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Frequency of Rh and Kell antigens among blood donors: A retrospective analysis from a tertiary care center in Eastern India

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ABSTRACT

Objectives: The phenotyping of Rh and Kell antigens plays a major role in preventing alloimmunization and adverse events in multi-transfused patients. This study tries to highlight the frequency of Rh and Kell antigens among the blood donors who donated blood at a tertiary care center located in Eastern India.

Material and Methods: The study was conducted in the Department of Transfusion Medicine and Blood Bank of All India Institute of Medical Sciences, Patna, from January 2021 to March 2023 on 10,032 whole blood donors of blood groups A, B, O, and AB by column agglutination technique using gel cards for blood grouping and phenotyping.

Results: A total of 10,032 whole blood donors of groups A, B, O, and AB were included in the study. The frequency of "D" antigen was 95.77% (n = 9,608), "C" was 90.47% (n = 9,076), "c" was 50.47% (n = 5,063), "E" was 15.9% (n = 1,595), "e" was 99% (n = 9,932), and "K" was 2.67% (n = 268). "c" antigen frequency was 45.81–49.48% in RhD positive blood groups and 100% in all the RhD negative blood groups while "C" antigen frequency was 92.04–94.68% in RhD positive blood groups, and 4–24.13% in RhD negative blood groups.

Conclusion: Since pre-transfusion phenotyping in multi-transfusion patients is not routinely practiced, transfusion of at least Rh and Kell phenotyped donor red cells can lead to a great decrease in the risk of alloimmunization and adverse events related to transfusion.

Keywords: Alloimmunization, Antigen, Frequency, Kell, Phenotype, Rh, Transfusion

INTRODUCTION

ABO blood group is considered the most important blood group system in the human body, followed by Rh blood group system for transfusion as well as solid organ or peripheral blood stem cell transplantation.[1,2] While Rh system antigens are highly immunogenic, the Kell blood group antigens are second to Rh antigens in immunogenicity, causing hemolytic transfusion reactions (HTR) and hemolytic disease in fetuses and newborns.[3] The main aim of blood transfusion services is to provide the recipient with viable donor red cells that survive in circulation for their lifetime and to give the recipient all the benefits of transfusion. However, transfusion is also associated with a wide range of adverse events, of which HTR and alloimmunization are the most lethal ones. To provide safe blood, pretransfusion testing is performed, which includes blood group determination of both donor and recipient, cross-matching, antibody screening, and identification, as well as blood group antigen phenotyping in both donors and recipients. These

investigations become even more crucial in the case of those patients who require multiple transfusions, such as cancer and dialysis patients, as well as those with thalassemia and sickle cell anemia. As per the World Health Organization, hemoglobinopathy carriers are found in approximately 4.5–5% of the world's population. In India, there are about 30 million carriers, while the mean prevalence of such hemoglobinopathies is 3.3%.[4,5] Sickle cell, hemoglobin D, and hemoglobin E are the most predominant abnormal hemoglobins, and their cumulative gene frequency in India has been found to be 5.35%.[6] Providing safe blood to these patients poses a great challenge to blood transfusion services, especially in government medical care centers with huge workloads. The provision of antigen-matched red blood cells (RBCs) reduces alloimmunization among multi-transfused patients, along with improved RBC survival and reduced frequency of transfusions.[7,8] Furthermore, pretransfusion minor blood group antigen phenotyping of such patients is not routinely practiced everywhere. Providing minor antigen-phenotyped red cells puts a huge financial burden, especially in resource-constrained settings. In such a scenario, phenotyping for only Rh and Kell blood group antigens can cause a great decrease in the risk of alloimmunization and avoid adverse events related to subsequent transfusions.

Since there are very few studies about frequencies of Rh and Kell blood group antigens available in eastern parts of India, the study aimed to present the frequency of these antigens in whole blood donors who donated blood at our center. Furthermore, the frequency of these antigens was calculated separately in different blood groups.

MATERIAL AND METHODS

Study setting and design

This was a cross-sectional retrospective observational study conducted at the Department of Transfusion Medicine and Blood Bank of an apex tertiary care center in Eastern India for a period of 27 months, from January 2021 to March 2023. The study population included the whole blood donors of A, B, O, and AB blood groups screened according to the Standards of Blood Banking and Drug and Cosmetics Act 1940.[9,10] Those donors who were positive for transfusion-transmitted infections (HIV 1 and 2, HBV, HCV, Syphilis, and Malaria) and positive indirect antiglobulin test (IAT) were excluded from the study. After the determination of the blood group, phenotyping for Rh and Kell antigens was done.

