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Original Article

Pathology Section

Importance of Phenotyping Minor Blood Group Antigen in Renal Transplant Donors: A Retrospective Cohort Study

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ABSTRACT

Introduction: Various studies have implicated minor antigens with increased graft rejection in the long term, especially in alloimmunised patients. Recipient's antibody testing is done exhaustively during each case of rejection. However, without data on donors' minor antigen profile, this significant cause of graft failure may be overlooked.

Aim: To highlight the necessity of testing and creating a database by documenting the prognostically important minor blood group antigen in kidney transplants donors.

Materials and Methods: This was a retrospective cohort study done on 650 kidney transplant donors from Kolkata, West Bengal, India. Data was collected from August 2014 to July 2022, a period of 10 years and analysed from August 2022 to December 2022. Blood group antigens were identified by immunoserological tests and the phenotypes were recorded with specific importance to minor blood groups. Genotype confirmation was done, where possible. The prevalance percentage of minor blood group phenotype was analysed using statistical analysis {Statistical Package for the Social Sciences (SPSS) build 1.0.0.1275 version 26.0}.

Results: In 650 donors, mean age was 33±7 years and male: female ratio was 1.1:1. The prevalence of clinically important minor blood group phenotypes like Kidd {Jka-499 (76.8%), Jkb-449 (69.1%)}, Duffy {Fya-559 (86%), Fyb-396 (60.9%)}, Lewis {Le-a-112 (17.2%), Le-b-402 (61.8%)} and MNS {M-567 (87.2%), N-266 (40.9%), S-375 (57.7%), s-572 (88%)} in the kidney transplant donor group was documented. Lewis Le(a-b+) -402 (61.85%), Duffy Fy(a+b+) -306 (47.08%), Kidd Jk(a+b+) -298 (45.84%) and MNS (M+N+) -301 (46.30%), (S+s+) -298 (45.85%) were the most prevalent minor antigens. The phenotypes Lewis Le(a+b+), Kidd Jk(a-b-) had null percent in population and Duffy Fy(a-b-), MNS(S-s-) were present 0.15% and 0.15%, respectively in the population.

Conclusion: The result of the present study emphasises the importance of testing, registering a database for immunogenic significant minor blood group antigens in transplant donors. This will aid in investigation of graft failure in alloimmunised patients.

Keywords: Graft rejection, Kidney transplantation, Minor phenotype

INTRODUCTION

In all cases of blood transfusion, there should be compatibility between the donors and recipients' Red Blood Cells (RBC) which is achieved by grouping as well as cross-matching by serological and other methods. Blood group plays the major role for compatibility [1]. International society for blood transfusion has recognised 43 blood group systems and documented the presence of 348 red cell antigens. Around 35 minor blood groups have also been identified in the population which plays a role in transfusion reactions [2,3].

Kidney transplantation requires extensive and thorough compatibility between various parameters of the donor and recipient. Anti A/Anti B antibodies plays a major role in renal allograft rejection and their role have been well-established [4]. But the implication of non ABO system antigens especially Kidd, MNS, Duffy and Lewis group, of which there is evidence of renal expression, have not been studied in detail. These antigens have the capacity for development of innate/acquired antibodies which increases the immunological risk of the patient manifold [5]. They act as minor histocompatibility antigens which have been proved both clinically and experimentally leading to Antibody Mediated Rejection (AbMR), especially in alloimmunised patients and those who are dependent on chronic transfusions. During kidney transplant, the donors and recipients cross-matching is done and compatibility checked before transplant. Whenever features of graft rejection occurs the recipients' antigen as well as antibody profile is analysed in detail. At that junction if the data on donors' minor blood group is available, all cases of mismatch can be meticulously checked for antigen antibody incompatibility leading to graft failure. This will ensure early investigation and better management of immunological risks presented by these in each and every case of graft rejection and renal injury.

Thus, aim of present study was: i) To emphasise the importance of serological and molecular testing for these minor RBC antigen groups in the renal transplant donor cohort: ii) To estimate the prevalence of these clinically significant antigens; iii) To lay the foundation for creating a donor antigen database for reference in each and every case of graft rejection by documentation which can be retrieved while investigating for graft rejection.

