Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology,
E-ISSN (2090-2506)
Volume 32 (4), October, 2025
Pages: 69–83.
www.Ejimmunology.org
https://doi.org/10.55133/eji.320409

Role of human leucocyte antigen (DQ) in acute renal allograft rejection: A single center study

Tarek T. H. ElMelegy, Sohair K. Sayed, Maha G. Abd El-Kadr, and Abeer A. Mokhtar

Department of Clinical Pathology, Faculty of Medicine, Assiut University Hospital, Assiut, Egypt.

Corresponding author: Maha G. Abd El-Kadr, Department of Clinical Pathology, Faculty of Medicine, Assiut University Hospital, Assiut, Egypt.
Email: mahagamal@aun.edu.eg

Abstract

Human leukocyte antigen (HLA) system is a very polymorphic gene complex encoding for cell surface proteins. Kidney transplantation (KT) is considered the optimal renal replacement therapy. Donor-specific antibodies (DSA) against HLA class II antigens are more common than class I. This study aimed to determine the role of HLA (DQ) in acute rejection of renal allograft. This study included 43 KT recipient donor pairs. HLA typing (A, B, DR) of donor and recipient and flowcytometry cross matching results pre transplantation were collected from patients' files. Panel Reactive Antibody (PRA) classes I and II were done to recipients pre transplantation. PRA classes I and II and HLA-DQ genotyping were done to recipients 15-16 weeks post-transplantation. Rejection occurred in 9.3% recipients. Recipients with positive PRA class II had a statistically significant higher percentage of rejection (25.0%) compared to (0.0%) of those with negative PRA class II (p=0.029). Recipients with positive anti HLA-DR antibodies (Abs) and anti HLA-DQ Abs had statistically significant higher percent of rejection (28.6%) compared to (0.0%) of those with negative anti HLA-DR Abs and anti HLA-DQ Abs (p= 0.016). Recipients with positive anti HLA-DQ4 Abs or anti HLA-DQ5 Abs had a statistically significant higher percent of rejection (50%) compared to (5.1%) of those without anti HLA-DQ4 Abs or anti HLA-DQ5 Abs (p=0.037). Recipients with positive anti HLA-DQ9 Abs had a statistically significant higher percent of rejection (30%) compared to (3.0%) of those without anti HLA-DQ9 Abs (p=0.034). Recipients who received kidney from HLA-DQ mismatched donors had higher incidence (57%) of anti HLA-DQ5 antibodies and anti HLA-DQ6 antibodies compared to those with HLA-DQ matched donors (0.0%) (p= 0.018). In conclusion, anti HLA-DQ antibody was one of the most prevalent post-transplant PRA detected. Regarding acute rejection, there was no risk association between its occurrence and HLA-DQ mismatching.

Keywords: HLA, Major Histocompatibility Complex, KT, DSA, PRA

Date received: 23 January 2024; **accepted:** 26 July 2025

Introduction

The human leukocyte antigen (HLA) system encodes for cell surface proteins that are encoded by the major histocompatibility complex (MHC) in humans. These cell-surface proteins are responsible for the regulation of the immune system.[1]

HLA complex contains more than 200 identified loci located close together within the

short arm of chromosome 6.[2] The distinctness of HLA is used by the human immune system to differentiate self from non-self. The presentation of "foreign" peptides, or antigens, to immune competent cells is the responsibility of HLA. When foreign antigens interact with HLA molecules, T cells can only identify them.[1]

Three class I HLA (A, B, and C) are found on all nucleated cells in humans, but six class II HLA (DPA1, DPB1, DQA1, DQB1, DRA, and DRB1) are only found on lymphocytes and antigen-presenting cells. The majority of the immunogenicity of mismatched antigens is caused by three of the seven heterodimers (HLA-A, -B, and -DRB1), hence previous HLA-typing techniques have mostly concentrated on these alleles.[3]

The gold standard therapeutic approach for treating renal dysfunctions that provides patients with end-stage renal disease (ESRD) with the highest chance of survival is kidney transplantation (KT).[4, 5] KT is associated with 68% lower risk of death than dialysis.[6] Over the past ten years, there was an improvement in kidney transplant patient survival and graft.[7]

The immune system's response, particularly against the transplant's HLA proteins, is what determines if KT is successful. Antibodies reactive to HLA may develop in patients who have previously been exposed to non-self HLA through transplants, blood transfusions, or pregnancy.[8]

The outcome in KT was improved when HLA matching was done[9, 10] and still part of the kidney graft allocation. HLA-DR matching has a much more effect on graft outcomes if compared with matching at the HLA-A or -B locus.[11]

HLA-DQ is not yet a determinant of graft allocation; however, its relative significance is becoming more widely acknowledged. The percentage of acute rejection, renal glomerulopathy, and renal graft loss is greater in recipients who have *de novo* anti-DQ donor-specific antibodies.[12, 13]

Uncertainty surrounds the impact of broad antigen HLA-DQ mismatching on KT. Data from the Australia and New Zealand Dialysis and Transplant Registry revealed that HLA-DQ mismatching affects outcomes,[14] despite earlier research indicating no significant link between the condition and graft outcomes.[14, 15] The present study aimed to determine the role of human leucocyte antigen (DQ) in acute rejection of renal allograft.

Subjects and Methods

The present study included 43 donors and 43 recipients with ESRD from the Transplantation Unit in Assiut University Urology Hospital, during the period from September 2018 to September 2022. All transplants required a negative flowcytometric crossmatch for IgG, T cell and B cell, and ABO blood group compatibility between donor and recipient.

Exclusion criteria

Recipients with pre-transplantation desensitization protocols and recipients with history of previous transplants or pregnancy were excluded from the study.

