International*. 27:253-261.
37. Von Glehn Ponsirenas, R., Cazarote, HB., De Almeida Araújo, S., et al. (2018). Anti-HLA donor-specific IgG subclasses and C1q-binding evolution in posttransplant monitoring. *Transplantation Direct*. 4(9):35-49.
38. Khovanova, N., Daga, S., Shaikhina, T., et al. (2015). Subclass analysis of donor HLA-specific IgG in antibody-incompatible renal transplantation reveals a significant association of IgG4 with rejection and graft failure. *Transplant international*. 28(12):1405-1415.
39. Hamdani, G., Goebel, JW., Brailey, P., et al. (2018). IGG 3 anti-HLA donor-specific antibodies and graft function in pediatric kidney transplant recipients. *Pediatric Transplantation*. 22(5):e13219.
40. Lowe, D., Higgins, R., Zehnder, D., et al. (2013). Significant IgG subclass heterogeneity in HLA-specific antibodies: implications for pathogenicity, prognosis, and the rejection response. *Human immunology*. 74(5):666-672.
41. Valenzuela, NM., Schaub, S. (2018). The biology of IgG subclasses and their clinical relevance to transplantation. *Transplantation*. 102(1S):S7-S13.
42. Viglietti, D., Loupy, A., Vernerey, D., et al. (2017). Value of donor-specific anti-HLA antibody monitoring and characterization for risk stratification of kidney allograft loss. *Journal of the American Society of Nephrology*. 28(2):702-715.
43. Riella, LV., Safa, K., Yagan, J., et al. (2014). Long-term outcomes of kidney transplantation across a positive complement-dependent cytotoxicity crossmatch. *Transplantation*. 97(12):1247-1252.
44. Loupy, A., Lefaucheur, C., Vernerey, D., et al. (2013). Complement-binding anti-HLA antibodies and kidney-allograft survival. *New England Journal of Medicine*. 369(13):1215-1226.