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Original Research Article

An Evaluation of Phenotypic Characteristic of Subjects Having Blood Transfusion Reactions

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Abstract

Background: Out of 35 blood group system and over 600 different blood group antigens discovered so far, some are considered as important i.e., ABO, Rh, Kell, Kidd, Duffy, MNS, Lewis, Lutheran and P. Few of them are clinically significant and are associated with transfusion reactions.

Materials and methods: This are a prospective, cross -sectional analytical study conducted at the Blood Bank, Department of Pathology, Fakhruddin Ali Ahmed Medical College & Hospital. (FAAMCH), Barpeta, Assam, for a period of one year from 17/05/2016 to 16/05/2017. A total of 240 samples (120 from recipients having blood transfusion reaction and 120 from respective donors) were typed for the presence of antigens of Rh, Kell, Kidd, Duffy, Lutheran, Lewis, MNS and P blood group system.

Result & Observation: Rh D antigen was found to be the most common antigen observed in both the recipient and donor. For the Kidd blood group system, 85% recipient and 87.5% donors were typed as JKa antigen positive. Thus, screening and identification of red cell antigens and their phenotype frequencies of various blood group systems for patient having blood transfusion reaction as well as their respective donors have shown some differences in antigenic phenotype.

Conclusion: Red blood cell alloimmunization occurs due to genetic disparity between donor and recipient antigen particularly in multitransfused cases. Leucoreduced packed RBC's or irradiated blood products can help to reduce the incidence of transfusion reaction, still reaction can occur due to alloimmunization to other potent blood group antigens. Study on antigen frequency of various blood group system in local donor population and also knowledge on frequency of alloimmuzitation and type of alloantibodies in multitransfused patient may have an impact on the incidence of transfusion related reaction as corresponding

antigen negative blood from donor database may be transfused to the patient to reduce the rate of adverse blood transfusion reaction and thereby improve blood safety to a great extent.

Keywords: Red Cell Antigen, Alloimmunization, Blood Transfusion Reaction.

Introduction

Almost 35 blood group system[1] and over 600 different blood group antigens[2] are discovered so far. Nine of these are considered as a major blood group systems i.e.- ABO, Rh, Kidd, Duffy, MNS, Lewis, Lutheran and P. [3,4] . Out of which ABO and Rh are the most important blood group systems in blood transfusion service. These antigens are immunogens that can lead to the development of antibodies in the recipient after transfusion [5].

The main aim of blood transfusion service is to provide adequate and safe blood to the recipient in relation to transfusion-transmitted infection and compatibility testing so that the donor red cells survive after transfusion and serve the functions for the actual benefit of the patient. That unit of blood should be selected for transfusion after compatibility testing where donor red cells are compatible with those of recipients blood and for that purpose there should not be antigen on donor cells for the antibodies detected in the recipients serum during antibody detection and identification.[6] Alloimmunization is due to red cells antigenic difference between donor and recipient .The alloantibodies encountered during compatibility testing are mainly against antigen related to Rh,[7,8,9,10] Kell,[11] Kidd,[12] Duffy[13] and MNS[14] blood group system. These alloantibodies are responsible for hemolytic transfusion reactions (HTRs) and are called clinically significant antibody.[15] Phenotype of a blood group of an individual is the observable expression of antigens on red blood cells which can be determined by serological test with antisera.[16] Transfusion of ABO compatible blood of unknown phenotype with clinically significant antigens result in alloimmunization specially in multi transfusion patients due to development of alloantibodies in their blood. So, these alloimunized patient should receive antigen negative compatible blood to prevent the development of transfusion reaction. It is essential to know frequencies of the various antigens in the donor blood unit to avoid blood transfusion reaction in patient who have developed multiple allantibodies. But compatible blood is provided in most part of India by random matching of the blood units only.

Very little information is available regarding the distribution of these blood group systems and its relation to blood transfusion reaction in Northeast India. This study was performed with the aim of screening and identifying red all antigens and their phenotypic expression in patients having blood transfusion reaction as well as the concerned donors so as to find out the relationship of Rh, Kell, Kidd, Duffy, Lutheran, Lewis, MNS and P in the development of blood transfusion reaction.

Materials and Methods

This is a prospective, cross–sectional analytical study conducted at the Blood Bank, Department of Pathology, Fakhruddin Ali Ahmed Medical College & Hospital, (FAAMCH), Barpeta, Assam, for a period of one year from 17/05/2016 to 16/05/2017 after obtaining approval from the Institutional Ethical Committee. Patients developing blood transfusion reaction at FAAMCH, Barpeta were included in this study population. A detailed clinical and transfusion history

was taken using a set proforma which included name, age, sex, identification number, diagnosis, blood group, transfusion till date, transfusion in the study period and result of serological test like Direct Antiglobulin test.