Study specimens

Venous blood samples (5 ml) were collected from recipients both pre transplantation and 15-16 weeks post transplantation under complete aseptic conditions into plain tube. The blood was left to clot for 30 min at 37℃ and then centrifuged at 1509g for ten minutes. Sera were separated, divided into aliquots and kept frozen at -20℃ until used.

In addition, venous blood samples (2 ml) were collected from donors and recipients 15-16 weeks post transplantation under complete aseptic conditions into EDTA containing tubes. DNA was extracted using commercial kits (Cat. No. 51304, QIAamp DNA Mini Kit, QIAGEN, Germany), according to manufacturer's instructions and kept frozen at -20℃ for further HLA- DQ typing. Finally, 24 hours urine sample was collected 15-16 weeks post transplantation from each recipient.

Pre transplant investigations

Results of ABO blood grouping, HLA typing (A, B, DR) of donor and recipient and flowcytometry cross matching prior to transplantation were collected from patients' files at the hospital.

Panel Reactive Antibody (PRA) classes I and II were done to recipients of transplant pre transplantation using LABScreen™ PRA Class I (Cat. No. LS1PRA 0000661856, ONE LAMBDA, USA) and LABScreen™ PRA Class II (Cat. No. LS2PRA 0000659944, ONE LAMBDA, USA) on LABScan3D (Luminex® FLEXMAP 3D®) (ONE LAMBDA, USA), according to the manufacturer's instructions.

Investigations for recipients 15-16 weeks post-transplantation

Urea and creatinine tests and 24 h urinary protein tests were performed using an automated chemistry analyzer (ADVIA 1800 chemistry Auto-Analyzers, Siemens Healthineers, USA), according to the manufacturer's instructions.

Detection of PRA IgG antibodies to HLA class I & class II in serum

This was done using Luminex microbead method as mentioned before in pre-transplant investigations

DNA typing of HLA Class II alleles (HLA DQ)

This was done for 14 kidney transplant recipient/donor pairs when PRA was positive against HLA-DQ. This was done using LABType™ SSO (Cat.No. RSSO2Q 0000277911, ONE LAMBDA, USA) on LABScan3D (Luminex® FLEXMAP 3D®) (ONE LAMBDA, USA), according to manufacturer's instructions.

Kidney biopsy: This was done post transplantation for recipients who developed proteinuria together with increased level of serum creatinine. The results were collected from patients' files at the hospital.

Statistical Analysis

Data was analyzed using the Statistical Package for Social Science (SPSS), version 26.0 for Windows. Qualitative data are presented as frequencies and percentages. Quantitative data were checked for normality by the Shapiro Walk test and expressed as mean ± standard deviation, median and range according to the distribution of data. Independent Sample T test compared the mean difference between groups. The Chi square test/ Fisher Exact test was used to compare proportion between groups. The Mcnemar test was used to compare proportions pre- and post-transplantation in PRA. The Odds ratio and confidence interval was calculated, and *p* value of <0.05 was considered significant..

Results

Table 1 shows the characteristic features of kidney transplant recipients and donors. Table 2 shows the causes of ESRD of kidney transplant recipients and serum urea and creatinine level at 15-16 weeks post transplantation.

Table 1. Features of kidney transplant recipients and donors.

Variable	kidney transplant recipients (n=43)	kidney transplant donors (n=43)
Age (years)		
Mean ± SD	30.51±8.62	40.93±10.54
Median (range)	37 (16.0-58.0)	39 (21.0-57.0)
Gender		
Male	39 (90.7%)	15 (34.9%)
Female	4 (9.3%)	28 (65.1%)
Degree of relation		
First degree (parents),	19 (44.2%)	
Second degree (brothers, sisters)	20 (46.5%)	
Third degree (aunts, uncles)	3 (7%)	
No degree of relation (Husband)	1 (2.3%)	
Blood group	n=43	%
Identical	28	65.1%
Compatible	15	34.9%

Table 2. Causes of end-stage renal disease (ESRD) among kidney transplant recipients and kidney function tests 15-16 weeks post transplantation.

Causes of ESRD among kidney transplant recipients	Kidney transplant recipients (n=43)	%
Congenital causes	3	7.0%
Neglected GN	24	55.8%
Neglected chronic PN	14	32.6%
Diabetic nephropathy	1	2.3%
HTN nephropathy	1	2.3%
Variables	kidney transplant recipients (n=43) (15-16 weeks post transplantation)	
Serum Urea (RR: 2.5- 7.1 mmol/l)		
Mean ± SD	7.28 ± 4.75	
Median (range)	6.50 (2.2-31.7)	
Serum Creatinine (RR: 71- 115 µmol/l)		
Mean ± SD	131.77 ± 107.94	
Median (range)	94.0 (37.0-613.0)	

RR=Reference range; GN= Glomerulonephritis; PN= Pyelonephritis; HTN= Hypertension.

Serum creatinine increased in 34.9% (15/43) of recipients at 15-16 weeks post transplantation. When comparing recipients with increased serum creatinine levels to those with normal creatinine level, different variables did not show significant difference between the two groups. These include donor and recipient age, ABO group, causes of ESRD, HLA class I and HLA class II (DR) mismatch, PRA class I and class II results. Recipients of kidney from female donors had a statistically higher frequency of increased creatinine level (50.0%) compared to recipients from male donors (6.7%) (p=0.004). However, this was not associated with significant difference in the rate of acute rejection during 15-16 weeks post transplantation follow-up period.

The frequency of positive PRA class I (41.9%) and PRA class II (37.2%) were significantly higher in recipients at 15-16 weeks post transplantation follow up period than pre transplantation (9.3% and 4.7%, respectively) (p=0.003 and p=0.001, respectively) (Table 3).

