Original Article

Assessment of ABO, Rhesus, and Kell Blood Group Antigens, Phenotype, and Their Allelic Frequencies in Voluntary Blood Donors

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Abstract

Background: The Rhesus system is clinically the most important blood group system next to the ABO system. Even after proper blood grouping and cross-matching, there is a possibility of alloimmunization in recipients against Rh or minor blood group antigens like Kell, MNS, Duffy, etc. The determination of the prevalence of Rh antigens can play a major role in preventing alloimmunization and adverse events in multi-transfused recipients.

Materials and Methods: This study was conducted at the Department of Transfusion Medicine, Indira Gandhi Institute of Child Health, Bangalore, in 1,000 voluntary blood donors who were tested for red cell antigens of ABO, Rh (D, C, c, E, e), and Kell (K) blood group systems using column gel agglutination technology.

Results: The most common blood group was O (41.1%), followed by B (28.9%), A (23.6%), and AB (6.4%). Rh (D) positivity was 93.9%, while Rh (D) negative blood donors accounted for 6.1%. Among other Rh antigens, the most common antigen found was "C" (82.5%), followed by "e" (77.3%), "c" (49.3%), and "E" (36.2%). The prevalence of the "K" antigen was 0.2%. The most frequent Rh phenotype was "DCe" (30.8%), followed by "DCce" (23.4%), "DCE" (12.5%), "DCcEe" (5.9%), and "Dce" (4.8%).

Conclusion: Assessment of ABO and Rh (D) antigen status is routinely performed in all blood centers. However, phenotypic profiling and assessment of allelic frequencies help in evaluating the distribution of antigens in voluntary blood donors and, at the same time, assist in estimating the chances of obtaining compatible blood units for multi-transfused patients who have developed antibodies.

Keywords:

ABO, Rhesus, Kell, Alleles, Phenotypes

Introduction

Different types of blood groups are inherited and determined based on the presence of surface antigens on the red blood cells (RBCs), and these groups play an important role during blood transfusion [1]. The ABO blood group system is the most clinically important blood group system, discovered by Karl Landsteiner in 1900. The AB blood group was discovered and named by Alfred von Decastello and Adriano Sturli in 1902 [2]. The Rhesus (Rh) system was described by Karl Landsteiner and A.S. Weiner in the year 1940 [3]. Depending on the presence or absence of the Rh antigen on the RBC membrane, Rh phenotypes are of two types: Rh positive and Rh negative [2].

The Kell (K) blood group system was discovered by Mrs. Kellner in 1946 and consists of 27 antigens. The K antigen is very immunogenic [4]. The Rh blood group is one of the most complex and polymorphic blood group systems. The development of a stillborn fetus [hemolytic disease of the newborn (HDN)] associated with an immune reaction to paternal antigens led to insights into the complexities of the Rh blood group system [5]. It is important to know the frequency of ABO and Rh blood groups for safe and effective blood transfusion services and also to prevent erythroblastosis fetalis in an Rh-negative mother carrying an Rh-positive fetus. Apart from this, certain blood groups have been found to be associated with diseases like duodenal ulcer, diabetes mellitus, Rh incompatibility, and ABO incompatibility of the newborn [1,2].

The common antigens of the Rhesus and Kell blood group systems are also frequently tested in blood donors and patients because of their clinical relevance [6]. The Rh blood group system is one of thirty-five current human blood group systems. It is the second most important blood group system after ABO and consists of 50 defined blood group antigens, among which the five antigens—D, C, c, E, and e—are the most important [7]. The transfusion of ABO-compatible but unknown phenotype blood may result in alloimmunization, especially in patients who require multiple transfusions in hematologic disorders and malignancies. The most important red blood cell alloantibodies are directed towards the Rh (anti-D, -C, -E, -c, and -e) and Kell (anti-K) [4].

The blood transfusion services aim to ensure adequate and safe blood to minimize the development of transfusion-transmitted infections and transfusion reactions [4]. Phenotypic profiling and assessment of allele frequencies help in evaluating the distribution of antigens in a particular population and, at the same time, help in estimating the chances of getting compatible blood units for patients who have developed multiple antibodies [5]. This has significance when a policy needs to be devised for transfusing patients of a multi-transfused group, like thalassemia. Although large-scale studies are available from European and other countries on ABO, Rh phenotypic profiling, and allele frequencies, only a few studies are available from the subcontinent of India on the prevalence of ABO, Rh, and Kell antigens. Limited literature is available on antigen distribution, phenotyping, and assessment of allelic frequencies of Rh and Kell blood groups, particularly from India [5].

