



Red cell antigen phenotypes in blood donors & thalassaemia patients for creation of red cell antigen-matched inventory

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Background & objectives: Patients with thalassaemia are at a risk of alloimmunization and the presence of RBC alloantibodies further complicates transfusion therapy. Matching for the critical antigens of Rh, Kell, Kidd and Duffy blood group systems has been shown to minimize alloimmunization. The aim of the present study was to create a database of extensively typed donors for clinically significant and common blood group antigens of Rh, Kidd, Kell and Duffy systems for transfusion therapy of multitransfused thalassaemic patients.

Methods: Five hundred O group regular blood donors were phenotyped for Rh, Kell, Duffy and Kidd blood group antigens using haemagglutination technique. Eighty four non-alloimmunized and 15 alloimmunized thalassaemia major patients with known antigenic profiles (determined by polymerase chain reaction with sequence-specific primers) were selected for this study.

Results: By analyzing antigen profiles of 500 O group regular donors, a database of 193 donors matching perfectly for Rh, Duffy, Kell and Kidd antigens was prepared for 15 alloimmunized patients. For non-alloimmunized 84 thalassaemic patients, a database of 405 donors was created.

Interpretation & conclusions: A database of 500 regular blood donors phenotyped for common antigens of Rh, Duffy, Kell and Kidd blood group systems was created, which would be useful in providing extended antigen-matched RBCs for thalassaemia patients. This will improve the quality and effectiveness of transfusion therapy.

Key words Alloimmunization - antigen-matched blood - blood groups - blood phenotypes database - inventory - multitransfused thalassaemics

Blood transfusion is a common practice in the treatment of patients with anaemia, thalassaemia, sickle cell anaemia and other haematological disorders and malignancies. Many such patients require repeated blood transfusions during their illness. In majority of the blood banks, only ABO and RhD blood group status of blood

donors and recipients are matched when red blood cells (RBCs) are transfused. Although antibody screening and identification is performed in many blood banks, RBCs are not routinely tested for other minor blood group antigens unless the recipient has previously undergone immunization. The presence of RBC alloantibodies

creates the potential for serologic incompatibility, makes the selection of appropriate units for future transfusion difficult, delays the use of a potential lifesaving therapy and presents risk of haemolytic transfusion reaction (HTR) and delayed HTR.

The rate of alloimmunization or the production of antibodies that may potentially destroy foreign or donor RBCs among multitransfused individuals is significantly higher (8-76%) in patients receiving multiple transfusions such as sickle cell disease and thalassaemia and it increases with repeated transfusions¹. Hence, it is advocated that transfusions given to patients who are likely to become transfusion dependent over a period of time should also be matched for antigens other than ABO and RhD. Use of phenotyped matched RBC units for transfusion offers an advantage especially to patients with alloantibodies and prevents further alloimmunization to other antigens. It also helps reduce the incidence of alloimmunization in non-alloimmunized multitransfused patients².

Available reports on antibody screening and identification in multitransfused patients and multiparous women have shown that most antibodies are directed against Rh, Duffy, Kell and Kidd blood group antigens and cause HTRs or haemolytic disease of the foetus and newborn (HDFN)³⁻⁵. The frequency and specificity of irregular red cell antibodies vary among different ethnic groups according to the genetic diversity of the population. Provision of antigen-matched blood is known to reduce alloimmunization among multitransfused patients⁶. Also, there was improved RBC survival and diminished frequency of transfusions⁷.

As multitransfused thalassaemic patients and blood donors are not phenotyped for minor blood group antigens in a pretransfusion setting, it is difficult to obtain compatible units due to lack of pre-typed donors. Hence, in the present study, 500 regular blood donors were typed for the common and clinically important blood group antigens of Rh, Duffy, Kell and Kidd blood group systems to determine their frequency and common phenotypes in the population and to create a database of donors to be used for provision of antigen-matched blood units to multitransfused thalassaemics.