Table 3. Panel Reactive Antibody (PRA) distribution among kidney transplant recipients pre and post transplantation.

Variable	Pre-transplantation (n=43)	15-16 weeks post-transplantation (n=43)	p-value*
PRA Class I			
Positive	4 (9.3%)	18 (41.9%)	0.003
Negative	39 (90.7%)	25 (58.1%)	
PRA Class II			
Positive	2 (4.7%)	16 (37.2%)	0.001
Negative	41 (95.3%)	27 (62.8%)	

*$p \leq 0.05$ is significant.

The 16 Recipients with post-transplant positive PRA class II were further classified into 2 recipients (12.5%) with positive PRA against DR only, 2 recipients (12.5%) with positive PRA against DQ only, 10 recipients (62.5%) with combined positive PRA DR and DQ and lastly 2 recipients (12.5%) with combined positive PRA against DR, DQ and DP.

Of the 43 recipients, 25 recipients (58.1%) developed at least one post-transplantation PRA, 2 (4.7%) recipients had DQ PRA alone (DQ-only), 11 (25.6%) recipients acquired an A, B, and/or DR antibody in the absence of a DQ antibody (not DQ), whereas 12 (27.9%) patients also developed a DQ antibody with non DQ antibodies (DQ + non DQ) (Table 4).

During the follow-up period, the overall incidence of biopsy-proven acute rejection was 9.3% (4/43). Recipients in the DQ + non DQ groups had statistically significant higher percent of acute rejection (30.8%) compared with the other groups (0%) (p=0.0171; Table 4).

Table 4. Biopsy findings according to Panel Reactive Antibody (PRA) groups.

Total patients (43)	DQ only (n=2) 4.7%	DQ + non-DQ (n=12) 27.9%	Non DQ (n=11) 25.6%	No PRA (n=18) 41.8%	p-value*
No rejection	2 (100.0%)	8 (66.7%)	11 (100.0%)	18 (100.0%)	0.0171
Rejection	0 (0.0%)	4 (30.8%)	0 (0.0%)	0 (0.0%)	
Acute AMR only	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Chronic AMR only	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
TCMR only	0 (0.0%)	2 (16.67%)	0 (0.0%)	0 (0.0%)	NS
AMR+TCMR mixed rejection	0 (0.0%)	2 (16.67%)	0 (0.0%)	0 (0.0%)	NS

*Chi square test was used to compare proportion between groups; NA (non-applicable for calculation of significance); n= number. AMR= Antibody mediated rejection; TCMR= T- cell mediated rejection $p > 0.05$ is not significant (NS).

Fourteen recipients had PRA against HLA-DQ 15-16 weeks post-transplantation. The distribution of these HLA-DQ reactive antibodies and their mean fluorescence intensity (MFI) are shown in Table 5.

Table 5. Types of DQ and their mean fluorescence intensity (MFI) among kidney transplant recipient 15-16 weeks post transplantation.

Variable	Positive DQ Panel Reactive Antibody (PRA) (n=14)	%
DQ2 Positive	1	7.1
MFI		
<3000	1	7.1
DQ4 Positive	4	28.6
MFI		
<3000	1	7.1
>3000	3	21.4
DQ5 Positive	4	28.6

Table 5. Continued.

Variable	Positive DQ Panel Reactive Antibody (PRA) (n=14)	%
MFI		
<3000	1	7.1
>3000	3	21.4
DQ6 Positive	4	28.6
MFI		
<3000	3	21.4
>3000	1	7.1
DQ7 Positive	5	35.7
MFI		
<3000	3	21.4
>3000	2	14.3
DQ8 Positive	6	42.9
MFI		
<3000	4	28.6
>3000	2	14.3
DQ9 Positive	10	71.4
MFI		
<3000	7	50.0
>3000	3	21.4

Data expressed as frequency (%); MFI= Mean fluorescence intensity; n= number.

Table 6 shows the association of different variables with biopsy proven acute rejection. Recipients with positive PRA class II had a statistically significant higher percentage of rejection (25.0%) compared to (0.0%) of those with negative PRA class II (p=0.029). Recipients with positive anti HLA-DR Abs or anti HLA-DQ Abs had a statistically significant higher percent rejection (28.6%) compared to (0.0%) of those with negative anti HLA-DR Abs or anti HLA-DQ Abs (p=0.016).

Recipients with positive anti HLA-DQ4 Abs or anti HLA-DQ5 Abs had statistically significant higher percent of rejection (50%) compared to (5.1%) of those without anti HLA-DQ4 Abs or anti HLA-DQ5 Abs (p=0.037). They had 18.5 times increased risk of rejection. Recipients with positive anti HLA-DQ9 Abs had a statistically significant higher percent of rejection (30%) compared to (3.0%) of those without anti HLA-DQ9 Abs (p= 0.034). They had 13.71 times increased risk of rejection (Table 6).

There was no statistically significant relation between acute rejection and several transplant recipient/donor variables. These include age, donor gender, degree of relation between donors and recipients, ABO, causes of ESRD, HLA class I (A, B) matching, HLA class II (DR) matching, PRA class I, anti HLA-DQ2, anti HLA-DQ6, anti HLA-DQ7 and anti HLA-DQ8 (Table 6).

Table 6. Association between rejections in kidney transplant and different variables.

