Original Article

Access this article online
Quick Response Code:

Website: www.ajts.org
DOI: 10.4103/ajts.AJTS_34_19

ABO, Rh, and kell blood group antigen frequencies in blood donors at the tertiary care hospital of Northwestern India

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Abstract:

BACKGROUND: This study was performed to provide information on frequencies of ABO, Rh & Kell antigens/alleles, phenotype in blood donors at Blood Bank, SMS hospital, Jaipur and to compare them with other races.

METHODOLOGY: This study was conducted on blood donors from April 2016 to March 2017 using a fully automated system for ABO,Rh & Kell typing of blood cells. D, C, c, E, e & K antigens were typed using monoclonal antisera from Immucor The data were collected and calculations done to determine the antigen/allele, phenotype. The chi square test 3 degree of freedom with $P < 0.001$ (S) was used for comparisons between the results of our study and those of other studies.

RESULTS: A total of 8067 donors were included in this study. Maximum donors was of B blood group (39.4%) of age 18-25(35.5%) with 60-69kg weight (65%). The most common Rh antigen found was e(99.3%) followed by D (93.8%), C (85.4%), c (60.1%), E (17.5%). R1r (DCCee) was the most common phenotype in our study (39.5%). Kell (K+) antigen was present in 2.7% of donors.

CONCLUSION: We have determined the prevalence of Rh antigens and Rh phenotypes in blood donor at our hospital and derived the allele frequencies in the same population.

Keywords:

ABO, antigen frequencies, Kell, phenotyping, Rh

Introduction

Primary goal of transfusion is to provide safe blood to the patient. The concept of safe blood started with the discovery of the ABO blood group in 1901[1] and Rh blood group in 1939–1940.[1] Other blood group systems such as Kell,[2] Kidd, Duffy,[3] and MNSs[4] were discovered later. ABO blood group remains the most important to transfusion as well as transplantation.[5] Rh blood group system is the second-most important system after ABO.[6] It has 50 antigens and 5 most important antigens include D, C, c, E, and e. D is most immunogenic, and its antibody (anti-D) leads to hemolytic disease of the fetus and newborn (HDFN). The Kell blood group system is the third-most important immunogenic blood group system. It was discovered in 1946 following the discovery of antiglobulin test.[7] There are 25 antigens in the Kell blood group system. Anti K is associated with HDFN. Antibodies of all blood group systems are lethal and can cause alloimmunization, HDFN, and hemolytic transfusion reactions. When red cells positive for a particular antigen are transfused to a patient who lacks that antigen, the possibility of production of the corresponding antibody develops. This depends on the immunogenecity

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Submission: 01-04-2019
Accepted: 05-01-2019
Published: 19-12-2020

How to cite this article: Prinja N, Narain R. ABO, Rh, and kell blood group antigen frequencies in blood donors at the tertiary care hospital of Northwestern India. Asian J Transfus Sci 2020;14:179-84.

of the antigen. The prevalence and antigenicity of various antigens determine the frequency of occurence of that corresponding antibodies. Antibodies against high-frequency antigens are uncommon, whereas alloantibodies against antigens of low/intermediate frequency with high antigenicity are frequent. Hence, blood grouping, cross matching, antibody screening, antibody identification, and phenotyping of red cells are important for safe transfusion. These procedures have added to the cost of blood banking. Keeping in view the heavy financial burden of phenotyping of all clinical significant antigens, the phenotyping of Rh and Kell can play a major role in preventing the alloimmunization and avoiding adverse events in subsequent transfusions in multitransfusion cases.[7] This was the first study from Northwestern India, which was done to record the phenotypic frequency of Rh and Kell. This study contributed to the development of blood bank data for the constitution of panel of blood donors, particularly for multitransfused, alloimmunized patients. The aim of our study is to determine the frequencies of ABO, Rh, and Kell antigens in blood donors to generate the blood bank database and to compare the results with other ethnic groups/populations.

Materials and Methods

Study design
This is a descriptive type of the cross-sectional study.

Study setting
This study was conducted in the blood bank of the tertiary care hospital of Northwestern India.