MATERIALS AND METHODS

This was a retrospective cohort study which was retrospective in nature. The testing procedures were the routine protocol and the data was assimilated from the record section. The study was conducted in Kolkata, West Bengal, India after approval by the Institutional Ethics Committee (IEC) (IEC no. ADP/IEC/2022/03). Data was collected from August 2014 to July 2022 (10 years) and results were analysed during the period August 2022 to December 2022. The declaration of Helsinki principles was followed in thoroughness. The anonymity of the subjects was maintained. The written informed consent was given by the donors as part of the transplant protocol. Thorough history and relevant demographic details like sex, age, geographical distribution and Body Mass Index (BMI) were noted down.

All the donors were stratified in accordance to their sex, age, and geographic distribution, Live Related Donor (LRD), Live Unrelated Donor (LURD) and BMI. The present study analysed 650 kidney donors. The donor cohort was determined according to the criteria laid down by Transplantation of Human Organs and Tissues Rules,

2014 [6]. The unrelated donors were approved and permitted by the regional authority as per the Government norms [7]. Thorough and detailed urological examination was done.

Inclusion criteria: Live kidney donors and recipients whose cardiological evaluation, pulmonary status, mental and psychiatry abilities, dental health, gynaecological check-ups, premalignant or malignant state and chronic conditions were within normal limit were included in the study.

Exclusion criteria: Cadaveric donors (as number of cases was minimal) as well as those donors with any systemic disorders were excluded from the study.

Study Procedure

A 3 mL of blood was collected in EDTA vacutainer from the 650 kidney donors. All the immunohaematological tests performed in this study used Column Agglutination Technology (CAT). ABO and RhD groups were determined by forward and reverse grouping using monoclonal antisera (Diamed, Switzerland ABO/D+). The tests were run in an automated instrument system (1H500, Biorad, Cressier, Switzerland). Any discrepancy in forward (cell) and reverse typing observed were tested for presence of minor blood group antigens. The RhD negative were tested for weak D and the 'O' blood group were tested for anti H lectin (to rule out minor Rh phenotypes like C,c, E,e and Bombay blood group, respectively). Immunoglobulin (IgG) (monoclonal antisera) anti D and anti H (Tulip Diagnostics, Goa, India) were the reagents used.

Extended phenotyping for other minor blood group antigens like Kell (Kp^a,Kp^b), Duffy (Fy^a,Fy^b), Kidd (Jk^a,Jk^b), Lewis (Le^a, Le^b) and MNS (M,N,S,s) were performed. They were carried out by gel card method (DIAMED, Bio-Rad Laboratories, Switzerland). The procedure for the testing was referred from American Association of Blood Banks (AABB) and Directorate General of Health Services (DGHS) Technical manual [8,9]. A 50 µL of 5% red cell suspension in bromelain (Diamed, Biorad) was loaded in gel column along with positive/negative control. Both forward and reverse grouping was done. The gel column was observed for 4+,3+,2+,1+, negative agglutinate.

Further testing included Adsorption Elution Technique where red cells and reagent were washed, incubated and eluted. Secretory status was checked by performing the inhibition technique. Supernatant of saliva mixed with antibody was tested with 50 µL of 5% suspension of known 'O' red cells.

Quality control: The donors whose sample was negative were cross checked by a combination of inhouse polyspecific check cells comprising IgG+C3d Coombs reagent (Diamed, Switzerland). The reagents were cross checked for their quality by grouping them with known positive/negative red cells panels (commercially available).

Genotype confirmation of the minor blood groups was facilitated especially in samples with discrepancies in serology. They were referred for molecular testing identifying the gene mutations by assessment of Deoxyribonucleic Acid (DNA) extraction and simple sequence length polymorphisms ({Simple Sequence Length Polymorphism (SSLP)} studies).

Approximately, 0.2 g of tissue was taken for automated DNA isolation using the BioSpring 96 work station (Qiagen). The quality and quantity of extracted DNA were evaluated by spectrophotometer at 260 nm and the purity was checked by checking 260:280 ratios. An analysis of DNA integrity was performed by gel electrophoresis. All markers were chosen from NCBI gene databases. A 20 µL of Polymerase Chain Reaction (PCR) was carried out. A 1X reaction buffer was used which was composed of 20 mMTris pH8.4, 50 mM KCl, 3 mM $MgCl_2$ and one Unit Taq DNA polymerase. The reactions were set-up under the following conditions as shown in [Table/Fig-1].