In this present study, the phenotypic prevalence of ABO, Rh, and Kell antigens was studied along with their allelic frequencies in voluntary blood donors to gain insight into their distribution and ensure the supply of antigen-negative compatible blood without delay to prevent the development of transfusion reactions in alloimmunized patients.

Materials and Methods

This prospective study was conducted at the Department of Transfusion Medicine, Shrimaan Harnaamdas Kapoor Blood Centre, Indira Gandhi Institute of Child Health, Bangalore. One thousand eligible voluntary first-time blood donors who donated blood between November 2021 and October 2022 were enrolled in the study after obtaining written informed consent. Voluntary blood donors who were in the age group of 18–60 years, weighed >45 kg (to donate 350 mL of blood) and >55 kg (to donate 450 mL of blood), with blood pressure (100/80–140/89), regular pulse rate (60–100/min), normal local and systemic examination, and hemoglobin levels of >12.5 g/dL, were included in the study.

All 1,000 voluntary blood donors were tested for red cell antigens of the ABO, Rh (D, C, c, E, e), and Kell (K) blood group systems using column gel agglutination technology. All Rh-negative samples were confirmed by D antigen weak expression test (Du testing) using the Matrix Coombs Anti-IgG card.

The samples were collected in 2 mL tripotassium ethylenediamine tetraacetic acid (K3EDTA) vacutainers. The CC-1200 Matrix gel card centrifuge was used. The Matrix ABO/Rho(D) Forward and Reverse Grouping Card with Autocontrol and the Matrix Rh

Phenotype Card with Anti-K were used for the identification of antigens on the red cell surface. The Matrix ABO/Rho(D) Forward and Reverse Grouping Card with Autocontrol contains six microtubes prefilled with a gel in a suitable buffer containing monoclonal Anti-A, Anti-B, and Anti-D (IgM) from microtubes 1 to 3. Microtubes 4 to 6 contain neutral gel, where microtube 4 (Ctrl) is the negative control, and microtubes 5 and 6 (A1 & B) serve for reverse grouping. The Matrix Rh Phenotype Card with Anti-K contains six microtubes prefilled with a gel in a suitable buffer containing monoclonal Anti-C, Anti-c, Anti-E, Anti-e, Anti-K, and neutral gel in appropriate microtubes. Additionally, Matrix Diluent-2 LISS (low ionic strength saline) was used for the preparation of red cell suspension.

All the reagents used were from TULIP Diagnostics. A positive test reaction was defined as agglutinated red blood cells forming a clear line on the surface of the gel column or agglutinates dispersed in the gel column, with the reaction strength graded from 4+ to 1+. A negative test reaction was defined as non-agglutinated red blood cells settled at the bottom of the microtube, forming a compact button.

Descriptive statistical analysis was used to determine the prevalence and frequencies of various blood groups and their antigenic phenotypes using Microsoft Excel. Ethics: This was a prospective study undertaken after obtaining Institutional Ethics Committee approval.

Results

This study was conducted at the Department of Transfusion Medicine, Shrimaan Harnaamdas Kapoor Blood Centre, Indira Gandhi Institute of Child Health, Bangalore, in 1,000 voluntary blood donors. The most common blood group was O (41.1%), followed by B (28.9%), A (23.6%), and AB (6.4%). Of 1,000 donors, Rh (D) positive donors were 939 (93.9%), and Rh (D) negative blood donors were 61 (6.1%). All the Rh-negative samples were confirmed by Du testing.

Among male voluntary blood donors, the most common blood groups were O (36.8%), followed by B (25.6%), A (21.8%), and AB (6.2%). Rh (D) positive donors were 847 (84.7%), and Rh (D) negative blood donors were 57 (5.7%). All the Rh-negative samples were subjected to Du testing.

Among female donors, the distribution of blood groups was similar to that of males, with the most common blood group being O (4.3%), followed by B (3.3%), A (1.8%), and AB (0.2%). Rh (D) positive donors were 92 (9.2%), and Rh (D) negative blood donors were 4 (0.4%), which were confirmed by Du testing.

Apart from the “D” antigen (93.9%), the “C” antigen was found to be more frequent, being positive in 82.5% of the blood donors, followed by “e” (77.3%), “c” (49.3%), and “E” (36.2%). The prevalence of the “K” antigen was 0.2%, being present only in Rh (D) positive donors.