4ml of blood sample was collected in EDTA tube from patient having blood transfusion reaction after taking their consent. Also, another 4ml of blood sample was collected in a tube from blood bag of the respective concerned donor of that patient having transfusion reaction. A total of 240 samples (120 from patients and 120 from respective concerned donors) were typed for the presence of antigens of Rh, Kell, Kidd, Duffy, Lutheran, Lewis, MNS and P blood group system. Those donors who were eligible as per Drugs and Cosmetic Act, 1940 and Rules, 1945 were selected after obtaining informed consent. All the patients sample typed for extended red cell antigenic profile were selected after confirming that their Direct Antiglobulin Test (DAT) results were negative. Repeat ABO and Rh D blood group (Forward and reverse grouping) was done by conventional tube technique as per standard Operating Procedure using monoclonal reagents:

Anti D- negative cases were tested for the presence of weak D phenotype by an Indirect Antiglobulin test (IAT) according to our Standard Operating Procedure. Both the samples from patient and donor were tested for red cell antigen typing for blood group Rh (D,C,E,c,e), Kell (K,k), Duffy (Fya, Fyb), Kidd (Jka, Jkb), Lewis (Lea, Leb), P (P1), MNS s (M,N,S,s) and Lutheran (Lua, Lub) by conventional tube technique following manufacturer's instructions. Agglutination reaction in positive test results were recorded using agglutination viewer and were graded as 1+ to 4+. No agglutination indicates absence of corresponding antigen.

The antisera used for the study were from Diamed, Switzerland. The antihuman globulin reagent used were also from Diamed, Switzerland

The typing of D,C,c,E,e,K, Jka, Lea, Leb, P,M, and N antigens were done using monoclonal antisera, while that of k, Fya, Fyb, Jkb,S,s, Lua, Lub antigen were done using polyclonal antisera by I.A.T. False positive and false negative results were avoided by taking quality control measure at each step. The blood samples negative for a particular antigen in the IAT were confirmed by check cells (Coombs' control cell). Calculation of red cell antigens and phenotype frequencies of the various blood group system was done by totaling the number of sample positive for a particular antigen phenotype divided by the total number of sample screened. Results were expressed as percentage.

Results

A total of 240 samples were typed for the presence of antigens of Rh, Kell, Duffy, Kidd, MNSs, Lutheran, P and Lewis blood group system of which 120 samples were from patients having transfusion and another 120 were from respective concern donor of that patient. Age of the donors vary from 18 years to 50 years. Mean age of the donor was 34 years. Out of 120 donors 96 were male and 24 were female. Age group of patients having transfusion reaction ranges from 12 years to 68 years with a mean age of 40 years.

In patient and donor group, 119 (99.17%) were D+ and 1 patient (0.83%) was D negative. The most common Rh-antigen observed in the patient population was D (99.17%) followed by e (97.5%), C (91.67%), c (51.67%) and E (19.17%). Of the five antigen of Rh –system in donor group also, D antigen was found to be the most common antigen (99.17%) followed by e (97.5%), C (90%), c (54.17%) and E (25%).

Table 1: Distribution of Rh antigen (C,E,c,e) of patients and donors in the present study in Rh (D+) and Rh (D-) cases

Antigen	Antigen frequency in D+ patients		Antigen frequency in D-		Total Patient	
	Number	(%)	Number	(%)	Number	(%)
	N=119	(99.17%)	N=01	0.83%	N=120	
C	110	(92.44%)	00	0%	110	91.67%
c	61	(51.26%)	01	100%	62	51.67%
E	23	(19.33%)	00	0%	23	19.17%
e	116	(97.48%)	01	100%	117	97.5%
Antigen	**Antigen frequency in D+ donors**		**Antigen frequency in**		**Total donors**	
	Number	**(%)**	**Number**	**(%)**	**Number**	**(%)**
	N=119	99.17%	N=01	0.83%	N=120	
C	108	90.76%	0	0%	108	90%
c	64	53.78%	01	100%	65	54.17%
E	30	25.21%	0	0%	30	25%
e	116	97.48%	01	100%	117	97.5%

Table 2: Distribution of Rh antigen of different studies in Rh (D+) and Rh (D-) cases

	In Rh D Positives (%)			In RhD negatives (%)		
Antigen	Thakral et al	Kahar et al	Gundraju – kkupam et al	Thakral et al	Kahar et al	Gundraju-kuppam et al
C	90.15%	93.81%	92.5%	8.54%	16.67%	15.25%
c	49.48%	50.52%	52.1%	100%	88.89%	100%
E	18.9%	22.68%	19.4%	-	16.67%	8.47%
e	98.1%	100%	98.3%	100%	100%	100%