Material & Methods

This study was carried out at the department of Transfusion Medicine, ICMR-National Institute of Immunohaematology, Mumbai, India (2013 to 2016). A total of 500 consecutive O group regular blood donors visiting KEM Hospital, Mumbai, and various

blood donation camps, were enrolled for this study. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from all the participants. Donors were typed for common blood group antigens of Rh (C, c, D, E, and e), Duffy (Fy^a and Fy^b), Kell (K and k) and Kidd (Jk^a and Jk^b) using commercially available antiserum using the manufacturer's instructions (IMMUCOR, Inc., USA) by conventional tube technique. The antigen profiles were analyzed for providing antigen-matched blood to 84 non-alloimmunized and 15 alloimmunized, multitransfused thalassaemia major patients from our previous study⁸. Antigen profiles of these patients were determined by PCR using sequence-specific primer (PCR-SSP) and haemagglutination. For PCR, DNA was prepared from ethylenediaminetetraacetic acid (EDTA) blood using commercially available DNA extraction kit (Qiagen, Germany). The common alleles of Rh, Duffy, Kell, Kidd and MNS antigens were genotyped using PCR-SSP along with known controls in Veriti[®] 96-well Thermal Cycler (Applied Biosystems, USA) as described earlier⁸. The amplified products were separated electrophoretically on two per cent agarose gel containing ethidium bromide and visualized under ultraviolet transilluminator, Gel Doc system (Bio-Rad, USA). For selecting antigen-matched donors, antigen profile of the patient determined by PCR-SSP was considered. Frequencies of blood group antigens in blood donors were calculated as percentage.

Results

Five hundred O group regular blood donors were phenotyped for common antigens of Rh, Duffy, Kell and Kidd blood group systems. Of these, 94 per cent (n=470) were RhD positive. The frequency of Rh antigens (C, c, D, E and e) in the donor population is shown in the Figure. The frequency of e antigen was the highest (99.4 %), followed by D and C antigens. The frequency of the antigens was in the order: e > D > C > c > E. Eight different most probable Rh phenotypes were identified, with the most common being R₁R₁ (CDe/CDe) having a frequency of 44 per cent and the most rare being r'r (Cde/cde) with a frequency of 0.4 per cent. Seventy five per cent (n=375) of donors belonged to the R₁R₁ and R₁r phenotype. Rare Rh phenotypes R₂R₂ and r'r had frequency of <1 per cent. Seventy eight donors had Rh blood group phenotypes occurring with <10 per cent frequency. In the donor population, the apparent homozygosity for C, c, E and e antigens was found to be 44.0, 15.2, 0.6 and 82.4 per cent, respectively, and the heterozygosity for

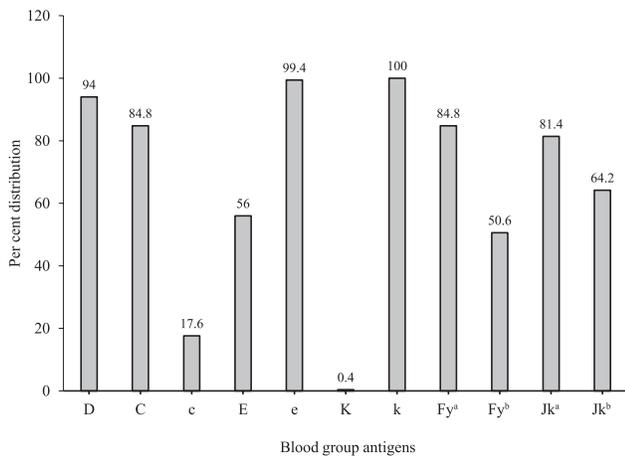


Figure. Per cent distribution of Rh antigens (D, C, E, c and e), Duffy (Fy^a and Fy^b), Kell (K and k) and Kidd (Jk^a and Jk^b) antigens in O group regular blood donors enrolled in the present study.

C/c and E/e was found to be 40.8 and 17.0 per cent, respectively.