A 15 µL of PCR product was resolved using 4% agarose gel (SLR Molecular biology grade), (120-150 min, 200 volt), ethidium bromide dye (0.5 µg/mL) and 0.5×TAE buffer. It was allowed to run for upto 10 cm from the wells. The gel was imaged using Biorad gel electrophoresis system.

Steps	Cycle	Temperature °C	Time
1	1	95	2 min
2	35	95	30 sec
		55	30 sec
		72	60 sec
3	1	72	5 min
4		4	Sample removed

[Table/Fig-1]: Steps of Polymerase Chain Reaction (PCR).

STATISTICAL ANALYSIS

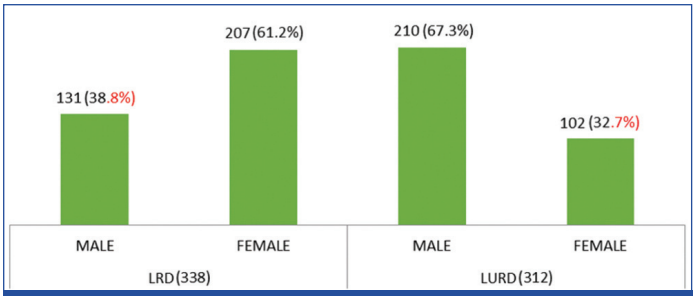
The IBM SPSS for windows (SPSS build 1.0.0.1275) version 26. (IBM corp, Armonk NY, USA) was used as the method of statistical data analysis to calculate the prevalence percentage. The period prevalence over a time was represented as percentage prevalence. It was calculated as frequency of participants in sample with characteristic feature divided by total number of participants in the sample. A frequency distribution was obtained by descriptive analysis of all variables. Categorical or qualitative variable were described as proportions or frequencies. Continuous variables were presented as mean and expressed as percentage.

RESULTS

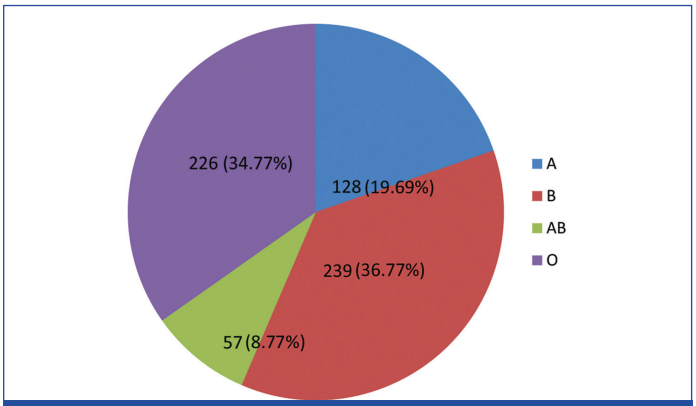
The sample size was 650 live renal transplant donors. The demographic details of the donors are given in [Table/Fig-2,3]. The age group was between 18-60 years with number of donors 341 (52.5%) being males and the rest 309 (47.5%) being females. The mean age was 33.5 years. Most of the donors in the present study were in the age group of 31-45 years (39.42%). Also, the related and unrelated donor frequencies were similar; however in related donors the female to male ratio was much higher compared to unrelated donors where the male population was much more. Donors from the home state had maximum representation whereas from other regions they were around 5-15%. Donors with BMI between 25-29 were 38.2% followed by those with BMI <25=36.2%. The distribution of the major blood groups A, B, O is shown in [Table/Fig-4].

Demography	n (%)
Age (years)	
18-40	321 (49.4)
41-60	329 (50.6)
Graft type	
LRD	338 (52)
LURD	312 (48)
Sex	
Male	341 (52.5)
Female	309 (47.5)
Geographic distribution	
West Bengal	371 (57.1)
Bihar/Jharkhand	76 (11.7)
South India	33 (5.1)
Bangladesh	79 (12.1)
Nepal	91 (14)
BMI (kg/m^2)	
<25	235 (36.2)
25<or≤29	248 (38.2)
≥30	167 (25.6)

[Table/Fig-2]: Baseline demographic characteristics (N=650).
LRD: Living related donor; LURD Living unrelated donor



[Table/Fig-3]: Proportion of male and female in Live Related Donors (LRD)/LURD (Live unrelated donors) (N=650).