Table 3: Reaction pattern with antisera, phenotype, probable genotype of the patients and donors in the present study and comparison of these Rh-Phenotype with other study

S.N.	Reaction with antisera					Phenotype	In Patients		In Donors		Most Probable genotype		Kahar *et al*	Thakral *et al*	Makroo *et al*	Sharma *et al*	White	Black
	C	c	D	E	e		Incidence	%	Incidence	%								
1	+	-	+	-	+	CCDee	56	46.67%	53	44.17%	R1R1	(CDe/Cde)	40.87%	43.80%	42.60%	41.00%	17.60%	29.00%
2	+	+	+	+	+	CcDEe	14	11.67%	17	14.17%	R1R2	(CDe/cDE)	13.91%	8.22%	14.50%	3.10%	11.80%	3.70%
3	+	+	+	-	+	CcDee	36	30%	34	28.33%	R1R0	(Cde/cDe)	23.48%	30.00%	32.20%	25.50%	31.10%	8.80%
4	-	+	+	+	+	ccDEe	5	4.17%	9	7.50%	R2R0	(cDE/cDe)	4.35%	8.95%	0.10%	5.50%	10.40%	5.70%
5	+	+	+	+	-	CcDEE	2	1.67%	2	1.67%	R2Rz	(cDE/CDE)	-	-	1.10%	3.30%	0.10%	-
6	-	+	+	-	+	ccDee	4	3.33%	2	1.67%	R0R0	(cDe/cDe)	0.87%	0.97%	1.30%	3.00%	3.00%	22.90%
7	-	+	-	-	+	ccdee	1	0.83%	1	0.83%	rr	(cde/cde)	11.30%	5.81%	4.60%	5.60%	15.00%	7.00%
8	+	-	+	+	+	CCDEe	1	0.83%	1	0.83%	R1Rz	(CDe/CDE)	0.87%	-	0.50%	2.20%	0.20%	-
9	+	-	+	+	-	CCDE	1	0.83%	1	0.83%	RzRz	(CDE/CDE)	-	-	-	1.50%	0.10%	-

N = Sample Size of Patient, % =Percentage.

Table 4: Comparison of red cell antigen frequencies of patients and donors in the present study with other studies

Sl. No.	Antigen	N =120	In Patients		In Donors		Kahar *et al*	Thakral *et al*	White	Black
			Number	%	Number	%				
1	Kidd	**N =120**								
	Jka		102	85%	105	87.5%	80.87%	82.65%	77%	92%
	Jkb		79	65.83%	74	61.67%	71.30%	66.56%	74%	49%
2	Duffy	**N =120**								
	Fya		92	76.67%	96	80%	46.96%	86.75%	66%	10%
	Fyb		47	39.17%	38	31.67%	13.91%	56.15%	83%	23%
3	Kell	**N =120**								
	K		4	3.33%	8	6.67%	6.09%	5.56%	9%	2%
	k		120	100%	120	100%	100%	100%	99.8%	100%
4	Lewis	**N =120**								
	Lea		23	19.17%	25	20.83%	16.52%	20.82%	-	-
	Leb		76	63.33%	74	61.67%	65.22%	60.57%	-	-
5	Lutheran	**N =120**								
	Lua		0	0%	2	1.67%	0%	0.95%	-	-
	Lub		116	96.67%	117	97.5%	97.39%	96.80%	-	-
6	MNSs	**N =120**								-
	M		95	79.17%	90	75%	76.52%	75.39%	78%	74%
	N		74	61.67%	77	64.17%	62.61%	61.51%	72%	75%
	S		47	39.17%	50	41.67%	51.30%	56.47%	55%	31%
	s		105	87.50%	108	90%	91.30%	87.38%	89%	93%
7	P1	**N =120**	79	65.83%	83	69.17%	64.35%	71.92%	-	-

Table 5: Comparison of Rh antigen frequencies of patients and donors in the present study with other studies

Antigen	In Patients		In donors		Kahar *et al*	Thakral *et al*	Sharma *et al*	Makroo *et al*	Gundraju-kuppam *et al*	White	Black
	Number N=120	%	Number N=120	%							
D	119	99.17%	119	99.17%	84.35%	93.39%	91.6%	93.6%	94.1%	85%	92%
C	110	91.67%	108	90%	81.74%	84.76%	84%	87%	88%	68%	27%
c	62	51.67%	65	54.17%	56.52%	52.82%	58.3%	58%	54.9%	80%	96%
E	23	19.17%	30	25.00%	21.74%	17.90%	25.6%	20%	18.8%	29%	22%
e	117	97.5%	117	97.50%	100%	98.30%	78.5%	98%	98.4%	98%	98%