Sample collection

Blood collected in pilot tubes (from the diversion pouch) during blood donation was used for the testing. No extra blood sample was collected from the donors.

Immunohematology

About 1% red cell suspension was prepared in a low ionic strength solution. For determination of blood group, forward and reverse group was performed on ABO gel cards (BIORAD). Rh and Kell phenotyping was performed using Rh and Kell phenotyping gel cards (BIORAD). IAT was performed on each donor sample. Only those samples that were IAT negative were undertaken for Rh and Kell antigen typing. All these procedures were performed using the column agglutination technique and according to the manufacturer's instructions.

Statistical analysis

Data were entered into a Microsoft Excel sheet; numerical values, percentages, mean, and standard deviation were calculated. Statistical analysis was performed using SPSS software (Version 25.0.0.0, Chicago, USA).

RESULTS

A total of 10,032 whole blood donors were included in the study out of which 97.96% (n = 9,828) were male while 2.03% (n = 204) were female. The mean age of the donors was 29.85 ± 8 years. The proportions of RhD positive as well as negative blood groups are shown in Figure 1.

Frequencies of Rh and Kell antigens were calculated of which frequency of "D" antigen was 95.77% (n = 9,608), "C" was 90.47% (n = 9,076), "c" was 50.47% (n = 5,063), "E" was 15.9% (n = 1,595), "e" was 99% (n = 9,932), and "K" was 2.67% (n = 268). This is shown in Figure 2.

The frequency of these antigens was compared with those in other major studies done in blood donor populations across India as well as with other ethnic groups worldwide [Table 1].

The frequencies of Rh Phenotypes were also calculated and compared with those of the Indian population as well as other racial groups [Table 2]. R_1R_1 phenotype was found to

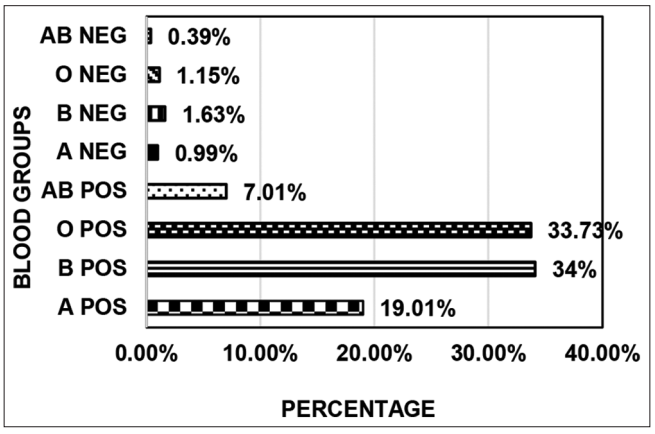

Figure 1: Proportion of different blood groups.

Table 1: Comparison of Rh and Kell antigens frequencies with other studies.

Antigen	Present study (*n*=10,032) (%)	Makroo *et al.* (*n*=3,037) (%)	Thakral *et al.* (*n*=1,240) (%)	Setya *et al.* (*n*=6,678) (%)	Caucasians (%)	Blacks (%)
D	95.77 (*n*=9,608)	93.6	93.4	93.8	85	92
C	90.47 (*n*=9,076)	87	84.7	88.25	68	27
c	50.47 (*n*=5,063)	58	52.8	52.62	80	96
E	15.9 (*n*=1,595)	20	17.9	19.77	29	22
e	99.16 (*n*=9,932)	98	98.3	98.38	98	98
K	2.67 (*n*=268)	3.5	5.56	5.14	9	2

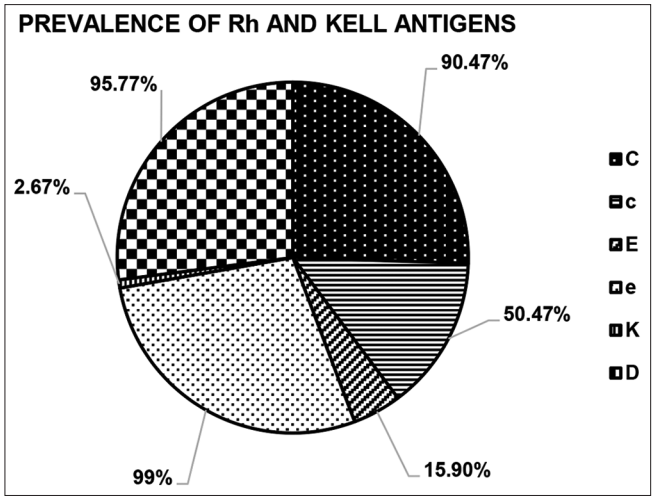

Figure 2: Frequency of Rh and Kell antigens.

be the most common, with a frequency of 49.4% (*n* = 4,957). Since genotype was not performed, these were the presumed Rh phenotype frequencies.

