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DENOVO HLA DQ ANTIBODIES IN RENAL TRANSPLANTATION

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ABSTRACT
Donor-specific antibodies (DSA) have proved awell established biomarker predicting antibody against the human leukocyte antigen (HLA)-A, -B, and -DR loci. It has detrimental effect on renal allograft outcomes including high incidence of antibody-mediated rejection, graft dysfunction, inferior graft survival and poor transplant outcomes. Inadequate data is available describing the incidence and impact of *denovo* HLA-DQ antibodies. 644 renal transplant recipients without pre-transplant donor-specific antibodies over the period of four years from the western part of India were examined. 23% (157/644) patients developed donor-specific antibodies, in which 17.8% (28) had a HLA-class I and 82.6% (129) had class II antibodies.55.8% (72) class II positive patients developed denovo DQ antibodies. The mean of serum creatinine and proteinuria was significantly higher in HLA-DQ antibodies developed patients than those without antibodies.18.05% (13/72) denovo positive patients rejected grafts. The study is conclusive that the donor-specific HLA-DQ antibodies were the most common type detected and these antibodies may contribute to poor graft outcomes.

KEYWORDS: Antibody-mediated Rejection (AMR), Kidney Transplantation, *denovo* Donor Specific Antibody (dnDSA), HLA-DQ Antibody.

INTRODUCTION
Studies over the last decade have established the role of post transplantation human leukocyte antigen (HLA) antibodies.Antibody-mediated rejection has been recognized as the leading cause of graft dysfunction and DSA are strongly associated and may be a cause of allograft loss[1,2] in kidney transplantation[3-8] DSAs identified before kidney transplant can cause early rejection, such as hyperacute rejection, accelerated acute rejection and early acute antibody-mediated rejection.[5,6,9-10] Some studies in primates have proved that, if left untreated, an immunologic reaction starting with DSA formation will leadto chronic rejection of the allograft[9,11-14]

Denovo DSA (dnDSA) formation of antibodies against donor HLA has been recognized as the risk factors for high HLA mismatches especially DQ, inadequate immunosuppression and non-adherence, and graft inflammation, such as viral infection, cellular rejection, or ischemia injury which can increase graft immunogenicit.[15-17] *Denovo* DSAs are predominantly directed to donor HLA class II mismatches and usually occur during the first year of kidney transplant, but they can appear anytime, even several years later.[15-17] They have been reported to be as frequent as 15-25% in 5 years'post-transplant patients.[18] These antibodies have been reported towards both Class I and Class II antigens.[19-21] DQ antibodies detected in conjunction with other class Iand class II antibodies were associated with significantly reduced graft survival. Importantly, Antibody mediated rejection and graft losses did not occur in patients with low levels of DQ-only antibodies; however, the role of DQ DSA alone is not well studied. This study deals with the DQ DSA incidence and actual 4-year post graft outcomes extensively.

MATERIALS AND METHOD
HLA typing methods
All 644 patients (Male: 547 and Female:97) enrolled in the study were from India.They were admitted in the Institute of Kidney Disease and Research Center, Ahmedabad, Gujarat. They were transplanted during the year 2013-2014. The data for all patients as age, gender, frequency and kind of RTx, history of blood transfusion and pregnancy was collected (Table 1) and was investigated for serum creatinine (mg/dl), glomerular filtration rate (eGFR) and urinary protein (g/day). The

patients were between the ages of 21-65 Years (Table 2). Donor and recipient typing were done by molecular methods according to their respective manufacturer's directions. All recipients and living donors were typed by a sequence-specific oligonucleotide probes (Labtype SSO, One Lambda) with positive hybridization detected by Luminex and data analyzed by Fusion software (One Lambda). As a back-up all patients and donors were also typed via sequence-specific primers (HLA-A/B/Cw/DR/DQ-SSP) (BAG Healthcare-Germany). The results obtained by electrophoresis were later analyzed by software.

Pre transplant cross-matches test of all patients was negative. HLA typing was carried out in patients and donor pre-transplant. Sequence specific primers [SSP] and Sequence specific oligo nucleotide [SSO] were used. Luminex-200 platform has used for HLA antibodies screening along with standard CDC, flow cytometry cross match.

Determination of post-transplant DSA

Recipient sera was screened for class I and class II HLA DSA through the use of single HLA antigen-charged polystyrene beads according to the manufacturer's instructions (LAB Screen; One Lambda, Canoga Park, CA) utilizing a multichannel flow array (Luminex, Austin, TX), and identified via Fusion software (LAB Screen; One Lambda). Patient serum was monitored for DSA at months 1, 3, 6, 9, and 12 post transplant, every 6 months thereafter, monitor/diagnosed for graft dysfunction or rejection. A positive *de novo* DSA was defined as a new antibody not present before transplant with donor specificity and a MFI of greater than 1000, which is at the lowest limit of detection by a flow cytometriccrossmatch in our center. For both pre-transplant and post-transplant DSA, only those at a MFI greater than 1000 were considered.