N = Sample Size of Patient, % = Percentage

Table 6: Comparison of antigen phenotypes of different blood group system of patients and donors with other studies

Sl. No.	Antigen	In Patients		In Donors		Kahar *et al*	Thakral *et al*	Nanu-Thapliyal et *al*	White	Black
		Number N=120	%	Number N=120	%					
1	Kidd									
	a+b+	61	50.83%	59	49.17%	52.17%	49.21%	48.37%	49.00%	34.00%
	a+b-	41	34.17%	46	28.33%	28.69%	33.44%	29.35%	28.00%	57%
	a-b+	18	15.00%	15	12.50%	19.13%	17.35%	21.74%	23.00%	0.90%
	a-b-	0	0.00%	0	0%	0.00%	0.00%	0.54%	VR	-
2	Duffy									
	a+b+	40	33.33%	32	26.67%	9.57%	42.90%	42.57%	49.00%	1.00%
	a+b-	52	43.33%	64	53.33%	37.39%	43.85%	40.80%	17.00%	9.00%
	a-b+	7	5.83%	6	5%	4.35%	13.25%	16.19%	34.00%	22.00%
	a-b-	21	17.50%	18	15%	48.69%	0.00%	0.44%	VR	68.00%
3	Kell									
	K+k+	4	3.33%	8	6.67%	6.09%	5.68%	4.04%	8.80%	2.00%
	K+k-	0	0.00%	0	0%	0.00%	0.00%	0.00%	0.02%	Rare
	K-k+	116	96.67%	112	93.33%	93.91%	94.32%	95.96%	91.00%	98.00%
4	Lewis									
	a-b-	21	17.50%	21	17.50%	18.26%	18.61%	23.98%	0.60%	22.00%
	a+b-	23	19.71%	25	20.83%	16.52%	20.82%	13.35%	22.00%	23.00%
	a-b+	76	63.33%	74	61.67%	65.22%	60.57%	61.04%	72.00%	55.00%
5	Lutheran									
	a+b+	0	0.00%	2	1.67%	0.00%	0.95%	ND	7.50%	NA
	a-b-	4	3.33%	3	2.50%	2.61%	3.15%	ND	VR	NA
	a+b-	0	0.00%	0	0%	0.00%	0.00%	ND	0.15%	NA
	a-b+	116	96.67%	115	95.83%	97.39%	95.9%	ND	92.35%	NA
6	MNSs									
	M+N+	49	40.83%	47	39.17%	39.13%	36.90%	43.08%	50.00%	44.00%
	M+N-	46	38.33%	43	35.83%	37.39%	38.50%	42.29%	28.00%	26.00%
	M-N+	25	20.83%	30	25%	23.48%	24.60%	14.63%	22.00%	30.00%
	S+s+	32	26.67%	38	31.67%	24.35%	43.85%	26.75%	44.00%	28.00%
	S-s-	0	0.00%	0	0%	0.00%	0.00%	1.16%	-	1.00%
	S+s-	15	12.50%	12	10%	8.69%	12.62%	10.00%	11.00%	3.00%
	S-s+	73	60.83%	70	58.33%	66.96%	43.53%	62.09%	45.00%	69.00%
	M+N+S+s+	17	14.17%	19	15.83%	10.43%	19.55%	10.72%	24.00%	13.00%
	M+N+S+s-	6	5.00%	4	3.33%	3.48%	3.47%	4.64%	4.00%	2.20%
	M+N+S-s+	26	21.67%	24	20.00%	25.22%	13.88%	27.83%	22.00%	33.40%
	M+N+S-s-	0	0.00%	0	0%	0.00%	0.00%	0.00%	0.00%	0.40%
	M+N-S+s+	7	5.83%	8	6.67%	5.22%	14.80%	13.33%	14.00%	7.00%
	M+N-S+s-	7	5.83%	7	5.83%	4.35%	7.90%	5.51%	6.00%	2.10%
	M+N-S-s+	32	26.67%	28	23.33%	27.83%	15.80%	22.61%	8.00%	15.50%
	M+N-S-s-	0	0.00%	0	0.00%	0.00%	0.00%	0.00%	0.00%	0.40%
	M-N+S+s+	8	6.67%	11	9.17%	8.69%	9.46%	3.48%	6.00%	4.50%
	M-N+S+s-	2	1.67%	1	0.83%	0.87%	1.26%	1.16%	1%	1.60%
	M-N+S-s+	15	12.50%	18	15.00%	13.91%	13.88%	9.27%	15.00%	19.20%
	M-N+S-s-	0	0.00%	0	0%	0.00%	0.00%	0.29%	0.00%	0.70%