[Table/Fig-4]: ABO blood group distribution in donors (N=650).
Rh blood group distribution in donors (N=650) showed Rh D+609 donors (93.6%) and RhD- 41 (6.4%)

[Table/Fig-5] tabulates the prevalence of minor blood group antigens Kidd, Duffy, Lewis and MNS in the kidney transplant donors.

During transplant work-up, the recipients' antigen and antibody prevalence was also analysed in detail and cross-matching done with donors. The 650 donor and recipients' minor antigen profile is given in [Table/Fig-6]. Lewis Le(a-b+)-402 (61.85%), Duffy Fy(a+b+)-306 (47.08)%, Kidd Jk(a+b+)-298 (45.84%) and MNS (M+N+)-301 (46.30%), (S+s+)-298 (45.85%) were the most prevalent minor antigens. The phenotypes Lewis Le(a+b+), Kidd Jk(a-b-) had null percent in population and Duffy Fy(a-b-), MNS(S-s-) were present 0.15% and 0.15%, respectively in the population. Among the recipients, Lewis Le(a-b+) with 409 (63%), Duffy Fy(a+b+) with 297 (45.7%), Kidd Jk(a+b+) with 304 (46.8%), M+N- with 280 (43.1%), and S+s+with 317 (48.8%) had maximum representation. The minor antigens Lewis Le(a+b+), Duffy Fy(a-b-), Kidd Jk(a-b-) and S-s- had null representation. Prevalence of clinically significant red cell phenotype in donors with potential for long term graft rejection with M, N, S antigens in their blood as observed in present study is depicted in details in [Table/Fig-7].

Minor blood group antigens	n (%)
Duffy	
Fya	559 (86)
Fyb	396 (60.9)
Kidd	
Jka	499 (76.8)
Jkb	449 (69.1)
Lewis	
Le-a	112 (17.2)
Le-b	402 (61.8)
MNS	
M	567 (87.2)
N	266 (40.9)
S	375 (57.7)
s	572 (88)

[Table/Fig-5]: Prevalence of clinically significant minor blood group implicated for graft survival in kidney transplant donors.

Minor blood group phenotype	Present study (donors-650) n (%)	Recipient-650 n (%)
Lewis		
Le(a-b-)	136 (20.92)	132 (20.3)
Le(a-b+)	402 (61.85)	409 (63.0)
Le(a+b-)	112 (17.23)	109 (16.7)
Le(a+b+)	0	0
Duffy		
Fy(a+b+)	306 (47.08)	297 (45.7)
Fy(a+b-)	253 (38.92)	249 (38.3)
Fy(a-b+)	90 (13.85)	104 (16.0)
Fy(a-b-)	1 (0.15)	0
Kidd		
Jk(a+b+)	298 (45.84)	304 (46.8)
Jk(a+b-)	201 (30.92)	202 (31.1)
Jk(a-b+)	151 (23.24)	144 (22.1)
Jk(a-b-)	0	0
MNS		
M+N-	266 (40.9)	280 (43.1)
M-N+	83 (12.8)	96 (14.8)
M+N+	301 (46.30)	274 (42.1)
S+s-	77 (11.85)	86 (13.2)
S-s+	274 (42.16)	247 (38)
S+s+	298 (45.84)	317 (48.8)
S-s-	1 (0.15)	0

[Table/Fig-6]: Prevalence of immunogenic potent minor blood group phenotype in renal transplant donors and recipients.

S. No.	Phenotype	n (%)
1	M+N-S+s-	29 (4.5)
2	M+N-S+s+	131 (20.2)
3	M+N-S-s+	110 (17)
4	M+N+S+s-	22 (3.4)
5	M+N+S+s+	101 ((15.5)
6	M+N+S-s+	151 (23.2)
7	M-N+S+s-	3 (0.4)
8	M-N+S+s+	45 (6.9)
9	M-N+S-s+	58 (8.9)
10	M+N-S-s-	0
11	M-N-S-s-	0

[Table/Fig-7]: Distribution of MNS blood group antigen phenotype in the donor transplant population.
MNS: Minor blood group system

The M+N+S-s+phenotype was 151 (23.2%) followed by M+N-S+s+131 (20.2%). M-N+S+s- and M+N+S+s- were rarely present and M+N-S-s- and M-N-S-s- were absent among the donor cohort. Minor blood group antigens like Kell, and Rh (C,c,E,e) were compiled as shown in [Table/Fig-8,9]. KellKp (a+b+)-636 (97.8%), K-k+-638 (98.2%) were most common while KellK+k- and K-k- had null representation. In Rh system, e+609 (100%), C+567 (93.1%) and e-41 (100%), c-36 (87.8%).