Study subjects
Healthy voluntary blood donors were selected as per the inclusion and exclusion criteria as per departmental Standard Operating Procedures (SOP).

Inclusion criteria
The inclusion criteria were as follows:
1. Age >18 years
2. Weight of donor >60 kg.(as per departmental SOP)
3. Medical examination and general conditions within the normal limits
4. Donor willing to participate in the study.

Exclusion criteria
The exclusion criteria were as follows**:**
1. Age <18 years
2. Weight of donor <60 kg
3. Donor not giving consent for the study
4. Various medical/surgical conditions as per departmental SOP.

Sample size
Eight thousand and sixty-seven voluntary donors were recruited in the study.

Ethics approval
This study was approved by the Institutional Ethics Committee. Donors were enrolled after that.

Written informed consent for the study was also obtained at the time of donor screening.

Study procedure
Blood donation was taken in the quadruple bag. It was done from the ante-cubital vein following all aseptic measures. All blood samples were phenotyped for ABO, Rh (C, c, E, e, and D), and Kell (K, k) antigens on a fully automated system (Galileo, Immucor). Units that tested positive for Rh D antigen were labeled as Rh positive. All units that tested negative for Rh D antigen were further tested for the presence of weak D by the tube technique.

Galileo is a robotic instrument that is programmed to move all the necessary micro plates, liquid reagents fluids, and blood sample fluids to the necessary area of processing for a given assay in the correct sequence, for example, incubator bays, microplate washing station, centrifuge, and reader. The microplate reader uses charged-coupled device cameras to capture an image of the microplate from underneath. The Galileo software then calculates a reaction value for each well based on multi-feature image analysis. A result and interpretation is then assigned to the wells based on the predefined criteria associated with the calculated reaction value. The mechanism and data processing of the Galileo is software driven.

Statistical analysis
Qualitative data were expressed in the form of percentage and proportions. A significance of difference was inferred by the Chi-square test. Quantitative data were expressed in the form of mean ± standard deviation. A significance of difference was inferred by the *t*-test. For significance, $P < 0.05$ was considered statistically significant. MEDCALC 12.2.1.0 version software (MedCalc Software Ltd, Acacialaan 22, 8400 Ostend, Belgium) was used for the statistical analysis.

Observation and Results

Nearly 39.5% of donors were B group followed by O (31.3%), A (21.4%), and AB (7.8%) [Table 1]. D antigen was present in 93.8% (7566) donors and absent in

Table 1: ABO blood group of donors

Blood group	Number of participants, *n* (%)	95% CI
A	1730 (21.4)	20.5-22.3
B	3182 (39.5)	38.3-40.5
O	2523 (31.3)	30.3-32.3
AB	632 (7.8)	7.3-8.4
Total	8067 (100.0)	

CI=Confidence interval

Table 2: Rh-D antigen of donors

D antigen	Number of participants, *n* (%)	95% CI
D positive	7566 (93.8)	93.3-94.3
D negative	501 (6.2)	5.7-6.7
Total	8067 (100.0)	

D antigen was present in 93.8% (7566) donors and absent in 6.2% (501) of the population. CI=Confidence interval

Table 3: Coexistence of D antigen and ABO blood group of donors

Blood group	A (*n*=1730), *n* (%)	B (*n*=3182), *n* (%)	O (*n*=2523), *n* (%)	AB (*n*=632), *n* (%)
D positive (*n*=7566)	1590 (19.7)	3022 (37.5)	2362 (29.3)	592 (7.3)
D negative (*n*=501)	140 (1.7)	160 (2)	161 (2)	40 (0.5)

χ^2=18.296 at 3 degree of freedom; *P*<0.001 (significant)

Table 4: Rh antigen frequency in the study population

Rh antigen	Number of participants, *n* (%)	95% CI
C	6892 (85.4)	13.8-15.3
c	4846 (60.1)	59-61.2
E	1410 (17.5)	16.6-18.3
e	8012 (99.3)	9.1-99.5