The prevalence of different Rh phenotypes in both Rh-positive and Rh-negative blood donors was determined by testing for all other Rh antigens. The most frequent phenotype was “DCe,” present in 30.8% of the donors, followed by “DCce” (23.4%). “DCE” was observed in 12.5%, closely followed by “DCcEe” (5.9%). “Dce” accounted for 4.8%.

Discussion

In this present study conducted in voluntary blood donors, blood group A was found in 23.6% of the donors, blood group B in 28.9%, AB blood group was seen in 6.4%, and O was found in 41.1% of the donors. 93.9% of the donors were Rh positive, while 6.1% were Rh negative. The Kell blood group was found in only 0.2% of the donor population.

Table 1: ABO blood groups in voluntary blood donors

Blood group	Total donors		Male donors		Female donors	
	N	(%)	N	(%)	N	(%)
O positive	386	38.6	346	34.6	40	4.0
B positive	272	27.2	240	24.0	32	3.2
A positive	221	22.1	203	20.3	18	1.8
AB positive	62	6.2	60	6.0	02	0.2
O negative	25	2.5	22	2.2	03	0.3
B negative	17	1.7	16	1.6	01	0.1
A negative	15	1.5	15	1.5	0	0
AB negative	02	0.2	02	0.2	00	0
TOTAL	1000	100	904	90.4	96	9.6

Table 2: Rh blood groups in voluntary blood donors

Blood group	Total donors		Male donors		Female donors	
	N	(%)	N	(%)	N	(%)
Rh (D) positive	939	93.9	847	84.7	92	9.2
Rh (D) negative	61	6.1	57	5.7	04	0.4
TOTAL	1000	100	904	90.4	96	9.6

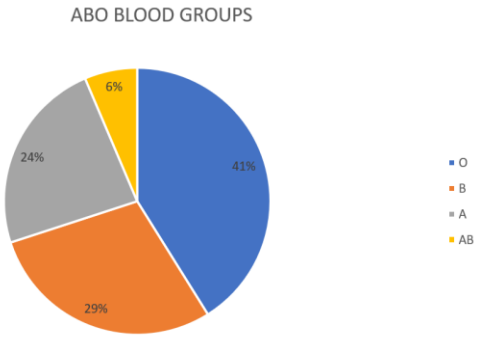

Figure 1: Pie chart showing ABO blood group distribution in voluntary blood donors

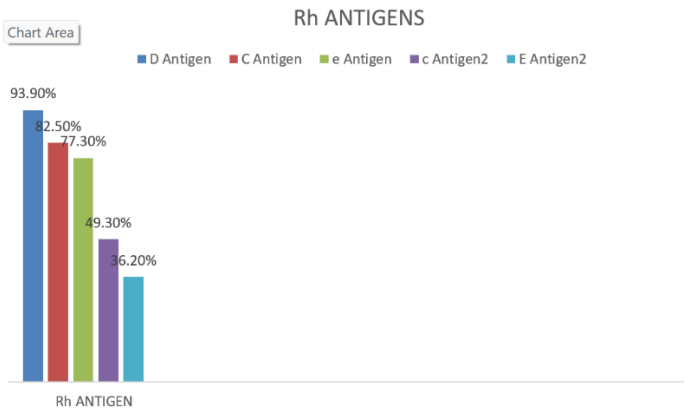

Figure 2: Rh Antigen prevalence in voluntary blood donors

Table 3: Rh phenotype prevalence in voluntary blood donors

SL. No.	Rh phenotype	Number	Percentage
1	DCe	308	30.8
2	DCce	234	23.4
3	DCE	125	12.5
4	DCcEe	59	5.9
5	Dce	48	4.8
6	DcEe	45	4.5
7	DcE	42	4.2
8	DCEe	36	3.6
9	ce	16	1.6
10	CE	15	1.5
11	DCc	15	1.5
12	DCcE	11	1.1
13	Ce	09	0.9
14	Cce	08	0.8
15	DEe	07	0.7
16	DE	06	0.6
17	Cc	04	0.4
18	CcE	03	0.3
19	CEe	02	0.2
20	DC	02	0.2
21	De	02	0.2
22	cE	02	0.2
23	Ee	01	0.1
	Total	**1000**	**100.0**

O blood group was found to be the most common, which was comparable to other studies from India, as depicted in Table 4 [8,9,10,11]. O was found to be the most common blood group even in international studies [2,12,13,14]. In a few studies from North India, blood group B was found to be the most common [1,4,6]. This could be attributed to regional and geographic differences among the populations studied.