Since the genotype study was costly and time consuming, only 12 donors underwent genotype analysis based on mutant gene amplification showing different polymorphic bands.

Results were obtained by oligonucleotide array with multiple probes. It included Jk (4 alleles, including 2 Jk null), FY (4 alleles), MNS (9 haplotypes), Le (4 alleles). Only two mismatch in genotype compared to phenotype assay were observed. It occurred in Kidd and Duffy blood groups. The mistyped Duffy Fy sample was later resolved as it occurred due to incorrect fragmentation sequence.

Minor blood group	n (%)
Kell	
K-k+	638 (98.2)
K+k+	12 (1.8)
K+k-	0
K-k-	0
Kp(a+b+)	636 (97.8)
Kp(ab+)	14 (2.2)

[Table/Fig-8]: Prevalence of Kell phenotype in the donor transplant group.

Antigens	Antigen frequency in D+Donors numbers (%) n=609	Antigen frequency in D- Donors numbers (%) n=41	Total donors numbers (%) N=650
C	567 (93.1)	7 (14.6)	573 (88.1)
E	133 (21.8)	7 (17.07)	140 (21.5)
c	304 (49.9)	36 (87.8)	340 (52.3)
e	609 (100)	41 (100)	650 (100)

[Table/Fig-9]: Distribution of Rh blood group systemantigens (C,E,c and e) in blood donors. (D+:-609) (D-:-41).

The cause for incorrect Kidd Kp^a/Kp^b scoring was unclear. The source of error could have been technical or clerical. MN and Ss and Lewis system had 100% concordance between genotype and serological phenotype.

Due to non compliance and majority of patients being lost in follow-up, there were reported 23 cases (3.5%) of acute rejection and 67 (10.3%) cases of chronic rejection. Clinical symptoms of chronic rejection cases presented with anaemia, mild breathlessness, oliguria with less urine output. Creatinine was high. Histopathological evaluation of the renal biopsy showed allograft rejection, Banff classification type A. There was moderate tubulitis and mild patchy interstitial inflammation comprising lymphocytic aggregate along with areas of haemorrhage.

However, data on their detailed immunological investigations with reports could be collected in only 32 patients. Of these minor blood group mismatches between donors and recipients were appreciated in 21 patients. Eighteen of them presented with lymphocytic leucocytosis, thus cellular cause of rejection remains a possibility for them. Three of the recipients with minor antibody mismatch were started on immediate immunosuppressants.

DISCUSSION

Various theories and evidence collected from studies on renal transplantation suggests that the presence of minor blood group antigens may lead to long term graft rejection due to inferior allograft quality [10]. During work-up for kidney transplantation, blood group antibody screening is mandatory especially in the recipients [11]. There may be evidence of pre-existing high titre antibodies either due to innate causes or due to exposure to foreign antigens. The reasons may be either following a blood transfusion or on a previous graft [12]. Thus, the compatibility between the donor and recipients' antigen antibody profile becomes mandatory. Studies on minor antigens which are expressed in kidney epithelium and become an imminent cause for poor prognosis of the graft survival are important [1,3].

Lewis- Lewis antigen is present primarily in the gut as well as on renal epithelial cells and glomerular cells. Studies have shown that when compared between Lewis positive and Lewis negative recipients; there was a significant difference between their graft survival outcomes [13]. Gratama JW et al., in their study on 1,111 recipients reported that, in patients receiving Human Leukocyte Antigen (HLA) mismatched kidney, if they were Lewis negative, they were at a far higher risk for graft failure [14]. According to a study by Lenhard V et al., on 167 cadaver kidney recipients, there was no significant effect on graft survival on cumulative incompabilities of red cell antigen without Lewis system. However, Lewis system presence showed decreased survival rates [15]. Oriol R et al., studied 1,300 North American cadaveric kidney transplant cases. They found a significant effect of Lewis antigen on graft survival [16]. Their study was correlated by other studies Spitalnik S et al., found eight cases of graft rejections in Lewis negative recipients of Lewis incompatible grafts. Wick MR and Moore SB showed Lewis antigen to be capable of both cell-mediated and humoral immune responses of a cytotoxic nature [17,18].