CI=Confidence interval

6.2% (501) of the population [Table 2]. D antigen was present maximally in B blood group donors followed by O, A, and AB blood group donors [Table 3]. Eight thousand and twelve (99.3%) donors had e antigen [Table 4]. This means that e was the most common (99.3%), followed by D (93.8%), C (85.4%), c (60.1%), and E (17.5%). Distribution of each antigen (C, c, E, and e) was followed for each blood group (A, B, O, and AB). e antigen was maximum present in all the blood groups. e antigen was maximally present in both D-positive and D-negative donors. C antigen is more prevalent in D-positive donors. C antigen is more prevalent in D-negative donors. The difference among C/c/E Rh antigens in D positive and D negative groups was found to be statistically significant on the Chi-square test ($P < 0.001$–$P = 0.102$) [Table 5]. The most common Rh phenotype observed was DCCee followed by DCcee > DCcEe > ccee > DccEe > Dccee > DccEE > Ccee > DCCEe [Table 6]. Kell antigen is present in 214 (2.7%) of the blood donors of our study population, whereas 97.3% of the donor population is Kell negative [Tables 7 and 8].

Discussion

The antigen frequencies of the study population were compared within the country and abroad. The distribution varies regionally, ethnically, and from one population to another. In the present study, the ABO blood group antigen frequencies showed the prevalence as B > O > A > AB. Studies conducted by Agarwal *et al.*,[8]

Table 5: Coexistence of other Rh antigens in ABO blood group system

Antigen	Blood group				χ^2	*P*
	A (*n*=1730), *n* (%)	B (*n*=3182), *n* (%)	O (*n*=2523), *n* (%)	AB (*n*=632), *n* (%)		
C	1417 (17.6)	2721 (33.7)	2189 (27.1)	565 (7)	28.863	<0.001 (significant)
c	1153 (14.3)	1844 (22.9)	1529 (19)	320 (4)	60.925	<0.001 (significant)
E	328 (4)	594 (7.4)	427 (5.2)	61 (0.8)	33.127	<0.001(significant)
e	1723 (21.4)	3148 (39)	2509 (31.1)	632 (7.8)	13.929	0.004 (significant)

Table 6: Incidence of various Rh phenotypes in the study population

Phenotype	Number of participants, *n* (%)	95% CI
DCCee	3188 (39.5)	38.4-40.6
DCcee	2772 (34.4)	33.3-35.4
DCcEe	904 (11.2)	10.5-11.9
ccee	480 (5.9)	5.4-6.4
DccEe	457 (5.7)	5.2-6.2
Dccee	176 (2.2)	1.8-2.6
DccEE	55 (0.7)	0.5-0.9
Ccee	21 (0.3)	0.1-0.4
DCCEe	14 (0.2)	0.1-0.3
Total	8067 (100.0)	

Most prevalent phenotype was DCCee and least prevalent was DCCEe. CI=Confidence interval

Table 7: K antigen in donors

Antigen	Number of participants, *n* (%)	95% CI
K positive	214 (2.7)	2.3-3
K negative	7853 (97.3)	97-97.7
Total	8067 (100.0)	

CI=Confidence interval

Table 8: Coexistence of K antigen with ABO Blood group system

Blood group	A (*n*=17302523), *n* (%)	B (*n*=31822523), *n* (%)	O (*n*=25232523), *n* (%)	AB (*n*=6322523), *n* (%)
K positive (*n*=214)	41 (2.4)	93 (2.9)	47 (1.9)	33 (5.2)
K negative (*n*=7853)	1689 (97.6)	3089 (97.1)	2476 (98.1)	599 (94.8)

χ^2=23.678 with 3 degrees of freedom; *P*<0.001 (significant)