Rh positivity was detected in 93.9% of donors in the present study and is comparable to other studies [1,2,4,6,8,9,10,11,12,13,14]. Among other Rh antigens, the most common antigen was found to be "C" (82.5%), followed by "e" (77.3%), "c" (49.3%), and "E" (36.2%) (Table 5), in contrast to other studies where the most common antigen was "e," closely followed by "C" [8,14,16]. These differences can also be attributed to the geographic and regional differences among various populations.

On the basis of the expression of different antigens of the Rh system, the most common phenotype was found to be "DCe," present in 30.8% of the donors, followed by "DCce" (23.4%). "DCE" was present in 12.5%, followed by "DCcEe" (5.9%). "Dce" accounted for 4.8% of blood donors. Rh phenotypes differ across different world populations. Phenotypes observed in blood donors would also be prevalent in patients. Knowledge of the distribution of the Rh (D) antigen in the blood donor population also helps in pre-transfusion testing.

In our study, the prevalence of the "K" antigen was 0.2%, being present only in Rh (D) positive donors. This rate is lower compared to other studies, as depicted in Table 6 [14,15,16].

Table 4: Comparison of frequency distribution of ABO and Rh blood groups among different regions in India and International studies

Place of study	A (%)	B (%)	AB (%)	O (%)	Rh positive (%)	Rh negative (%)
Present study	23.60	28.90	6.40	41.10	93.90	6.10
Indian studies						
Tamil Nadu, Chitra M (2021) [8]	18.0	30.0	6.0	46.0	91.0	9.0
Andhra Pradesh, Bhavani C et al (2016) [9]	20.00	35.80	7.30	36.90	96.28	3.72
Karnataka, Anushree CN et al (2017) [10]	21.40	34.00	5.00	38.80	97.10	2.90
Assam, Islam Barbhuiya FG et al (2017) [11]	21.60	29.30	4.80	44.30	98.50	1.50
Uttarakhand, Kumar S et al (2018) [1]	30.39	31.68	11.70	26.24	93.51	6.49
Delhi, Garg N et al (2015) [4]	22.30	39.20	8.90	29.60	93.80	6.20
West Bengal, Basu D et al (2018) [6]	25.13	33.77	9.03	32.07	96.60	3.40
International studies						
Ethiopia, Woldu B et al (2022) [2]	26.44	21.71	4.81	47.04	94.24	5.76
Pakistan, Sabir A et al (2021) [12]	22.58	29.79	14.83	32.78	81.01	18.99
Kenya, Githiomi R (2017) [13]	24.25	18.50	5.50	51.75	93.00	7.00
China, Zhao Y et al (2024) [14]	30.87	27.07	8.50	33.56	99.40	0.60

Table 5: Comparison of Rh antigen frequency distribution in different populations

Place of the study	C (%)	c (%)	E (%)	e (%)
Present study	82.5	49.3	36.2	77.3
North India, Mangwana S et al (2021) [15]	86.28	58.57	19.66	98.79
South India, Chitra M (2021) [8]	84.0	67.0	25.0	98.0
Jaipur, Amran Z et al (2017) [16]	70.8	65.0	11.6	87.4
Morocco, Zahid H et al (2016) [17]	69.67	85.07	17.18	99.14
China, Zhao Y et al (2024) [14]	88.77	53.63	44.94	92.61
Caucasians, Reid ME et al (2012) [18]	68.00	80.00	28.00	98.00
Blacks, Reid ME et al (2012) [18]	27.00	96.00	28.00	98.00

Table 6: Comparison of the prevalence of Kell phenotype in various populations.

Place of study	Percentage (%)
Present study	**0.2**
North India, Mangwana S et al (2021) [15]	**2.57**
Delhi, Garg N et al (2015) [4]	**1.6**
Jaipur, Amran Z et al (2017) [16]	**2.9**
Morocco, Zahid H et al (2016) [17]	**7.0**

Conclusion

Multi-transfused patients develop alloantibodies against some of the rare antigens (C, c, E, e, and Kell). Therefore, it is important to type the rare antigens before issuing the blood. Antigen typing of the various blood group systems will help in the preparation of indigenous screening cells and identification panels. Because of its simplicity and efficacy, the gel-card test is a rapid and practical method for population studies. Knowledge of the Rh antigen profile of a particular population may be helpful in devising more cost-effective ways of providing maximum benefits with the least resources. However, considering the regional differences

in antigen frequency of various blood group systems due to ethnic heterogenicity, extensive studies need to be conducted in this field so that treatment decisions can be made in a more scientific, population-specific, and cost-effective manner.