7	P									
	P1	79	65.83%	83	69.17%	64.35%	71.92%	62.86%	79.00%	94.00%

N = Sample Size of Patient, % = Percentage

No sample was reported as Du variant in both donor & patient groups. 100% D negative samples from patient and donor had both c and e antigen on their red cells. Thus, there is a strong association of c antigen and e antigen with D negative sample in both the groups (Table –I). Since genotyping was not done, the presume Rh-phenotype frequencies in both patient and donor population are shown in Table III. We have found nine probable phenotypes in both the study population group. In order of descending frequency, the most common phenotypes in patient group were DCCee-46.67%, DCcee-30%, DCcEe-11.67%, DccEe-4.17%, Dccee-3.33%, DCcEE-1.67 %, DCCEe-0.83%, DCCEE-0.83% and dccee-0.83% and in donor group were DCCee-44.17%, DCcee-28.33%, DCcEe-14.17%, DccEe-7.5%, Dccee-1.67%, DCcEE- 1.67%, DCCEe-0.83%, DCCEE-0.83% and dccee-0.83%. DCCee (46.67%) and DCCee (44.17%) were the commonest phenotype in patient and donor population respectively.

Genotype denotes the gene complex in a particular individual. Genotypes cannot be defined by results of typing of antigens in blood group system. In our study, the most common probable genotype in the patient population was DCe/DCe (R1R1) 46.67% followed by DCe/Dce (R1R0) 30%, DCe/DcE (R1R2) 11.67%, DcE/Dce (R2R0) 4.17%, Dce/Dce (R0R0) 3.33%, DcE/DCE (R2Rz) 1.67%, dce/dce (rr) 0.83%, DCe/DCE (R1Rz) 0.83% and DCE/DCE (RZRZ) 0.83%. Similarly, in order of descending frequency, the most common genotypes in donor group were DCe/DCe (R1R1) 44.17%, DCe/Dce (R1R0) 28.33%, DCe/DcE(R1R2) 14.17%, DcE/Dce (R2R0) 7.5%, DcE/DCE (R2Rz)1.67%, Dce/Dce (R0R0) 1.67%, dce/dce (rr) 0.83%, DCe/DCE (R1RZ) 0.83% and DCE/DCE (RZRZ) 0.83%.

Distribution of red cell antigens and phenotype frequencies of various blood group systems (Rh, Kell, Duffy, Kidd, MNSs, Lutheran, P and Lewis) found in the present study for patient and donor are depicted in Table IV, V & VI.

For the Kidd blood group system, 85% patients and 87.5% donors were typed as Jka antigen positive. Jk(a+b+) was the most common phenotype (50.83%) in both patients and (49.17%) donors respectively. However, Jk (a-b-) phenotype was not found in both the population.

In the present study, 76.67% patients and 80% donors were typed as Fya antigen positive and Fy(a+b-) was the most common phenotype in patients (43.33%) and donors (53.33%) respectively for Duffy blood group system. Fy (a-b+) phenotype was found only in (5.83%) patients and (5.0%) donors respectively.

In the Kell blood group system, (3.33%) patients and (6.67%) donors were typed as K-antigen positive. All the patients and donors were 100% k-antigen positive. (K-k+) was the most common phenotype in both patients (96.67%) and donors (93.33%). (K+k-) phenotype was not found in any of the patient and donor group.

Frequency of two Lewis antigens for patients and donors were Lea-19.17%, Leb -63.33% and Lea 20.83% and Leb – 61.67% respectively. Le (a-b+) was the most common phenotype for patients (63.33%) and donors (61.67%). No Le (a+b+) phenotype was observed.

In our study, 96.67% patients and 97.5% donors were reported as Lub antigen positive, out of which Lu (a-b+) was the most common phenotype observed in patients (96.67%) and donors (95.83%). No Lu (a+b-) phenotype was observed in both the group.

Frequencies of M-79.17%, N-61.67%, S-39.17% and s-87.5% of patient population and M-75%, N- 64.17, S-41.67% and s-90% of donor population are depicted in Table-IV. M+N+ and S-s+ were the most common phenotypes observed in the MNSs blood group system both for patient (40.83% and 60.83% respectively) and donors (39.17% and 58.33% respectively).