Variables	kidney transplantation		*p*-value*	OR (95% CI)
	Rejection (n=4)	No rejection (n=39)		
Donor age: Mean ± SD	40.50±9.03	40.97±10.78	NS	CI (-11.78-10.83)
Median (range)	41.5 (30-49)	43.0 (21-57)		
Recipient age: Mean ± SD	26.25±7.89	30.95±8.66	NS	CI (-13.83-4.43)
Median (range)	23.0 (21-38)	30.0(16-58)		
Donor gender				
Female donor (n=28)	3 (10.7%)	25 (89.3%)	NS	1.68 (0.15-17.72)
Male donor (n=15)	1 (6.7%)	14 (93.3%)		
Degree of relation				
second degree (n= 20)	2 (10.0%)	18 (90%)	NS	1.16 (0.14-9.14)
Non second degree (n=23)	2 (8.7%)	21 (91.3%)		
ABO blood group				
Identical (n=28)	4 (14.3%)	24 (85.7%)	NS	NA
Compatible (n=15)	0 (0.0%)	15 (100%)		
Causes of ESRD				
Congenital causes (n=3)	1 (33.3%)	2 (66.7%)		
Neglected GN (n=24)	2 (8.3%)	22 (91.7%)		
Neglected PN(n=14)	1 (7.1%)	13 (92.9%)	NS	NA
Diabetic nephropathy(n=1)	0 (0.0%)	1 (100%)		
HTN nephropathy(n=1)	0 (0.0%)	1 (100%)		
HLA class I				
Mismatch (n=39)	4 (10.3%)	35 (89.7%)	NS	NA
Match (n=4)	0 (0.0%)	4 (100%)		
HLA class II (DR)				
Mismatch (n=34)	4 (11.8%)	30 (88.2%)	NS	NA
Match (n=9)	0 (0.0%)	9 (100%)		
PRA class I				
Total positive (n=18)	2 (11.1%)	16 (88.9%)	NS	1.44 (0.18-11.29)
Total negative (n=25)	2 (8.0%)	23 (92.0%)		
Class I A:				
- Positive (n=18)	2 (11.1%)	16 (88.9%)	NS	1.44 (0.18-11.29)
- Negative (n=25)	2 (8.0%)	23 (92.0%)		
Class I B:				
- Positive (n=13)	1(7.7%)	12 (92.3%)	NS	0.75 (0.07-7.96)
- Negative (n=30)	3 (10.0%)	27 (90.0%)		
Class I C:				
- Positive (n=2)	0 (0.0%)	2 (100.0%)	NS	NA
- Negative (n=41)	4(9.8%)	37(90.2%)		
PRA class II				
Total positive (n=16)	4 (25.0%)	12 (75.0%)	0.029	NA
Total negative (n=27)	(0.0%)	27(100%)		
Class II DR:				
- Positive (n=14)	4 (28.6%)	10 (71.4%)	0.016	NA
- Negative (n=29)	0 (0.0%)	29 (100%)		

Table 6. Continued.

Variables	kidney transplantation		*p*-value*	OR (95% CI)
	Rejection (n=4)	No rejection (n=39)		
Class II DP:				
- Positive (n=2)	(50.0%)	1(50.0%)	NS	12.67 (0.62-257.1)
- Negative (n=41)	(7.3%)	38 (92.7%)		
Class II DQ:				
- Positive (n=14)	(28.6%)	10 (71.4%)	0.016	NA
- Negative (n=29)	0 (0.0%)	29 (100%)		
PRA class II HLA-DQ alleles				
DQ2				
Positive (n=1)	0 (0.0%)	1 (100%)	NS	NA
Negative (n=42)	4 (9.5%)	38 (90.5%)		
DQ4				
Positive (n=4)	2 (50.0%)	2 (50.0%)	0.037	18.50 (1.64-208.46)
Negative (n=39)	2 (5.1%)	37 (94.9%)		
DQ5				
Positive (n=4)	2 (50.0%)	2 (50.0%)	0.037	18.50 (1.64-208.46)
Negative (n=39)	2 (5.1%)	37 (94.9%)		
DQ6				
Positive (n=4)	1 (25.0%)	3 (75.0%)	NS	4.0 (0.13-51.29)
Negative (n=39)	3 (7.7%)	36 (92.3%)		
DQ7				
Positive (n=5)	1 (20.0%)	4 (80%)	NS	2.19 (0.24-35.12)
Negative (n=38)	3 (7.9%)	35 (92.1%)		
DQ8				
Positive (n=6)	1 (16.7%)	5 (83.3%)	NS	2.26(0.19-26.27)
Negative (n=37)	3 (8.1%)	34 (91.9%)		
DQ9				
Positive (n=10)	3 (30.0%)	7 (70.0%)	0.034	13.71 (1.23-152.14)
Negative (n=33)	1 (3.0%)	32 (97.0%)		

Data are expressed as frequency and raw % or mean ± SD. *Independent Sample T test compares the mean difference between groups, Fisher Exact test compares proportions between groups. n=number; SD=standard deviation; OR=Odds ratio; CI=confidence interval; ESRD=End stage renal disease; HLA=Human leucocyte antigen; PRA=Panel reactive antibody; $p > 0.05$ is not significant (NS). NA= not applicable for calculation of significance.

Recipients who had anti HLA-DQ antibodies (n=14) were subjected to HLA-DQ genotyping together with corresponding donors. Recipients who received kidney from HLA-DQ mismatched donors had higher incidence (57%) of anti HLA-DQ5 antibodies compared to those with HLA-DQ matched donors (0.0%) (p=0.018). Recipients who received kidney from HLA-DQ mismatched donors had higher incidence (57%) of anti HLA-DQ6 antibodies compared to those with HLA-DQ matched donors (0.0%) (p=0.018). There was no statistically significant difference in urea, creatinine, proteinuria, acute rejection between matched and mismatched DQ (Table 7).

Table 7. Effect of human leukocyte antigen (HLA)-DQ matching status on post transplant outcome.