Strength of DSA were defined as the following; weak 1000–4000 MFI, moderate >4000–8000 MFI, strong >8000–15,000 MFI, and very strong >15,000 MFI. In our system, 4000 MFI is the approximate lower limit of a positive B-cell flow cross match, while 8000 and greater MFI antibodies are associated with increasingly positive AHG cytotoxicity cross matches. Peak-MFI DSA was defined as the highest MFI of a single DSA in each individual recipient.

RESULTS

644 patients were transplanted during the year of 2013-2014. 24.37% (157/644)developed *denovo* post-transplant DSA. 45.8% (72/157) patients had developed DQ *denovo* DSA (DQ-only). The pre transplant have monitored different parameters as: chronic kidney disease (CKD in 201), Chronic Glomerulo Nephritis (CGN in 150), hyper tension (HTN in 129), diabetic and hyper tension (DM+HTN in 30) these are the causes of original (native) kidney failure and other causes also monitored (Table 3). Only 4 recipient was found different in ALPORT.

All recipients were segregated in two different groups, DSA positive 157(24.37%) and DSA negative 487(75.63%) based on the time of DSA appearance. Of the total 157 DSA positive patients, 45.8%(72) were DQ *denovo* DSA at high risk. Most DSA were directed to class II(76), whereas 28(4.34%) were directed to class Ipatients. Thus a majority of DSA were directed to class II 76 (11.80 %) (DR, DQ, DR+DQ) and class I & class II 53(8.22%) (A, B, CW, DR, DQ). Patient were prospectively monitored for DSA at months 1, 3, 6, 9, and 12 post-transplant, every 6 months thereafter. dnDSA, proteinuriaand serum creatinine was monitored in post-transplantpatients.

During the study period, HLA typing was carried out by PCR methods (A, B, CW, DR and DQ-SSP) in all patients and the donor. 5-10 antigens matched in 276 patients and9-10 antigens matched in only 11-11 patients. 2 and 6 antigens matched in moderate from 70 to 81 patients (Table 4). Most DSA was directed to class II (*n*=76; 11.80 %), class I & II (n=53; 8.22%) and class I (n=28; 4.34%) out of these class II, DR (*n*=32), DR+DQ (*n*=53), DQ (*n*=72). In contrast, a majority of non-DSA were directed to class I (*n*=10) (Figure1).

STATISTICAL ANALYSIS

Patients were categorized into one of the four following groups based on the presence and type of DSA. 75.63% (487) patients were DSA negative;72 patients have DQ-only (those with only a DQ DSA); 28 patients have non DQ (those with DSA directed against an HLA-A, -B, and -DR, but without a DQ DSA), and 87 patients have DQ+ non DQ DSA (those with DSA against HLA-A, -B, and/or -DR, as well as against DQ). Baseline categorical and continuous variables were compared (gender, ethnicity, retranslate status,induction agent, and rejection) and continuous variables (age, PRA, creatinine, and time to rejection), respectively.

DISCUSSION

The problem of clarifying that HLA antibodies develops at different post-transplant intervals could have different cytotoxicity and graft tissue damage. Increasing evidence highlights the significant impact of DSA against HLA -A, -B, and -DR antigens in kidney transplantation; however, the importance of DQ DSA alone is not well described. Our findings suggest that DQ antibodies are the most common *denovo* DSA detected post-transplant, and that these antibodies may be associated with a detrimental effect in terms of rejection and graft dysfunction. Furthermore, while we observed no difference in graft survival in patients with DQ antibodies alone, DQ antibodies detected in conjunction with other class I and class II antibodies were associated with significantly reduced graft survival. Importantly, AMR and graft losses did not occur in patients with low levels of DQ-only antibodies.

Here, we report an overall prevalence of *denovo* DSA of 18%, while the rate reported in the literature is highly variable ranging from 4 to 27% (9, 22-24). Most studies reporting on the impact of class II antibodies focus primarily on outcomes related to DR. Through the use of solid phase antibody detection and identification methods, improved reporting and better characterization of HLA-directed antibodies, including DQ antibodies, have been attained before and after transplant. Of the studies published that report the percentage of *denovo* DSA directed against DQ, the rate reported varies from 33 to 90%.[25-27] This variation may be attributed to differences in sample collection times, assays used to detect antibodies, and the MFI cutoff considered significant. In the cohort of patients reported herein, 77% developed a DQ DSA. The reason for the high rate of DQ DSA remains unknown, even recent data indicate that HLA-DR mismatches may be more immunogenic than DQ. This may be in part due to the fact that DR, but not DQ, matching is taken into consideration in the current UNOS allocation system.