Kidd- Kidd antigen is encoded by SLC14A1 gene on chromosome 18. The endothelial cells present on the vasa recta of the kidney expresses the Kidd antigen [1]. Study by Holt S et al., described acute cellular rejection, predominantly plasma cell-rich, due to Jk^b antibody binding to renal tissue [19]. Rourk A and Squires JE in a case report, demonstrated allograft rejection 10 years post kidney transplant. Their investigations led to the importance of anti-JK^b (Kidd) antigen, whose appearance in the patient's antibody screening resulted in the poor response [20]. However, another study by Hamilton MS et al., on 370 patients found only interstitial inflammation with Kidd antigen mismatches between donor and recipient [21]. Similarly, in another case of allogenic renal transplant, development of anti Kidd antibodies in an untransfused male patient leading to graft rejection was described in a case study by Sanford KW et al., [22].

MNS- MNS blood group rarely cause transfusion reactions. However, during the reperfusion of an ischaemic, hypothermic allograft in organ transplantation, MNS antigens have a tendency to bind to renal endothelial cells. This leads to increased chances of renal allograft rejection [3]. There was a study by Holt SG et al., on 149 patients whose antibody screening found anti-M antibodies [3]. Five of them underwent renal transplantation. These were however, no cases of rejection due to pretransplantation empirical therapy. Comparison of MNS phenotype with various other studies is depicted in [Table/Fig-10] [2,23-26].

Duffy: Anti Duffy antibodies are IgG and the genes responsible for encoding Duffy antigens are Fy^a and Fy^b on chromosome one. In kidney, the post capillary venules and the peritubular capillaries expresses the Duffy antigens on their endothelial cells. The chemokines Duffy Antigen Receptor Chemokines (DARC) have shown evidence of enhanced inflammatory response by chemokines and leucocytes causing delayed graft rejection [26]. A study by Akalin E and Neylan JF found Duffy (a-b-) patients with less rate of graft survival in renal transplants while Katznelson S et al., found the same effect on African American [27,28]. Duffy negative grafts caused tubulointerstitial fibrosis and arteriolar hyalinisation in allograft transplantation as appreciated by a study by Lerut E et al., [29]. They unmasked the role of minor blood group (specifically Kidd and Duffy) as minor histocompatibility antigens. They found Duffy to lead to more chronic lesions post transplantation while Kidd caused more interstitial inflammation. Mange KC et al., demonstrated Duffy (a-b-) patients to have lower allograft survival in presence of delayed graft function [30].

Donor population cohort for renal transplantation reflects the general population's characteristic distribution. The distribution of these minor blood groups in this geographical domain as found in present study donor group was similar to various studies on minor blood group prevalence [Table/Fig-11] [2,23-26,31]. Presence of minor blood group in the donor population in our geographic region of West Bengal was in accordance with various other phenotype frequencies from other parts of our country. Jha VK et al., analysed the role of donor's demographic characteristics on renal function post transplant and displayed their impact [32]. Though present study comprised predominantly serological method for antigen

S. No.	Phenotype	Present study %, Kolkata 2023	Subramaniyan R [2] %, Coimbatore, 2021	Thakral B et al., [23] % Chandigarh, 2010	Nanu A and Thapliyal RM [24] % New Delhi, 1997	Agarwal N et al., [25] %, New Delhi, 2013	Setya D et al., [26] % Rajasthan, 2020
1	M+N-S+s-	4.5	4.26	7.9	5.51	7.09	4.26
2	M+N-S+s+	20.2	19.68	14.8	13.33	14.96	19.68
3	M+N-S-s+	17	18.09	15.8	22.61	13.78	18.09
4	M+N+S+s-	3.4	2.13	3.47	4.64	5.12	2.13
5	M+N+S+s+	15.5	17.02	19.55	10.72	20.87	17.02
6	M+N+S-s+	23.2	23.4	13.88	27.83	28.74	23.4
7	M-N+S+s-	0.46	0.53	1.26	1.16	1.18	0.53
8	M-N+S+s+	6.9	6.38	9.46	3.48	3.15	6.38
9	M-N+S-s+	8.9	8.50	13.88	9.27	5.12	8.51
10	M+N-S-s-	0	0	0	0.29	0	0
11	M-N-S-s-	0	0	0	0	0	0

[Table/Fig-10]: Comparison of phenotypes in MNS blood group system in the present study (N=650) with other studies of India [2,23-26].