Variables	HLA DQ genotyping		*p*-value*	OR (95% CI)
	Mismatch (n=7)	Matched (n=7)		
Urea (> 7.1 mmol/l)				
Increase	3 (42.9%)	2 (28.6%)	NS	1.8 (0.20-17.26)
Normal	4 (57.1%)	5 (71.4%)		
Creatinine (> 115 µmol/l)				
Increase	4 (57.1%)	1 (14.3%)	NS	8.0 (0.59-106.93)
Normal	3 (42.9%)	6 (85.7%)		
Proteinuria				
Present	4 (57.1%)	3 (42.9%)	NS	3.33 (0.36-30.70)
Absent	3 (42.9%)	4 (57.1%)		
Rejection				
Occur	3 (42.9%)	1 (14.3%)	NS	4.50 (0.33-60.15)
Not occur	4 (57.1%)	6 (85.7%)		
PRA class II HLA-DQ alleles				
DQ2				
Positive	0 (0.0%)	1 (14.3%)	NS	NA
Negative	7 (100.0%)	6 (85.7%)		
DQ4				
Positive	3 (42.9%)	1 (14.3%)	NS	4.50 (0.33-60.15)
Negative	4 (57.1%)	6 (85.7%)		
DQ5				
Positive	4 (57.1%)	0 (0.0%)	0.018	NA
Negative	3 (42.9%)	7 (100.0%)		
DQ6				
Positive	4 (57.1%)	0 (0.0%)	0.018	NA
Negative	3 (42.9%)	7 (100.0%)		
DQ7				
Positive	1 (14.3%)	4 (57.1%)	NS	0.13 (0.10-1.67)
Negative	6 (85.7%)	3 (42.9%)		
DQ8				
Positive	2 (28.6%)	4 (57.1%)	NS	0.30 (0.03-2.76)
Negative	5 (71.4%)	3 (42.9%)		
DQ9				
Positive	5 (71.4%)	5 (71.4%)	NS	1.00 (0.09-10.16)
Negative	2 (28.6%)	2 (28.6%)		

Data are expressed as frequency and %. *Fisher Exact test compare proportions between groups. n=number; OR=Odds ratio; CI=confidence interval; $p > 0.05$ is not significant (NS). NA= not applicable for calculation of significance.

Discussion

The cell-mediated adaptive immune response is regulated by the MHC or HLA in humans.[16] KT 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 which may lead to graft rejection.[17, 18]

Serum creatinine is a well-known biomarker for renal function and an important indicator of graft status. The regular measurement of serum creatinine post transplantation could detect the graft dysfunction even before histological diagnosis.[19] In this study, serum creatinine was increased in 34.9% recipients during their post transplantation follow up; four of them had histological evidence of acute rejection. The study by Younespour et al., 2016, found that there was a strong association between graft dysfunction and elevated serum creatinine levels. However, changes in serum creatinine may not be equivalent to the degree of graft injury and it may change with other renal causes.[20]

Antibody mediated rejection (AMR) is defined as allograft rejection caused by recipient's Abs directed against donor class I and class II HLA (DSAs) and blood group antigens.[22] Preformed Abs or *de novo* Abs has become a biomarker for AMR graft loss. HLA Abs are risk factors for hyperacute, acute, and chronic allograft rejections.[23, 24]

In the current study, Class I and II PRA were studied pre transplantation and 15-16 weeks post transplantation in 43 kidney transplant recipients. The pre transplantation PRA was present in 6/43 recipients (14%). Rejection did not occur in five recipients of them. The sixth recipient had post-transplantation class I and II PRA MFI >10000 and acute active AMR with T cell mediated rejection. This agreed with finding of the study by Caillard et al., 2017, who reported that most preformed DSA disappeared after kidney transplantation,[25] but DSA which have been persistent after transplantation with high MFI values cause AMR. Therefore, researchers disputed that preformed DSAs above certain threshold become deleterious if they persist after transplantation. Moreover, Wang et al., 2019, demonstrated that preformed DSA with a high MFI that persist after transplantation were associated with severe early acute rejection and graft loss.[26] Also, Phillpott et al., 2022, reported that rising DSAs MFI titer was more considerable and of clinical significance than steady or declined titer.[27] However, Malheiro et al., 2017 and Callemeyn et al., 2021, stated that either preformed or *de novo* DSA were indicators of AMR, graft dysfunction and poor graft survival.[28, 29]

In the present study, out of the 37 recipients who had negative pre transplantation PRA, the PRA was still negative post transplantation in 40.5% of recipients and the remaining 59.5% recipients developed *de novo* PRA post transplantation, three of them developed acute rejection. *De novo* PRA were 21.6% class I, 16.2% class II and 21.6% mixed class I and II. This agreed with the findings of Chung et al., 2014, Ramon et al., 2017 and Cun et al., 2021 who reported that 13%-30% of kidney transplant recipients developed *de novo* DSA although they were non sensitized at transplantation time or even getting proper pre-transplantation desensitization program within 5 years post- transplantation.[30, 31,32] Moreover, Wiebe et al., 2012 and Yell et al., 2015, demonstrated that there was a very heterogenous graft outcome after appearance of *de novo* DSAs (ranging from no graft injury to rapid graft dysfunction and loss).[33,34] DSAs with the same MFI strength do not cause the same outcome. This could be explained by the findings of the study by Yoo et al., 2014, Tambur et al., 2015 and Lefaucheur et al., 2017, who found that the binding ability of DSAs to the beads (as determined by the Luminex solid phase assay) might not be as binding ability of DSAs to HLA antigen. They also reported that there are 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 that occur in extremely high DSAs titer).[35,36,37] Song et al., 2012 and Guidicelli et al., 2016, found that *de novo* DSA were mainly directed against donor class II HLA particularly if they were in high titer and this usually occurs during the first year after transplantation with 20% possibility of occurrence in the next four years.[38,39]