In our study, M+N-S-s+ (26.67%) and M+N+S-s+ (21.67%) were the most common whereas M-N+S+s- (1.67%) and M+N+S+s- (5%) were least common phenotypes in patients' group. In case of donors also, M+N-S- s+ (23.33%) and M+N+S-s+ (20%) were amongst the most common and M-N+S+s- (0.83%) was the least common phenotype. However, no M+N+S-s-, M+N-S-s-and M-N+S-s- phenotypes were found in the present study.

P1 antigen was observed in 65.83% of patient and 69.17% of donor population respectively.

Discussion

In routine practice, blood is matched for major blood group antigens (ABO and Rh D- antigen). There is a high probability that the donor may have other blood group antigens do not present in the recipients which may result in alloimmunization. The Knowledge of data on frequencies of antigens of various blood group system in a local donor population help in blood transfusion services, particularly in alloimmunized cases where clinically significant antibodies are identified in patients' serum and hence corresponding antigen negative blood can be given from donor database. In this study, extended antigen typing of various blood group system was done in multi transfused patients having transfusion reaction and their concern donors and the phenotype frequencies are compared with other studies in India and also in different population of the World, so as to find the relationship of these minor blood group system in the development of blood transfusion reaction.

Different studies have reported different incidence of D- antigen. In our study, D antigen was found to be present in 99.17% of both patients and donors, which is higher than reported in other studies in India, i.e., Thakral et al (93.39%) [17], Sharma et al (91.6 %),[18] Makroo et al (93.6%),[19] Pachaury et al[20] and Mangwana et al.[21] The frequency of D-negative phenotype is higher in White (15%),[22,23,24] however it is lower in the study conducted by Liu J. et al (1.02%)[25] in our neighboring country China. Sharma et al from Central India, Thakral et al from North India, Gundrajukuppam et al[26] from South India and Kahar et al [27] from South Gujrat have reported 8.4%, 6.61%, 5.9% and 15.65% Rh D- negative Phenotype respectively. We have found a marked difference in the frequency of C and c antigen in both the population as compared to White and Black population. The frequency of C antigen was found to be higher (91.67% in patients and 90% donors) than White (68%) and Black (27%) populations [22,23,24] whereas that of c antigen was lower (51.67% in patient and 54.17% in donors) than White (80%) and Black (96%) population. However, the frequency of C antigen was also higher than other studies reported in India i.e. Kahar et al (81.74%) Thakral et al (84.76%), Makroo et al (87%), Sharma et al (84%) and Garg et al (91.8%) [28]. But the c-antigen frequency in the present study was comparable with studies done in India by Kahar et al (56.52%), Thakral et al (52.82%), Makroo et al (58%) and Gundrajukuppam et al (54.9%).

Frequency of other Rh antigens in Rh-positive and negative is shown in Table-I. In the present study, C antigen was found to be more prevalent on D+ red cells both in patients (92.44%) and donors (90.76%) whereas c and e antigen was found in 100% of D negative red cells for both

group of population which are comparable to studies from Thakral et al (100%) and Gundrajukuppam et al (100%) as shown in Table I and II.

Though E is a strong immunogen, but due to its low frequency in population, it is considered as least effective. In our study, E antigen was found to be in 19.17% of patient and 25% of donor which is comparable to other studies done in India i.e. Kahar et al (21.74%), Sharma et al (25.6%), Makroo et al (20%) and Chitra M. et al (25%) [29]. Next to D-antigen, the most common antigen was e antigen (97.5% both in patients and donors), the frequency of which is comparable to other studies in India as reported by Kahar et al (100%), Thakral et al (98.30%), Gundrajukuppam et al (98.4%) and Makroo et al (98%) as shown in Table-V, except studies done by Sharma et al from Central India where it was low (78.5%). Mangwana et al from North India have also reported that e – antigen is the most common antigen (98.79%). For alloimmunized patient against e antigen, it would be difficult to find e antigen negative donor as the antigen frequency is high in population.

Rh phenotypes involving major antigen of both patient and donor population are compared with other studies in India and different races in Table III. R1R1 (CDe/ Cde) was the commonest phenotype in our study population (46.67% in patient and 44.17% in donor) as well as in the study by Kahar et al (40.87%), Thakral et al (43.8%), Makroo et al (42.6%) and Sharma et al (41%). However, this phenotype frequency was reported in only 17.6% of White and 29% of Black population. In contrast, most common phenotype in White is R1R0 (Cde/cDe) (31.1%) and in Black is R0R0 (cDe/ cDe) 22.9%. Least common phenotypes in Rh D positive sample for both the patient and donor population were R1Rz (CDe/ CDE), 0.83% and RzRz (CDE/ CDE), 0.83% respectively.