Minor blood group antigens	Present %, Kolkata, 2023	Subramaniyan R [2] % Coimbatore, 2021	Thakral B et al., [23] % Chandigarh, 2010	Agarwal N et al., [25] % New Delhi, 2013	Nanu A and Thapliyal RM [24] % New Delhi, 1997	Kahar MA and Patel RD [31] % Surat, 2014	Setya D et al., [26] % Rajasthan, 2020
Lewis							
Le(a-b-)	20.92	17.78	18.61	59.06	23.98	18.26	17.97
Le(a-b+)	61.85	61.11	60.57	24.81	61.04	65.22	66.85
Le(a+b-)	17.23	21.11	20.82	16.14	13.35	16.52	14.12
Le(a+b+)	0	0	0	0	0	0	0
Duffy							
Fy(a+b+)	47.08	47.87	42.9	48.03	42.57	9.57	44.38
Fy(a+b-)	38.92	39.9	43.85	36.22	40.80	37.39	40.85
Fy(a-b+)	13.85	12.23	13.25	15.36	16.19	4.35	14.76
Fy(a-b-)	0.15	0	0	0.39	0.44	48.69	0
Kidd							
JK(a+b+)	45.84	45.75	49.21	46.06	48.37	52.17	47.12
JK(a+b-)	30.92	37.23	33.44	30.71	29.35	28.69	37.09
JK(a-b+)	23.24	17.02	17.35	22.83	21.74	19.13	15.78
JK(a-b-)	0	0	0	0.39	0.54	0	0
MNS							
M+N-	40.9	41.49	38.5	35.83	42.29	39.13	37.96
M-N+	12.8	15.42	24.6	9.45	14.63	NA	24.11
M+N+	46.30	43.09	36.9	54.72	43.08	48.69	37.93
S+s-	11.85	6.92	12.62	13.39	10.00	NA	17.16
S-s+	42.16	50.53	43.53	47.63	62.09	66.96	40.34
S+s+	45.84	42.55	43.85	38.98	26.75	NA	42.5
S-s-	0.15	0	0	0	1.16	NA	0

[Table/Fig-11]: Comparison of clinically significant minor antigens for transplant with other donor population studies [2,23-26,31].

detection, genotypic confirmation was encouraged as and when feasible and required. Quirino MG et al., found Polymerase Chain Reaction Sequence-Specific Primer (PCR-SSP) as an economical method for genotyping and demonstrated serological methods to be more competent, fast and beneficial than molecular techniques [33]. However, it is a recognised fact that all the above methods employed for blood group antigen typing are very effective, comparatively safe and complementary in their actions.

Limitation(s)

The genotype results could not be tabulated in detail as the molecular test report was not retrieved in majority of the patients. Follow-up of the recipients with records detailing their antibody screening results, clinical vital parameters including creatinine value, kidney biopsy reports according to Banff classification, details of interstitial infiltration/fibrosis and acute or chronic allograft rejection could not be collected as a result of non compliance in many patients. Detailed Ag-Ab profile matching to determine exact cause of rejection could not be done exhaustively. Further studies are required on this. Other recipients are still in follow-up.

CONCLUSION(S)

With widespread increase in chronic kidney disease as well as renal transplant over the years it is mandatory to prevent graft rejection in each and every case by more thorough compatibility tests. All causes presenting an immunological risk for graft survival has to be investigated and documented. Minor blood group incompatibility between donors and recipients especially on alloimmunisation may lead to long term tubulointerstitial inflammation and graft failure. Recipients' detailed serological analysis is an accepted transplant protocol. However, immunological tests for clinically significant minor blood groups in donors with their documentation will help identification and management of graft rejection in all cases of antigen antibody mismatch. Thus, present study highlights the importance of documenting these minor blood groups in renal transplant donors for better transplant prognosis.

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