Our study showed that the most prevalent DQ PRA detected were DQ7 (35.7%), DQ8 (42.9%) and DQ9 (71.4%) while the least prevalent DQ PRA was DQ2 (7.1%). Lee et al., 2016, found that the most prevalent DQ-DSA were DQ6 (33.3%), DQ7 (23.5%), and DQ2

(23.5%).[40] Also, DeVos et al., 2012, found that the most prevalent DQ antibodies detected were DQ7 (25%), DQ2 (19%), and DQ4 (19%) [41]. This variation in detection rates of HLA-DQ antibodies may be due to the fact that various centers, particularly those dealing with Class II antibodies, have varying MFI cutoff levels.[41] The duration of follow-up after transplant, application of protocol biopsy, follow-up plan following transplant, various procedures, and assay utilized for detection are the other important factors. This difference in frequency of anti HLA-DQ antibodies could be also due to variation in racial and ethnic background of studies individuals'. The time taken for the *de novo* antibody to be detected differs from patient to patient but generally is formed 6-month posttransplant. However, there is a delay in the detection if antibodies other than DQ are also present. This cohort of DQ + non-DQ antibodies take around four months for detection.[40]

The current study showed that 58.1% of the 43 recipients developed at least one post-transplant PRA, 4.7% recipients had DQ PRA alone (DQ-only), 25.6% recipients developed A,-B, and/or -DR antibody in absence of a DQ antibody (non DQ), and 27.9% recipients developed a DQ antibody in addition to other non DQ antibodies (DQ + non DQ). Thus, 32.6% recipients had a DQ antibody making it the most common PRA detected. The study by López del Moral et al., 2022, reported that out of 400 kidney transplant recipients, 260 patients (65%) developed post-transplant DSA, 167 patients (64.2%) developed DQ DSA alone (DQ-only DSA), and 93 patients (35.8%) developed a DQ antibody in addition to other non DQ antibodies (DQ + non DQ). Thus, 260 patients (65%) had a DQ antibody; making it the most prevalent DSA detected [42]. Also, Lee et al., 2016, reported that 79 (30%) out of the 263 patients developed post-transplant DSA, 35 patients (13.3%) developed DQ DSA alone (DQ-only DSA), whereas 16 (6.1%) developed a DQ antibody in addition to other non DQ antibodies (DQ + non DQ) and 28 patients (10.6%) developed a donor-specific A,-B, and/or -DR antibody in absence of a DQ antibody (non DQ). Thus, 51 patients (19.4%) had a DQ antibody making it one of the most prevalent DSA detected.[40] Jennifer et al., 2012, found that 62 (18%) out of the 347 patients developed post-transplant DSA, 33 patients (10%) had DQ DSA alone (DQ-only DSA), 14 patients (4%) developed a donor-specific -A,-B, and/or -DR antibody without a DQ antibody present (non DQ), and 15 patients (4%) developed a DQ antibody in addition to other non DQ antibodies (DQ + non DQ). Thus, 48 patients (14%) had a DQ antibody making it the most prevalent DSA detected.[43] Also Willicombe et al., 2012, reported that 92 (18.2%) out of the 505 patients developed post-transplant DSA, 26 patients (5.2%) developed a DQ antibody in addition to other non DQ antibodies (DQ + non DQ), whereas 24 (4.8%) developed DQ DSA alone (DQ-only DSA) and 42 patients (8.3%) developed a donor-specific A,-B, and/or -DR antibody in absence of a DQ antibody (non DQ). Thus, 50 patients (10%) had a DQ DSA antibody. It is still unclear why DQ DSA rates are so high, however, HLA-DR mismatches might be more immunogenic than DQ. This could be partly because the current United Network of Organ Sharing (UNOS) allocation scheme considers DR matching, but not DQ matching.[44]

The present study showed that during the 15–16-week follow-up period, the total incidence of biopsy-proven acute rejection was 9.3% (4/43), with recipients in the DQ + non DQ groups had statistically significant higher percentage of acute rejection (30.8%) compared with the other groups (0%) (p= 0.0171). DeVos et al., 2012, reported that during the 26-month follow-up period, the overall incidence of biopsy-proven acute rejection was 15% (52/347), with a greater risk in the DQ + non DQ and non DQ groups than in the no-DSA group.[41] Also, Jalalzadeh et al., 2015, reported that during a one-year follow-up period, the total incidence of biopsy-proven acute rejection was 18.8% (125/663).[45] There's growing evidence that the sex of the recipient and donor influences how well a kidney allograft works. It has been demonstrated that allografts from female donors had greater incidence of acute rejection and allograft loss within a year.[45] Puoti et al., 2016, reported that the 5-year survival rate of female donor kidneys was lower than

that of male donor kidneys.[46] While Zeier et al., 2002 found that female recipients of male donors' kidneys had a more targeted survival advantage over male recipients of female donors' kidneys.[47] Some explanations for these observations include that the female kidney has fewer nephrons than the male kidney[48] and higher expression of human leukocyte antigens, which can cause increased immunogenicity.[46,47] However, the current study showed that recipients of kidney from female donors had a statistically higher frequency of increased creatinine level (50.0%) compared to recipients from male donors (6.7%%), but with no difference in rate of acute rejection during 15-16 weeks post transplantation follows up period (p = 0.999).