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Competing Interests: Nil

References

1. Kumar S, Modak PK, Ali SH, Barpanda SK, Gusain VS, Roy R. A retrospective study: ABO and Rh phenotype blood group distribution among blood donors in H.N.B. Base Hospital, Srinagar, Uttarakhand, India. J Family Med Prim Care. 2018;7:34–8.
2. Woldu B, Melku M, Shiferaw E, Biadgo B, Abebe M, Gelaw Y. Phenotype, allele and genotype frequency of ABO and Rhesus D blood groups of blood donors at the North Gondar District Blood Bank, Northwest Ethiopia. J Blood Med. 2022;13:11–9.
3. Farhud DD, Yeganeh MZ. A brief history of human blood groups. Iran J Public Health. 2013;42:1–6.
4. Garg N, Singh DK, Tomar R, Singh B. Phenotype prevalence of blood group systems (ABO, Rh, Kell) in voluntary, healthy donors – experience of a tertiary care hospital in Delhi, North India. J Blood Disord Transfus. 2015;6:297.
5. Pahuja S, Jain S, Nain M, Goel R, Sehgal S, Jain M. Assessment of Rhesus and Kell blood group antigens, phenotypes, and their allelic frequencies in North Indian blood donors. Asian J Transfus Sci. 2020;14:137–41.
6. Basu D, Datta SS, Montemayor C, Bhattacharya P, Mukherjee K, Flegel WA. ABO, Rhesus, and Kell antigens, alleles, and haplotypes in West Bengal, India. Transfus Med Hemother. 2018;45:62–6.
7. Pachaury R, Arya DR, Mahawar NL, Bharti A, Das PK. Frequency of Rh phenotypes in voluntary blood donors. JMSCR. 2017;5:83–8.
8. Chitra M, Jagannathan SY, Arumugam P, Ravishankar J. Prevalence of Rh antigens among voluntary blood donors in Chennai, Tamil Nadu, India. Int J Res Med Sci. 2016;4:5360–3.
9. Bhavani C, Sujeeva Swapna R, Neeraja M, Sravani P, Chaitanya B. Distribution of ABO blood groups and Rh (D) factor in and around Anatapuramu, Andhra Pradesh. Int J Med Res Rev. 2016;4:372–5.
10. Anushree CN, Sujatha R, Patil SB, Jaya Prakash HT. Distribution pattern of ABO grouping and Rhesus typing among blood donors: a study from a tertiary care teaching hospital blood bank of Dr. BR Ambedkar Medical College, Bangalore. Indian J Pathol Oncol. 2017;4:8–11.
11. Islam Barbhuiya FG, Rahman M, Ahmed SA. Frequency and distribution of ABO and Rh (D) blood groups among the Bengali Muslims of Cachar District of Assam, India. Asian J Multidiscip Stud. 2016;4:2017–20.
12. Sabir A, Iftikhar A, Ijaz MU, Hussain G, Rasul A, Iqbal RK, et al. Retrospective study of frequency of ABO and Rhesus blood group among population of Safdarabad and Faisalabad cities of Pakistan. BMC Res Notes. 2021;14:1–7.
13. Githiomi R, Waiganjo N, Muna K. The heterogeneity and distribution patterns of ABO and RH D phenotypes in the voluntary blood donors of Kenya. J Clin Immunol Immunopathol Res. 2017;8:1–7.
14. Zhao Y, Yao N, Lv Y, Cui D, Xie J. Analysis of Rhesus (Rh) antigen distributions in donors and multi-transfused patients for phenotype-matched transfusion. Indian J Hematol Blood Transfus. 2024;40:130–8.
15. Mangwana S, Simon N, Sangwan L. RH phenotype, ABO and Kell antigens, alleles and haplotypes frequencies in North Indian blood donor population. Glob J Transfus Med. 2021;6:81–5.
16. Amran Z, Mhera M, Agarwal P. Phenotype frequencies of blood group systems (ABO, Rh, Kell) among different patients in Jaipur, India. IJSRSET. 2017;3:1329–32.
17. Zahid H, Yahyaoui A, Uwingabiye J, Khazraji A, Labrini F, Hadef R, et al. Phenotype frequencies of Rh and Kell blood group systems in Blood Transfusion department of Avicenna Military Hospital, Marrakech, Morocco. IJMHR. 2016;2:1–10.
18. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. Academic Press; 2012 Nov 7.