Extended antigen matching between thalassaemic patients and blood donors: By analyzing antigen profiles of 500 O group regular donors, a database of 193 donors matching perfectly for Rh, Duffy, Kell and Kidd antigens was prepared for 15 alloimmunized thalassaemia major patients (Table I). Based on partial matching of donors and non-alloimmunized thalassaemic patients (n=84) for Rh antigens (C, c,

D, E and e), 206 R₁R₁ donors were identified for 47 patients with thalassaemia, 131 R₁r donors for 16 patients, 43 R₁R₂ donors for 13 patients, 10 R₀r donors for four patients, three R₂r donors for one patient and 12 rr donors for three patients. Approximately three donors matching for R₁R₁, R₁R₂, R₂r and rr phenotypes were identified per patient. On an average, eight donors matching for R₁r and only two donors for R₀r phenotype were identified per patient.

For non-alloimmunized 84 thalassaemia patients, database of 405 donors perfectly matching for common and clinically important antigens of Rh, Duffy, Kell and Kidd blood group systems was prepared (Table II). On an average, five donors matching perfectly for Rh, Duffy, Kell and Kidd antigens per thalassaemia patient were identified. For some relatively rare Rh phenotypes, three donors per patient were identified.

Discussion

In the present study, 500 regular O group blood donors were phenotyped for 11 common and clinically important antigens of Rh, Duffy, Kell and Kidd blood group systems. R₁R₁ was the most common Rh phenotype identified in the present study as compared to high prevalence of R₁r and Rh-negative phenotype in Caucasians and R₀r in Africans, (Table III)⁹⁻¹⁵.

Table I. Number of antigen-matched donors available for alloimmunized patients (n=15)

Patient number	Antigen profile (genotype)				Number of antigen-matched donors available
	Rh	Duffy	Kidd	Kell	
1	R ₁ R ₁	Fy(a+b+)	Jk(a+b+)	K-k+	17
2	R ₁ R ₁	Fy(a+b-)	Jk(a+b+)	K-k+	24
3	R ₁ R ₁	Fy(a+b-)	Jk(a-b+)	K-k+	8
4	R ₁ R ₁	Fy(a+b+)	Jk(a+b-)	K-k+	8
5	R ₁ R ₁	Fy(a-b+)	Jk(a+b-)	K-k+	2
6	R ₁ R ₁	Fy(a+b-)	Jk(a-b+)	K-k+	8
7	R ₁ R ₂	Fy(a+b+)	Jk(a+b+)	K-k+	5
8	R ₀ r	Fy(a+b-)	Jk(a+b+)	K-k+	1
9	R ₁ r	Fy(a+b+)	Jk(a+b+)	K-k+	11
10	R ₁ R ₁	Fy(a+b-)	Jk(a+b-)	K-k+	16
11	R ₁ R ₁	Fy(a+b-)	Jk(a+b-)	K-k+	16
12	R ₁ R ₁	Fy(a+b+)	Jk(a+b+)	K-k+	35
13	R ₂ r	Fy(a-b-)	Jk(a+b-)	K-k+	3
14	R ₁ r	Fy(a+b+)	Jk(a+b-)	K-k+	11
15	R ₁ R ₁	Fy(a+b-)	Jk(a+b-)	K-k+	28

Table II. Extended antigen-matched donors for common antigens of Rh, Duffy, Kell and Kidd blood group systems for thalassaemic patients (n=84)