The current study showed that recipients with positive anti HLA-DR Abs and anti HLA-DQ Abs had statistically significant higher percent of rejection (28.6%) compared to (0.0%) of those with negative anti HLA-DR Abs and anti HLA-DQ Abs (p-value: 0.016). This agreed with the study by Leeaphorn et al., 2018, who found that the production of donor specific HLA antibodies against class II correlated with the increased incidence of acute AMR, chronic graft dysfunction and graft loss. Also, DSA against class II or both class II and class I antigens had a strong association with graft loss.[49]

Our study showed that recipients who develop post transplantation PRA against HLA DQ4, DQ5 or DQ9, had a significant higher risk of acute rejection than other types of HLA DQ (Odds Ratio: 18.5, 18.5 and 13.71, respectively).These results are partially in agreement with those of Leeaphorn et al., 2018, who showed that when DQ5 was the donor mismatch, there was an increased risk of graft loss in receivers of living kidney donors, while demonstrated that when the kidney donor mismatch was DQ8, they observed a greater risk of acute rejection, independent of recipient DQ. Certain donor HLA-DQ mismatches, such as HLA-DQ4, did not result in a higher incidence of acute rejection. Thus, in order to improve both short- and long-term outcomes, a more sophisticated approach to DQ mismatching may be crucial.[49]

Our study found that there was no difference between matched and mismatched DQ regarding acute rejection, which means that the risk of acute rejection was not correlated with HLA-DQ mismatching. However, a short follow-up period (15–16 weeks) and a small number of pairings with HLA-DQ genotyping (only 14) may have constrained our study. Our results agreed with those of Freedman et al., 1997, who concluded that there was no impact of HLA-DQ mismatching on transplant survival.[50] Similarly, Sasaki and Idica, 2010, demonstrated that graft survival was unaffected by HLA-DQ.[51] The last two investigations[50, 51] were carried out when the HLA-DQ typing method was less sophisticated and accurate than it is now. It is possible that the small number of patients and brief follow-up period in their studies contributed to the absence of correlation between HLA-DQ mismatching and graft failure. Our study founding was not in agreement with that of Lim et al., 2016, who showed that, independent of HLA-ABDR mismatches and early immunosuppression, HLA-DQ mismatching was linked to an increased risk of acute rejection.[15] Moreover, the study by Leeaphorn et al., 2018, which included 93,782 patients showed a correlation between the likelihood of acute rejection and graft loss and HLA-DQ mismatching.[49]

In conclusion, preformed class I & II PRA may turn to negative post transplantation, but they become deleterious if they persist in high titer after transplantation. Anti HLA-DQ antibody was one of the most prevalent and detected post-transplant PRA. Recipients with post-transplant positive anti HLA-DR Abs and anti HLA-DQ Abs had higher frequency of acute rejection compared to those with negative anti HLA-DR Abs and anti HLA-DQ Abs. Recipients who develop post-transplantation PRA against HLA DQ4, DQ5 or DQ9 had a higher risk of acute rejection than other types of HLA DQ. Matched and mismatched DQ are not associated with increased risk of acute rejection. Recipients of kidney from female donors had higher frequency of increased creatinine level, but not associated with increased risk of acute rejection during the 15-16 weeks post transplantation follows up period.

Author Contributions

SKS; Project administration, conception and design of the study and supervision. TTHE; Methodology, analysis and interpretation of data, writing, review and editing. AAM; Analysis and interpretation of data, Writing, review and editing. MGA; Writing the original draft, review & editing, and practical work.

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.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The protocol of the study was reviewed and approved by the Institutional review board (IRB) of the Faculty of Medicine, Assiut University (IRB local approval no: 17200326, dated April 2019).

Informed consent

The importance of the study was explained to all participants and an informed consent was obtained from each participant or the parents before enrolling in this study.

References

1. Parija, S.C., (2023). Structure and Function of Immune System. In Textbook of Microbiology and Immunology (pp. 129-149). Singapore: *Springer Nature Singapore.*
2. Monos, D.S. and Winchester, R.J., (2019). The major histocompatibility complex. *In Clinical Immunology* (pp. 79-92). *Elsevier.*
3. Sullivan, H.C. and Alquist, C.R., (2022). Human Leukocyte Antigens. *Practical Transfusion Medicine*, pp.33-46.
4. Jan, M.Y., Patidar, K.R., Ghabril, M.S., et al. (2024). Optimization of Kidney Health in Liver Transplant Candidates: Pretransplant Considerations and Modalities. *Transplantation*, pp.10-1097.
5. Williams RC, Opelz G, McGarvey CJ., et al. (2016). The risk of transplant failure with HLA mismatch in first adult kidney allografts from deceased donors. *Transplantation* 100: 1094–1102
6. Hod T, Goldfarb-Rumyantzev AS., (2015). Clinical issues in renal transplantation in the elderly. *Clin Transplant;* 29: 167–175.
7. Kilduff, S., Steinman, B. and Hayde, N., (2024). Changes in graft outcomes in recipients< 10 kg over 25 years of pediatric kidney transplantation in the United States. *Pediatric transplantation*, 28(1), p.e14679.
8. Lakshmi, V.U., Balakrishnan, D., Narmadha, M.P. et al. (2024). Antibody-Mediated Rejection In Liver Transplantation-An Unresolved Puzzle. *J Liver Transplantation*, p.100200
9. Affdal, A., Ballesteros, F., Malo, M.F., et al. (2024). Canadian Kidney Transplant Professionals' Perspectives on Precision Medicine and Molecular Matching in Kidney Allocation. *Transplantation Direct*, 10(1), p.e1565.
10. Devos JM, Gaber AO, Teeter LD., et al. (2014). Intermediate-term graft loss after renal transplantation is associated with both donor-specific antibody and acute rejection. *Transplantation* 97: 534–540, 2014
11. Doxiadis II, de Fijter JW, Mallat MJ., et al. (2007). Simpler and equitable allocation of kidneys from postmortem donors primarily based on full HLA-DR compatibility. *Transplantation* 83: 1207–1213
12. Freitas MC, Rebellato LM, Ozawa M., et al. (2013). The role of immunoglobulin-G subclasses and C1q in de novo HLA-DQ donor-specific antibody kidney transplantation outcomes. *Transplantation* 95: 1113–1119
13. Tambur AR. Engen RM, Jedraszko AM., et al. (2021). Substituting imputation of HLA antigens for high-resolution HLA typing: evaluation of a multiethnic population and implications for clinical decision making in transplantation. *Am J Transplant.*; 21:344–352.
14. Do Nguyen HT, Wong G, Chapman JR., et al. (2016). The association between broad antigen HLA mismatches, eplet HLA mismatches and acute rejection after kidney transplantation. *Transplant* Direct 2: e120, 2016pmid:27990485
15. Lim WH, Chapman JR, Coates PT., et al. (2016). HLA-DQ mismatches and rejection in kidney transplant recipients. Clinical Journal of the *American society of Nephrology.* 11(5):875-883.
16. Robson K. J., Ooi J. D., Holdsworth S. R., et al. (2018). HLA and kidney disease: from associations to mechanisms. *Nature Reviews. Nephrology.* 14(10):636–655.
17. Joshi S, J. Gaynor J, Ciancio G., (2012). Review of ethnic disparities in access to renal