In Rh-D negative sample of both the population, rr (dce/dce), 0.83% was the only phenotype in the study population. But, other studies in India have reported 11.30%, 5.81% and 5.6% phenotype frequency of rr (dec/dec) by Kahar et al, Thakral et al and Sharma et al respectively. Different donor population with different Rh-phenotypic characteristic is responsible for the reported difference in frequencies of alloimmunization in patients. So, knowledge of frequencies of Rh-phenotype in a local population may be helpful in blood transfusion services in reducing the rate of alloimmunization, by matching the blood of Rh-system and thereby preventing transfusion reaction in multiple transfused case.

In the Kell blood group system, K-antigen frequency in this study (3.33% in patients and 6.67% in donors) was higher than that reported in Blacks (2%) but lower than that in Whites (9%). Tarek M.E. et al [30] and Owaidah et al [31] have reported K antigen frequency as 8.23% and 8% respectively. Studies done in India by Shah et al (2.4%)[32] and Pahuja et al (1.18%)[33] have observed lower frequency of K- antigen. In the present study, k-antigen was found to be 100% in both the study population which is comparable with studies done by Thakral et al (100%) and Kahar et al (100%). The most common phenotype was (K- k+) both in patients (96.67%) and donors (93.33%) which is comparable with that in the study by Kahar et al (93.91%), Thakral et al (94.32%) and Nanu and Thapliyal et al[34) (95.96%) . Frequency of (K+ k-) phenotype (0%) of both group of study population is comparable with other studies as shown in Table VI.

The frequencies of antigens of Kidd blood group system Jka-85%, Jkb-65.83% (in patients) Jka-87.5%, Jkb-61.67% (in donors) were similar to studies by Kahar et al (Jka-80.87%, Jkb-71.30%) and Thakral et al (Jka- 82.65%, Jkb-66.56%) and Makroo et al (Jka – 81.5% and Jkb –

67.4%). Studies done in other countries by Tarek M.E. et al (Jka – 83.88% and Jkb - 58. 75 %) and Owaidah et al (Jka – 86% and Jkb – 60 %) have also reported similar findings. But Romphruk et al[35) from Thailand and Halawani et al [36) from Saudi Arabia have reported it as (Jka – 74.14% and Jkb – 72.52 %) and (Jka – 90.64% and Jkb – 69.40%) respectively. Jka (a+b+) (50.83% in patients and 49.17% in donors) was the most frequent phenotype in Kidd system and is comparable to study done by Kahar et al (52.17%), Thakral et al (49.21%), Nanu and Thapliyal (48.37%) in the White population (49%). Similar observations were also reported by Romphruk et al (46.73%) and Halawani et al (52.45%). However, Jk (a+b-) was the commonest phenotype (57%) in Blacks.

In the present study, the antigen frequency of Duffy blood group system was found to be (Fya – 76.67% and Fyb – 39.17%) for recipients and (Fya – 80% and Fyb – 31.67%) for donors which appears to be unique compared to other studies done in India. The phenotype Fy (a+b-) with frequency of 43.33% in patients is comparable with that found by Thakral (43.85%) and Nanu and Thapliyal (40.8%). But in case of donors, Fy (a+b-) phenotype frequency of 53.33% is higher than the other studies as shown in Table VI. Fy (a-b-) phenotype frequency of 17.5% for patients and 15% for donor in our study was lower than the study by Kahar (48.69%) and black population (68%) but higher than Thakral (0%) and Nanu Thapliyal (0.44%). The Duffy glycoprotein is a receptor that binds cytokines released during inflammation. It also binds malaria parasite Plasmodium vivax. It is postulated that Plasmodium vivax Duffy binding protein interacts with Duffy antigens on RBC's to permit RBC infection [37]. Individuals with the Fy (a-b-) phenotype may have a selective advantage as their RBC's are resistant to P. Vivax infection. South Gujarat and Black population being an endemic zone for malaria may have high frequency of Fy (a-b-) in study by Kahar et al and in Black population. Barpeta district is a part of lower Assam in Northeast India, which is not a highly endemic District. But foothills of Bhutan is known for its high endemicity. F.A.A.M.C.H., Barpeta is the only Medical College in lower Assam and has a large catchment area including neigbouring district and population living in foothills of Bhutan. The observations in this study regarding the frequency of Fy (a-b-) (17.5% in patients and 15% in donors) can be explained plausibly by these endemicity scenario.