Chandra and Gupta,[9] and Gundrajukuppam *et al*.[10] also followed the same trend. In a study conducted by the Australian Red Cross society[11] and in the USA by Mollison *et al*.,[12] the most common blood group was O followed by A, B, and AB. Similar study was conducted by Hammed *et al*.,[13] in which the most common blood group was B. In all the studies,[14-17] the incidence of D positive is more common than D negative which varies from 93.7% to 97.8%. The frequency of Rh positive in our study was 93.8%, whereas only 6.2% were Rh negative. Comparing the results of our study with different regions of the world, it has been observed that there is a huge variation in the prevalence of D antigen globally. The variation of prevalence of D antigen has been seen from 60% to 80% in Southern France[18] to 60%–99% in Japan, Burma, and Malaysia.[18] This means that the least cases of HDFN are seen in Japan, Burma, and Malaysia. The frequency of D-negative person among the Taiwanese population is 0.3%, and hence, the development of Anti D is very less. Hence, D typing in the patients of Taiwan has been discontinued.[19] In India, the frequency of D-negative antigen varies from 5% to 10%, so D typing is essential for blood donors and patients requiring blood transfusion in Indian. Antigen frequency (AF) of other Rh antigens (C, c, E, and e) was compared to other Indian studies [Table 9]. The most prevalent antigen in our study is "e" with 99.3%, and the least common antigen was E (17.5%) which is similar to all other studies of India. The trend of the prevalence of other Rh antigens in all Indian studies is e > C > c > E. The prevalence of "E" antigen in our study is 17.5% which is least among all studies. The frequency of c antigen in our study is 60.1%. "e" antigen is most commonly seen in India and other regions globally.[25,26] Therefore, it is very difficult to find "e" antigen-negative donor for a patient with alloimmunization against this antigen. C is the second-most antigen found in India, China, and in Asia,[26] whereas in other population, c antigen is the second most common.[25] E is the least common antigen found in all regions worldwide. This AF in the given population helps in predicting the common alloantibodies in transfused patients. It helps in the selection of antigen negative blood for patients with alloantibodies.

Table 9: Comparison of antigen frequencies with different Indian studies

Indian studies	C	c	E	e
Thakral *et al*. (*n*=1240)[20]	84.8	52.8	17.9	98.3
Singh *et al*. (*n*=500)[21]	87.4	58.8	20.4	98.8
Singh *et al*. (*n*=1000)[22]	85.1	62.3	21.5	99.0
Kahar and Patel (*n*=115)[23]	81.74	56.52	21.74	100
Garg and Kumar Singh (*n*=2769)[24]	91.8	55.2	21.1	98.7
Present study (*n*=8067)	85.4	60.1	17.5	99.3

The prevalence of other Rh antigens in D positives and D negatives was compared with different Indian studies. The prevalence of Rh antigens in D positives in our study is similar to the other studies.[20,10] In D negative, the prevalence of c and e antigen is seen 100% in our study. The same is seen in other studies except in Kahar and Patel.[23] E antigen in D-negative donors is not seen in our study, which is similar to the study by Thakral *et al*.[20] C antigen in D-negative donors is least in our study [Table 10]. The most common phenotype was found to be R_1R_1 (39.5%) followed by R_1r (34.4%). Other studies such as D. Krishna *et al*. (43.4%), Thakral *et al*. (43.8%), Sharma *et al*. (41%), Garg and Singh (44.60%), and Kahar and Patel (40.87%) also found similar results. In our study, the most common phenotype among Rh positives (n = 2771) is R_1R_1 with 39.5%, and the least common phenotype is R_1R_2 with 0.2%. Among Rh negatives (n = 243), the most common phenotype is rr with a frequency of 5.9% [Table 11]. R_1r is the most common phenotype in whites (34.9%) and Caucasians (35.6%). The most common Rh phenotype in blacks is R_0r (45.8%). Differences were observed in the prevalence of Rh phenotypes among the Indian population and other Asian population, and the most common phenotype in Asians was R_1R_1 (51.8%) followed by R_1r (8.5%).[25]

Rh phenotype variation is seen in different populations around the globe. Blood donors and patients are genetically homogenous in a country so it can be said that same Rh phenotypes/genotypes that are observed in blood donors would be present in patients too. It is clinically important to know the common phenotypes/genotypes as it helps in the management of patients forming alloantibodies due to transfusions and also the prevention of alloimmunization in multitransfused patients.