transplantation. Clinical *Transplantation.* 26(4):E337-E343.

18. Phillips BL, Callaghan C. (2020). The immunology of organ transplantation. *Surgery* (Oxford). 38(7):353-360.
19. 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.
20. 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).
21. 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.
22. 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.
23. 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.
24. Morath C, Opelz G, Zeier M, et al. (2014). Clinical relevance of HLA antibody monitoring after kidney transplantation. *Journal of immunology research.* 14(3):94-106.
25. Caillard S, Becmeur C, Gautier-Vargas G, et al. (2017). Pre-existing donor-specific antibodies are detrimental to kidney allograft only when persistent after transplantation. *Transplant International.* 30(1):29-40.
26. Wang J, Wang P, Wang S, Tan J. (2019). Donor-specific HLA Antibodies in Solid Organ Transplantation: Clinical Relevance and Debates. *Exploratory Research and Hypothesis in Medicine.* 4(4):76-86.
27. 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.
28. 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 antigenincompatible kidney transplantation. *Transplant International*. 30(4):347-359.
29. 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.
30. 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.
31. 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.
32. 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.
33. 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.
34. 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.
35. 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.
36. 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.
37. 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.
38. Song EY, Lee Y-J, Hyun J, et al. (2012). Clinical relevance of pretransplant HLA class II donorspecific antibodies in renal transplantation patients with negative T-cell cytotoxicity crossmatches. *Annals of laboratory medicine.* 32(2):139-144.

39. Guidicelli G, Guerville F, Lepreux S, et al. (2016). Noncomplement–binding de novo donor-specific anti-HLA antibodies and kidney allograft survival. *Journal of the American Society of Nephrology.* 27(2):615-625.
40. Lee H, Min JW, Kim JI, et al. (2016): Clinical significance of HLA-DQ antibodies in the development of chronic antibody mediated rejection and allograft failure in kidney transplant recipients. *Medicine* (Baltimore); 95:e3094.
41. DeVos JM, Gaber AO, Knight RJ, et al. (2012). Donor-specific HLA-DQ antibodies may contribute to poor graft outcome after renal transplantation. *Kidney Int* 82: 598–604
42. López del Moral, C., Wu, K., Naik, M., et al. (2022). The natural history of de novo donor-specific HLA antibodies after kidney transplantation. *Frontiers in Medicine*, p.2683.
43. Jennifer M. DeVos, A. Osama Gaber, Richard J. Knight, et al. (2012). Donor-specific HLA-DQ antibodies may contribute to poor graft outcome after renal transplantation. *Kidney International*. 82, 598–604; doi:10.1038/ki.2012.190
44. Willicombe M, Brookes P, Sergeant R, et al. (2012). De novo DQ donorspecific antibodies are associated with a significant risk of antibody-mediated rejection and transplant glomerulopathy. *Transplantation* 94: 172–177.
45. Jalalzadeh, M., Mousavinasab, N., Peyrovi, S. et al. (2015). The impact of acute rejection in kidney transplantation on long-term allograft and patient outcome. *Nephro-urology monthly*, 7(1).
46. Puoti F, Ricci A, Nanni-Costa A, et al. (2016). Organ transplantation and gender differences: a paradigmatic example of intertwining between biological and sociocultural determinants. *Biol Sex Differ.* 7:35.
47. Zeier M, Dohler B, Opelz G, et al. (2002). The effect of donor gender on graft survival. *J Am Soc Nephrol.* 13:2570–6.
48. Kolonko A, Chudek J, Wiecek A., (2013). Nephron underdosing as a risk factor for impaired early kidney graft function and increased graft loss during the long-term follow-up period. *Transplant Proc.* 45:1639–43.
49. Leeaphorn, N., Pena, J. R. A., Thamcharoen, N., et al. (2018). HLA-DQ mismatching and kidney transplant outcomes. *Clinical journal of the American Society of Nephrology*: CJASN, 13(5), 763.
50. Freedman BI, Thacker LR, Heise ER, et al. (1997). HLA-DQ matching in cadaveric renal transplantation. *Clin Transplant* 11: 480–484
51. Sasaki N, Idica A. (2010). The HLA-matching effect in different cohorts of kidney transplant recipients: 10 years later. *Clin Transpl* 261–282