The M+N+ phenotype was the most frequent in our study for both patients 40.83% and donors 39.17% and is comparable to the study by Kahar (39.13%), Thakral (36.9%) and Nanu (43.08%), but is less than Whites (50%). The phenotype frequency of S-s+ for patients, 60.83% and donors 58.33% are higher than Thakral (43.53%) and Whites (45%) but it is comparable to Nanu and Thapliyal (62.09%). The Phenotype M+N-S-s+(26.67% for patients and 23.33% for donors) and M+N+S-s+ (21.67% for patients and 20% for donors) were more common than 15.8% and 13.88% reported by Thakral but are comparable to study done by Kahar (27.83%) and (25.22%) respectively. M+N+S+s+ and M+N+S-s+ was the commonest phenotype reported by Thakral (19.55%) and Nanu and Thapiyal (27.83%) respectively. So, there is phenotypic variation of MNSs system in different population.

The most common phenotype in the Lewis system was Le (a-b+), 63.33% for patients and 61.67% for donors, which is comparable with that of Kahar (65.22%), Thakral (60.57%) and Nanu and Thapiyal (61.04%). Le (a-b-) phenotype (17.5% for both patients and donors) are higher than that reported in Whites (6%).

Phenotype frequency of Lu (a-b-) in our study of 3.33% for patients and 2.5 % for donors is comparable to other studies in India by Kahar (2.61%) and Thakral (3.15%) and was very rare in White. However, no Lu (a+b+) phenotype was found in patients whereas it was 1.67% in donors, but Lu (a+b+) was reported to be 7.5% in Whites. The Lu (a-b+) phenotype was the most frequent in patients 96.67% and donors 95.83% in Lutheran system and it is comparable to the study by Kahar (97.39%) Thakral (95.9%) and also in Whites (92.35%).

From these studies, we have found that some patients and their donors show antigenic difference other than the ABO & Rh blood group system. Although current standard pre-transfusuion testing protocol include detection and identification of clinically significant antibodies in patients' serum to prevent adverse reaction caused by blood transfusion, pre transfusion antibody screening in patients sample is not a routine practice in F.A.A.M.C.H., Barpeta, as multiple transfused patients might have received minor antigen mismatched blood leading to formation of multiple alloantibodies. Previous studies by Thakral et al [38] (61%) and Dhawan et al [39] (52%) have reported that most common alloantibodies detected was against Rh system. As all the donors and patients in our study were k- antigen positive, the likelihood of finding alloantibody is negligible in Kell blood group system. In Indian population also probability for development of alloantibody against k-antigen is negligible as almost 100% population are k-antigen positive [17, 27]. However, in studies done by Thakral et al[38] and Rakesh et al[40] no anti K antibody was detected, but some other studies in India have detected anti K antibody.[38,39,41,42] This variation may be due to different distribution of blood group antigen in different population of India. Another way of reducing the risk of alloimmunization is by typing the donors' and patients' clinically significant antigen. But due to different distribution of blood group antigens in patient and donor, managing inventory by extended typing of these blood group antigen and cross matching will be difficult task, So, blood bank should have donor database of local population on antigen frequency of various blood group system. These databases may be useful for formulating in house red cells (screening and panel cells), which will help in detection and identification of clinically significant antibodies in patient serum (in cases of multiple transfusion) and also providing corresponding antigen negative blood to alloimmunized patient.

Conclusions

The most common Rh-antigen observed in both patients and donors was Rh-D-positive (99.17%) contrary to most of the other studies in India where e-antigen was reported as common antigen.

CCDee were the commonest phenotype in both patient (46.67%) and donor (44.11%). Phenotype frequencies of Fy (a-b-) [17.5% in patients and 15% in donors] is not as high as reported in some malaria endemic area and also not very low as in non-endemic area. Fy (a+b-) phenotype frequency of 53.33% is higher than other studies. No K+k- phenotype was reported and K-k+ was the most common phenotype in both patient and donor group. Frequency of S-antigen (39.17% in patient and 41.67% in donor) is found to be lower than other studies.

Routine antibody screening of all patients before transfusion may not be possible in many centers in our country due to cost involved in it. So, for economic purpose, blood banks can match the blood for antibodies which are common in that population which will help to reduce transfusion associated reactions and thus improve blood safety to a great extent. There are possibilities that the antibodies against some other antigens may go undetected with the use of commercially

available screening cell panel prepared from foreign donors. An indigenous homemade screening and panel red cells from local donor population have some definite advantage over the other as they have representation of antigens from local donors in screening and panel red cells for antibody detection and identification, much cheaper than commercial source, can be used immediately, no issue of transportation and also longer shelf life as transit time of transportation is avoided.

The sample size of this study was relatively small as the samples were taken from blood transfusion reaction cases and their respective concerned donors. Therefore, a population-based study in different region with adequate sample size is required to find out regional difference in red cell antigen phenotype frequencies of various blood group system and also for deriving a valid conclusion regarding antigen frequency and their phenotypes in a defined population in future.

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