Table 10: Comparison of Rh phenotypes with other Indian studies

	R1R1	R1r	R1R2	rr	R2r	R0r	R2R2	r'r	R1RZ
Gundrajukuppam *et al.* (*n*=1000)[10]	43.4	31.2	10.7	4.7	0.5	1.2	0.7	0.6	1.3
Thakral *et al.* (*n*=1240)[20]	43.8	30	8.22	5.81	8.95	0.97	1.45	0.56	-
Sharma *et al.* (*n*=1000)[28]	41	25.5	3.1	5.6	5.5	3.0	4.7	1.3	2.2
Garg and Singh (*n*=2769)[24]	44.60	32.60	14	0.07	5.90	1.30	0.80	-	-
Kahar and Patel (*n*=115)[23]	40.87	23.48	13.91	11.30	4.35	0.87	-	-	0.87
Present study (*n*=8067)	39.5	34.4	11.2	5.9	5.7	2.2	0.7	0.3	0.2

Table 11: Worldwide comparison of the prevalence of various Rh phenotypes

Nomenclature		Percentage prevalence of phenotypes				
Weiner	Fisher - race	White[25]	Black[25]	Asian[25]	Caucasian[25]	Present study
R_1R_1	DCCee	18.5	2	51.8	19.5	39.5
R_1r	DCcee	34.9	21	8.5	35.6	34.4
R_1R_2	DCcEe	13.3	4	30	12.5	11.2
rr	ccee	15.1	6.8	0.1	15.1	5.9
R_2r	DccEe	11.8	18.6	2.5	11.3	5.7
R_0r	Dccee	2.1	45.8	0.3	1.7	2.2
R_2R_2	DccEE	2.3	0.2	4.4	2	0.7
r'r	Ccee	0.8	*	0.1	0.8	0.3
R_1Rz	DCCEe	0.2	*	1.4	0.2	0.2

*Nomenclature and percentage prevalence of phenotypes among different ethnic groups

The percentage prevalence of Kell antigen varies from 1.60 by Garg and Singh *et al*.[24] to 6.09 by Kahar and Patel.[23] The K (KEL 1) AF in our study was 2.92% which is similar to the study conducted by Singh *et al*.(2.8%).[22] Other studies done by Thakral *et al*. (5.68%),[20] Singh *et al*. (4.4%),[21] and Kahar and Patel (6.09%)[23] had different results. This difference is seen as our study includes a large number of donors as compared to the other studies. Hence, there is a need to perform more studies on the prevalence of kell antigen with a large study population sample size to know the accurate prevalence of frequency of Kell antigen in the population. Cellano antigen is highly prevalent in our population, and hence, anti-k is not formed. Kell antigen is highly antigenic and present in low frequency. Hence, it is responsible for the frequent occurrence of anti-Kell antibody. The expression of Kell antigen varies from 0% in the Chinese population[26] to 9% in whites and Caucasians.[25] The prevalence of K antigen was found to be 2.7% in our study, which is similar to the Black population.

R1R1 is the most common genotype/phenotype in the Indian population followed by R1r, and hence, the most common alloantibodies among Rh blood group system would be antiE and antic. This has been proved in different studies,[27] which proved that the most common alloantibodies are antiE and antic in thalassemics[28] and chronic renal failure patients.[29]

Summary and Conclusion

Nearly 39.5% of donors were of B group followed by O (31.3%), A (21.4%), and AB (7.8%). The prevalence of blood group in our study population was B > O > A > AB. D antigen was present in 93.8% of total donor population. D antigen was absent in 6.2% of total donor population. D antigen was present in 95% of B blood group donors followed by AB (93.7%), O (93.6%), and A (91.9%) blood group donors. e antigen was found to have the highest prevalence, it is present in 99.3% of the study population. About 85.4% of the donors studied showed C positivity and 60.1% were positive for c antigen. E antigen was found to be present in 17.5%. The most common Rh phenotype observed was DCCee followed by DCcee > DCcEe > ccee > DccEe > Dccee > DccEE > Ccee > DCCEe. The prevalence of K antigens observed in our study population was 2.7%, whereas 97.3% population is Kell negative.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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