Rh phenotype	Duffy, Kidd and Kell phenotype			Number of thalassaemia patients	Number of antigen-matched donors
R ₁ R ₁	Fy(a+b+)	Jk(a+b-)	kk	7	35
	Fy(a+b-)	Jk(a+b-)	kk	7	28
	Fy(a+b+)	Jk(a+b+)	kk	7	35
	Fy(a+b-)	Jk(a-b+)	kk	7	36
	Fy(a+b-)	Jk(a+b+)	kk	17	56
	Fy(a+b+)	Jk(a-b+)	kk	1	8
	Fy(a-b+)	Jk(a+b-)	kk	1	8
R ₁ r	Fy(a+b-)	Jk(a+b+)	kk	3	29
	Fy(a-b+)	Jk(a+b-)	kk	3	21
	Fy(a+b-)	Jk(a-b+)	kk	2	11
	Fy(a+b-)	Jk(a+b-)	kk	2	23
	Fy(a+b+)	Jk(a+b+)	kk	4	28
	Fy(a+b+)	Jk(a-b+)	kk	1	8
	Fy(a+b+)	Jk(a+b-)	kk	1	11
R ₁ R ₂	Fy(a+b+)	Jk(a+b+)	kk	3	11
	Fy(a+b-)	Jk(a+b+)	kk	3	10
	Fy(a-b+)	Jk(a+b+)	kk	2	6
	Fy(a-b+)	Jk(a+b-)	kk	2	7
	Fy(a-b+)	Jk(a-b+)	kk	1	3
	Fy(a+b-)	Jk(a-b+)	kk	2	6
R ₀ r	Fy(a+b+)	Jk(a+b-)	kk	1	3
	Fy(a+b+)	Jk(a+b+)	kk	1	2
	Fy(a-b+)	Jk(a+b+)	kk	1	2
	Fy(a+b-)	Jk(a+b+)	kk	1	3
rr	Fy(a+b-)	Jk(a+b+)	kk	1	5
	Fy(a-b+)	Jk(a-b+)	kk	1	3
	Fy(a+b+)	Jk(a+b+)	kk	1	4
R ₂ r	Fy(a-b+)	Jk(a+b-)	kk	1	3

The frequency of Rh phenotypes were, however, comparable with other Indian studies except for the Rh-negative phenotype rr, which was reported to be highest (11.3%) in South Gujarat (Table III)⁹⁻¹⁵.

Comparison of frequency of Duffy, Kell and Kidd phenotypes in different ethnic groups and Indian populations is illustrated in Table IV^{9,10,13-16}. The frequency of K+k+ phenotype in this study was lower than that of the Caucasians and Africans. K-k+ was the most common phenotype (99.6%). No K (K1) antigen homozygous donors were identified. The incidence of Duffy phenotypes varied among

different ethnic groups with Fy(a+b-) as the most common (49.4%) phenotype in our study. Phenotypes Fy(a+b-) and Fy(a-b+) were reported to be higher in Chinese and Africans and as compared to our study. Fy(a-b-) phenotype was not identified in this study, but has high frequencies in Africans (68.0 %). In Kidd blood group system, Jk(a+b+) phenotype was the most common phenotype in the present study (45.4 %) and also in Caucasians and Chinese. In Africans, Jk(a+b-) was the most common phenotype. No Jk(a-b-) phenotype was identified in the present study (Table IV)^{9,10,13-16}.

Table III. Comparison of Rh phenotype frequencies with different ethnic groups and parts of India

Phenotype	North		South	East	West	Caucasians	Africans	Chinese	
	Thakral <i>et al</i> , 2010 ⁹ (Chandigarh) (n=1240)	Makroo <i>et al</i> , 2013 ¹⁰ (New Delhi) (n=3015)	Gundrajukuppam <i>et al</i> , 2016 ¹¹ (Tirupati) (n=1000)	Nag and Das 2012 ¹² (West Bengal) (n=3850)	Kahar and Patel 2014 ¹³ (South Gujarat) (n=115)	Present study (Mumbai) (n=500)	Reid and Lomas-Francis 2004 ¹⁴	Reid and Lomas-Francis 2004 ¹⁴	(Lin-Chu <i>et al</i> , 1998) ¹⁵
Wiener									
Fisher-race									
	Percentage proportion								
R ₁ R ₁	43.8	42.6	43.4	49.4	40.87	44.0	18.5	2.0	47.5
R ₁ r	30.0	32.2	31.2	27.0	23.48	30.2	34.9	21.0	8.1
R ₂ r	8.95	0.1	0.5	3.0	4.3	6.8	11.8	18.6	2.0
R ₁ R ₂	8.22	14.5	10.7	11.0	13.91	10.2	13.3	4.0	34.4
R ₂ R ₂	1.45	0.8	0.7	1.0	-	0.6	2.3	0.2	5.9
R ₀ r	0.97	1.3	1.2	2.7	0.87	2.2	2.1	45.8	0.3
rr	5.81	4.6	4.7	4.3	11.30	5.6	15.1	6.8	0.2
r ^s r	0.56	0.3	0.6	0.6	-	0.4	0.8	0	0.4