The frequency of these antigens was also calculated in different blood groups. While "c" antigen frequency was found to be 45.81–49.48% in RhD-positive blood groups, it was 100% in all the RhD-negative blood groups. Moreover, the frequency of "C" antigen in RhD-positive groups was 92.04–94.68% and 4–24.13% in RhD-negative blood groups. This is shown in Figures 3a and b for RhD positive and RhD negative blood groups, respectively.

DISCUSSION

It is very important for blood transfusion services to know about various blood group antigens and different phenotype frequencies in a population, and this information plays a pivotal role in areas such as antenatal serology, selection of compatible red cells in difficult transfusions, and paternity testing.[11] These frequencies have been well studied worldwide in Caucasian and Black races as well as in some parts of India.[11-13] Extensive polymorphism is shown by the Rh blood group system, and their allelic frequencies are different in different populations.[14]

In the present study, frequencies of Rh and Kell antigens were determined in the blood donor population of the eastern part of India. "D" is the most common Rh antigen worldwide, as indicated by various studies, and its frequency in this study was 95.77%, which was similar to those studies.[11-13] The frequency of "e" antigen was found to be 99%, which was comparable to that of Caucasians, Blacks, and a few studies done in the Indian population, as shown in Table 1, and it was the most common Rh antigen found worldwide as shown by various studies.[12,13,15-17] In Georgia, this frequency has been reported to be 100%.[18] The second most common Rh antigen was "C," with a frequency of 90.47%, similar to other Indian studies.[15-17] However, in Caucasians and Blacks, "C" was the third most common antigen with a frequency of 68% and 27%, respectively, while "c" was the second most common Rh antigen. In the present study, "c" was the third most common Rh antigen, similar to other Indian studies, except for that from northern India by Makroo *et al.*, where the frequency of "c" antigen was slightly higher than ours.[12,13,15-17] The least common Rh antigen found in our, as well as other populations, was "E," as shown in Table 1.

The frequency and variability of antigens affect their immunogenicity. The antigens with low frequency in a population are highly immunogenic and vice versa. Since "E" is a low-frequency antigen, it is highly immunogenic, while "C" and "e" are high-frequency antigens and, hence, have less immunogenicity. "c" has been described to be highly immunogenic next to "D." Many studies have explained anti-c and anti-E to be the most common alloantibodies formed in multi transfused patients.[16,19-23]

"K" antigen has also been described to be highly immunogenic apart from Rh antigens, followed by "c" and "E."[24] The frequency of "K" in the present study was 2.67%, which was somewhat similar to that determined in other studies which were 2–9%.[12,13,15-17] Due to its low prevalence, it has high immunogenicity, and hence, the formation of anti-K alloantibody is very common in multi-transfused patients. Another member of the Kell blood group system, cellano (k), is highly prevalent, and hence, anti-k alloantibody is rarely reported.

Table 2: Rh phenotype frequencies in Indian population and other ethnic groups.

Rh phenotype (Weiner)	Rh phenotype (Fischer-race)	Present study (*n*=10,032) (%)	Makroo *et al.* (*n*=51,857) (%)	Basu *et al.* (*n*=1,476) (%)	Setya *et al.* (*n*=6,678) (%)	Caucasians (%)	Blacks (%)
R_1R_1	CCDDee	49.4 (*n*=4,957)	40.95	49.02	43.71	18.5	2
R_2R_2	CcDDEE	0.83 (*n*=84)	0.78	0.72	1.55	2.3	0.2
R_1r	CcDdee	29.19 (*n*=2,929)	30.91	27.75	29.56	34.9	21
R_2r	CcDdEe	3.67 (*n*=369)	3.7	3.4	8.32	11.8	18.6
R_0r	CcDdee	1.4 (*n*=140)	1.15	0.98	1.12	2.1	45.8
R_zR_z	CCDDEE	0 (*n*=0)	0.002	0.26	0	0.01	0
R_1R_z	CCDDEe	0.16 (*n*=16)	0.32	0.72	0	0.2	0
R_2R_z	CcDDEE	0 (*n*=0)	0.4	0	0	0.1	0
R_1R_2	CcDDEe	11.12 (*n*=1116)	14.53	13.74	9.5	13.3	4
Rr	Ccddee	3.59 (*n*=361)	4.76	2.75	5.14	15.1	6.8
r'r	Ccddee	0.52 (*n*=52)	2.32	0.52	0.7	0.8	-
r"r	CcddEe	0.04 (*n*=04)	0.05	0.13	0.37	0.43	-
r'r'	CCddee	0 (*n*=0)	0.05	0	0	0.01	-
r"r"	CcddEE	0 (*n*=0)	0.004	0	0.01	0.002	-
r"r^y	CcddEE	0 (*n*=0)	0.002	0	0.01	-	-
r'r"	CcddEe	0.04 (*n*=04)	0.075	0	0	0.05	-