In this report, we found that the majority of DSA occur within 6 monthsto one-yearpost transplant, with no difference in time to DSA development between all groups. Cooper *et al*. found that 91% of DSA were detected within 6 months of transplant, while Zhang *et al*. found that 63% of patients developed DSA within 1 month after transplant and remaining 37% develops within 6 months'post-transplant.[22-23] We did notice a slight delay in detection in the DQ+ nonDQ DSA group, with a median time of detection around 12 months compared with the other groups at 6 months. This may be the result of a higher rate of detection 'for cause' as opposed to predetermined screening time points, specifically for episodes of graft dysfunction. Consequently, this group had a higher rate of AMR, in many cases as a result of noncompliance. *De novo* DSA have been reported towards both Class I and Class II HLA antigens, however found to be predominantly towards Class II.[20-21] Among Class II antibodies, DQ antigens have been found to be most frequent.[21]

Overall, our findings suggest that development of *denovo* DQ DSA may have clinically relevant implications. With an understanding of the significance of *denovo* DSA, future efforts to determine potential mechanisms to remove these and other antibodies in order to decrease the risk of acute and chronic rejection should be prioritized. Research of investigational therapies such as intravenous (IV) immune globulin, plasma exchange, and/or depleting antibodies, such as rituximab, are imperative to determine their ability to remove DSA and potentially alleviate long-term consequences of all classes of DSA, including DQ. It may also be beneficial to determine whether or not DQ antibodies are complement fixing in the context of AMR, implying that they are capable of forming a membrane attach complex leading to cell destruction in situ.DQ antibodies are more resistant to treatment comparing to DR or class I antibodies.

CONCLUSION

Through prospectively monitoring DSA, we found *denovo* DQ DSA more frequently compared with HLA-class I or -DR DSA in kidney transplant recipients with chronic AMR.DQ antibodies individually, particularly those expressed at higher MFIs, resulted in graft outcomes that were inferior compared with patients without DSA, and similar to those seen with more established *denovo* HLA antibodies. These data suggest patients with antibodies to DQ may require similar interventions as those with *denovo* -A, -B, and -DR antibodies.Screening for HLA-DQ DSA after kidney transplantation seems favorable to obtain better long-term outcomes of kidney allografts.

DECLARATIONS

Conflict of Interest

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

Table:1 - All patients enrolled in the study [644] were Asian [Indian] origin among them 547 patients were male and 97 were Female between the age of 15 to 62 years.

Recipients Characteristics	No
Gender	547 Male / 97 Female
Origin	Asian
Age [Years]	15-62 Years

Table 2: All patients having living related donor and majority of them were parents and wife between the age of 21-65 Years.

Donor's Characteristics	Total no of Cases: 644
Living	644
Age [Years]	21-65
Gender	189 Male / 455 Female
Donor Relation to Patient's	
Parents	333
Son /Daughter	6
Siblings	63
Husband to Wife	30
Wife to Husband	139
KPD	59
Extended Family	14
Baseline S.Creatinine	0.8 ± 1.25 mg /.dl

Table 3: Majority of patients were diagnosed chronic kidney disease and chronicglomerulo nephritis, hyper tension, diabetes etc. cause of native kidney failure.

Basic disease n=644	No
ADPKD	10
ALPORT	4
DM+HTN	30
FSGS	5
CGN	150
CIN	28
IgA Nephropathy	8
HTN	129
CKD	201
OBSTRACTIVE	18
MPGN	5
REFLUX	6
OTHERS	50

Table: 4 HLA typing was carried-out by PCR methods [A-B-Cw-DR and DQ] in all patients and donor, As majority of them were their parents hence 5 antigen match is majority in number.

HLA Matching [A ,B, Cw, DR and DQ]	Total no of Cases: 644
0	27
1	46
2	70
3	44
4	28
5	276
6	81
7	35
8	15
9	11
10	11

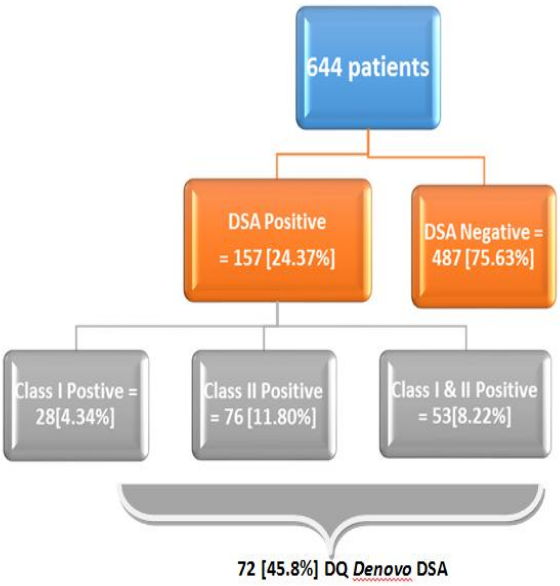

Figure 1: Antibodies detected in DQ*denovo* DSA-positive patients recognized a total of 72 (45.8%) out of 644 a total patients.

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