The frequency of Fy(a+b-) was higher (49.4 %) in the present study as compared to other Indian studies^{9,10,16}. Frequencies of Duffy phenotypes were similar to other Indian studies except in a report from South Gujarat where the frequency null phenotype of Fy(a-b-) was significantly high (48.69%) and of Fy(a+b+) was lower¹³. In Kell blood group system, K-k+ was the frequent phenotype among all the studies. The frequency of K+k+ was significantly low (0.4%) as compared to South Gujarat and Chandigarh^{9,13}.

The information on different blood group antigens in any given population is necessary to predict the availability of blood units that lack the corresponding antigen(s)¹⁰. Our study showed some variation between blood group antigen and phenotype frequency in different parts from India. Such a variation in antigenic frequencies was expected as the donors in the present study were from cosmopolitan city of Mumbai.

Singer *et al*¹⁷ has shown a decrease in alloimmunization rates from 33 to 2.8 per cent by providing phenotype-matched blood for Rh and Kell antigens. Patients who were given antigen-matched RBC units had improved RBC survival and diminished frequency of transfusions^{18,19}. As most of the antibodies are produced against common Rh, Kell, Duffy and Kidd blood group system antigens, genotyping only for these antigens and giving a partial matched blood is the need of the hour and will substantially minimize the incidence of alloimmunization.

To provide antigen-negative and antigen-matched blood to these multitransfused patients, large-scale screening of regular donors for clinically important antigens is essential. In the present study, a database of 500 regular O group donors phenotyped for common Rh, Duffy, Kell and Kidd blood group antigens was prepared. Of these, 193 donors were identified for 15 alloimmunized patients matching perfectly for above mentioned blood group antigens for prophylactic transfusion therapy. As 60-70 per cent of antibodies are produced against Rh antigens, at least partial matching of these antigens in thalassaemia patients will reduce the alloimmunization to a large extent²⁰. Based on partial matching for Rh antigens (C, c, D, E and e), donors matching for all common phenotypes in patients were identified. We also analyzed data for extended antigen matching between donors and 405 O blood group regular donors matching perfectly for D, C, c, E, e, Fy^a, Fy^b, Jk^a, Jk^b, K and k antigens were identified in 84 thalassaemia patients. This was an initial attempt

Table IV. Comparison of Duffy, Kell and Kidd phenotypes with different Indian populations

Phenotype	Indian population					Caucasians (Reid and Lomas- Francis 2004) ¹⁴	Africans (Reid and Lomas- Francis 2004) ¹⁴	Chinese (Lin Chu <i>et al</i> , 1998) ¹⁵
	Thakral <i>et al</i> , 2010 ⁹ (Chandigarh) (n=1240)	Makroo <i>et al</i> , 2013 ¹⁰ (New Delhi) (n=3015)	Agarwal <i>et al</i> , 2013 ¹⁶ (New Delhi) (n=508)	Kahar and Patel 2014 ¹³ (South Gujarat) (n=115)	Present Study (Mumbai) (n=500)			
	Percentage proportion							
Duffy								
Fy(a+b-)	43.85	42.1	36.22	37.39	49.4	17.0	9.0	91.0
Fy(a-b+)	13.25	12.3	15.36	4.35	15.2	34.0	22.0	0.3
Fy(a+b+)	42.90	45	48.03	9.57	35.4	49.0	1.0	8.9
Fy(a-b-)	0	0.3	0.39	48.69	0	Rare	68.0	0
Kidd								
Jk(a+b-)	33.44	32.5	30.71	28.69	35.8	28.0	57.0	23.2
Jk(a-b+)	17.35	18.5	22.83	19.13	18.6	23.0	9.0	26.8
Jk(a+b+)	49.21	48.9	46.06	52.17	45.6	49.0	34.0	49.1
Jk(a-b-)	0	Rare	0.39	0	0	Rare	Rare	0.9
Kell								
K+k+	5.68	3.5	1.97	6.09	0.4	8.8	2.0	0
K-k+	94.32	96.5	98.03	93.91	99.6	91.0	98.0	100
K+k-	0	Rare	0	0	0	0.2	Rare	Rare

to identify antigen-matched donors for multitransfused patients. The matched donors used for transfusions will drastically reduce the incidence of alloimmunization in these patients.