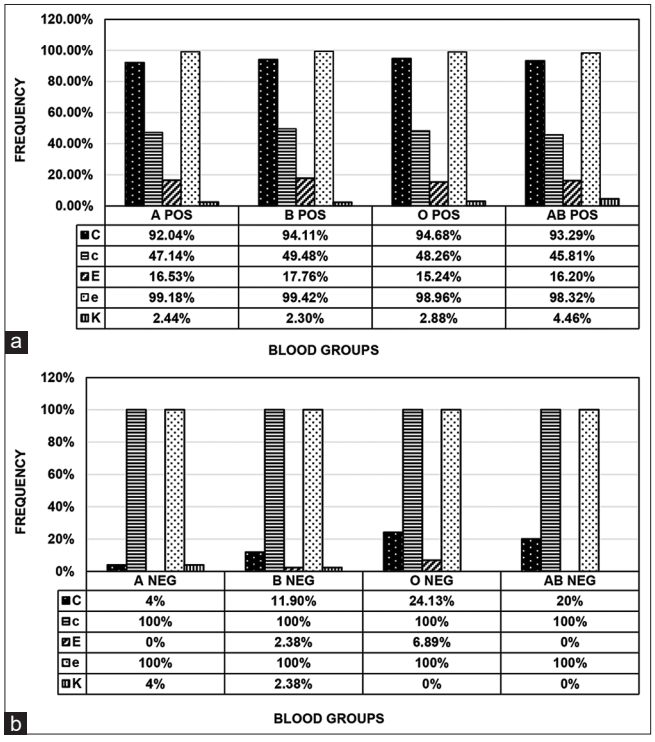

Figure 3: Frequency of Rh and Kell antigens in: (a) RhD positive blood groups and (b) RhD negative blood groups.

Due to genotypic homogeneity in both donor and patient populations in our country, common Rh phenotypes were also studied as they help in managing serologically those patients in whom alloantibodies have been formed due to repeated transfusions. The comparison of common Rh phenotypes is shown in Table 2. R_1R_1 was the most common, and R_1r was the second most common phenotype in our study, similar to that observed by Basu *et al.*[25] However, the frequency of R_1R_1 was a bit higher than that observed by Makroo *et al.*[26] On the other hand, the most common Rh phenotype in Caucasians was R_1r, while that in Blacks was R_0r.[12,13] This high prevalence of the R_1R_1 phenotype explains the common occurrence of anti-c and anti-E alloantibodies in our population.

This study also tried to determine the frequency of Rh and Kell antigens in various blood groups. The frequency of these antigens in RhD-positive blood groups was similar to that of the entire population included in the study. However, this picture was somewhat different in the RhD-negative population, where the frequency of "C" was <50% and that of "c" was 100% in all the RhD-negative blood groups.

In a resource-constrained country like India, the practice of providing at least Rh and Kell antigens matched red cells can lead to a great decrease in alloimmunization rates and increased red cell survival, leading to reduced frequency of transfusions and better clinical outcomes. Singer *et al.* observed a decreased rate of alloimmunization from 33% to 2.8% by providing Rh and Kell-matched blood.[20] Hence, it is the need of the hour to make Rh and Kell's antigen phenotyping a necessary part of routine pre-transfusion testing, even in small blood centers.

The strength of the study was a robust sample size. The limitation of the study was the lesser number of RhD-negative donors for validation of the antigens' frequency calculation in RhD negative blood groups.

CONCLUSION

Due to the high immunogenicity of Rh and Kell blood group antigens, the multi-transfused patients are at a high risk of

forming various alloantibodies due to repeated transfusions, which can lead to transfusion-related adverse events and increased frequency of transfusions due to poor red cell survival in the recipient. In low-to-middle-income countries like ours, pre-transfusion phenotyping in such patients is not routinely practiced. Transfusion of at least Rh and Kell phenotyped donor red cells can lead to a great decrease in the risk of alloimmunization and related adverse events, as well as better clinical outcomes.

Ethical approval

The research/study was approved by the institutional ethics committee (IEC) [AIIMS/Pat/IEC/2022/1096 dated 10th August 2023].

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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