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Conflicts of Interest: None.

References

- Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. *Br J Haematol* 2012; 159 : 394-404.
- Bhuva DK, Vachhani JH. Red cell alloimmunization in repeatedly transfused patients. *Asian J Transfus Sci* 2017; 11 : 115-20.
- Gupta R, Singh DK, Singh B, Rusia U. Alloimmunization to red cells in thalassemics: Emerging problem and future strategies. *Transfus Apher Sci* 2011; 45 : 167-70.
- Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multitransfused thalassemics of Indian origin. *Hematology* 2010; 15 : 174-7.
- Chou ST, Jackson T, Vege S, Smith-Whitley K, Friedman DF, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood* 2013; 122 : 1062-71.
- Osman NH, Sathar J, Leong CF, Zulkifli NF, Raja Sabudin RZ, Othman A, *et al*. Importance of extended blood group genotyping in multiply transfused patients. *Transfus Apher Sci* 2017; 56 : 410-6.
- Vichinsky EP, Luban NL, Wright E, Olivieri N, Driscoll C, Pegelow CH, *et al*. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: A multicenter transfusion trial. *Transfusion* 2001; 41 : 1086-92.
- Kulkarni S, Choudhary B, Gogri H, Patil S, Manglani M, Sharma R, *et al*. Molecular genotyping of clinically important blood group antigens in patients with thalassaemia. *Indian J Med Res* 2018; 148 : 713-20.
- Thakral B, Saluja K, Sharma RR, Marwaha N. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in North Indian blood donors. *Transfus Apher Sci* 2010; 43 : 17-22.
- Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd MNSs blood group antigens in the Indian blood donor population. *Indian J Med Res* 2013; 137 : 521-6.
- Gundrajukuppam DK, Vijaya SB, Rajendran A, Sarella JD. Prevalence of principal Rh blood group antigens in blood donors at the blood bank of a tertiary care hospital in Southern India. *J Clin Diagn Res* 2016; 10 : EC07-10.
- Nag I, Das SS. ABO and rhesus blood groups in potential blood donors at Durgapur Steel city of the District of Burdwan, West Bengal. *Asian J Transfus Sci* 2012; 6 : 54-5.

13. Kahar MA, Patel RD. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in blood donors of south Gujarat, India. *Asian J Transfus Sci* 2014; 8 : 51-5.
14. Reid ME, Lomas-Francis C. *The Blood Group Antigen FactsBook*, 2nd ed. San Diego, CA: Academic Press; 2004.
15. Lin-Chu M, Broadberry RE, Chang FJ. The distribution of blood group antigens and alloantibodies among Chinese in Taiwan. *Transfusion* 1988; 28 : 350-2.
16. Agarwal N, Thapliyal RM, Chatterjee K. Blood group phenotype frequencies in blood donors from a tertiary care hospital in North India. *Blood Res* 2013; 48 : 51-4.
17. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. *Blood* 2000; 96 : 3369-73.
18. Castilho L, Rios M, Bianco C, Pellegrino J Jr., Alberto FL, Saad ST, *et al*. DNA-based typing of blood groups for the management of multiply-transfused sickle cell disease patients. *Transfusion* 2002; 42 : 232-8.
19. Bakanay SM, Ozturk A, Ileri T, Ince E, Yavasoglu S, Akar N, *et al*. Blood group genotyping in multi-transfused patients. *Transfus Apher Sci* 2013; 48 : 257-61.
20. Gogri H, Kulkarni S, Vasantha K, Jadhav S, Ghosh K, Gorakshakar A. Partial matching of blood group antigens to reduce alloimmunization in Western India. *Transfus Apher Sci* 2016; 54 : 390-5.

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