



## “FREQUENCIES OF ABO, RH, KELL ANTIGENS IN BLOOD DONORS AT A NORTH INDIAN TERTIARY BLOOD CENTER: A CROSS-SECTIONAL STUDY. “

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### Abstract-

**Context:** It is important to assess the ethnic distribution of Red Blood Cell (RBC) antigen phenotype frequencies in the population as it helps in establishing a comprehensive donor data bank. It also helps in formulating indigenous cell panels and ensuring the availability of specific antigen negative compatible blood.

**Aims:** To assess the prevalence of ABO, Rh, and Kell antigens among replacement blood donors from diverse ethnic groups in Northern India.

**Settings and Design:** This prospective observational cross-sectional study was conducted over a one-year period from November 2022 to October 2023 at a tertiary care hospital of north india.

**Methods and Material:** 500 random samples were collected from healthy voluntary and replacement blood donors, representing diverse ethnic groups and were phenotyped for A,B, Rh (D,C, E, c, e) and Kell (K) antigens using column agglutination technology glass bead based cassettes.

The data was compiled into Microsoft Excel and was then analyzed through IBM SPSS Statistics version 27. Chi-Square ( $\chi^2$ ) test was utilized to evaluate the significance of relationships

**Results:** This study involved phenotyping 500 samples collected from donors of diverse ethnic groups, including Dogras, Non-Gujjar Muslims (NGM), Gujjar Muslims (GM), Sikhs, Kashmiri Pandits (KPs), and Christians. Nearly 35.4% (n= 177) donors were B group, O = 28.2% (n= 141), A = 25.6% (n= 128), and AB = 10.8% (n= 54). On extended phenotyping for Rh antigens, frequency of ‘e’ antigen was 97.6% (n=488), ‘D’ antigen 94.6% (n=473), ‘C’ antigen 86.6% (n=433), ‘c’ antigen 62% (n=310) and ‘E’ antigen 19.4% (n=97).

It was observed that R1R1 phenotype was highest among Sikhs (43.33%), R1r among Christians (50%), R1R2 among Gujjar Muslims (20.93%), R2r among Kashmiri Pandits (16.66%), R2R2 among Christians (50%), R0r among Gujjar Muslims (4.65%), rr among Gujjar Muslims (11.62%) and r’r (0.55%) among Dogra population.

The overall frequency of the Kell (K) antigen was 2.6%, highest among Gujjar Muslims (4.65%). The prevalence of Kell (K) antigen was higher among 'A' blood group donors (3.9%), followed by the 'AB' group (3.7%), 'O' group (2.83%), and 'B' group (1.12%).

**Conclusions:** Red cell antigen frequencies across diverse ethnic populations helps in establishing a robust donor database and development of in-house cell panels and fulfils the need of antigen negative compatible blood. Phenotyping aids in diminishing the likelihood of RBC antigen allo-immunization and the related complications. This can further contribute to the development of a rare blood group registry at both regional and national levels.

**Key-words:** Phenotype, Alloimmunization, Antibodies, Rh antigens, ABO antigens, Kell antigens

### **Introduction**

The blood transfusion service aims to meet patients' needs for blood and blood products while minimizing the risk of transfusion-related complications. In particular, during red blood cell transfusions, it is crucial that the transfused RBCs have a high survival rate and do not cause significant harm to the recipient's own erythrocytes.

While ABO blood group system holds paramount importance in transfusion and organ transplantation, in addition to it, the common antigens within the Rhesus and Kell blood group systems are frequently screened in both blood donors and patients owing to their significant clinical relevance<sup>1</sup>.

Antibodies across all blood group systems can be lethal, leading to Allo-Immune Hemolytic disease of the newborn (HDFN), and Hemolytic transfusion reactions (HTRs). The likelihood of production of antibodies relies on the immunogenicity of the particular antigen. High-frequency antigens rarely induce antibodies, while low/intermediate-frequency antigens with high antigenicity often lead to Allo-antibody formation<sup>2</sup>. Therefore, practices such as blood grouping, cross-matching, antibody screening, identification, and red cell phenotyping are essential for ensuring safe transfusions.

Recognizing the financial burden associated with phenotyping all clinically significant antigens, focusing on just ABO, Rh and Kell phenotyping, especially in chronic transfusion recipients (like thalassemic patients, patients on dialysis, patients with cancers and multi transfused antenatal patients) can significantly influence in preventing alloimmunization and avoiding adverse events<sup>3</sup>.

Identifying and understanding the presence of various blood group antigens is of utmost significance for the formulation of robust policies within facilities dedicated to blood transfusion services. The presence of ABO, Rh, and Kell antigens exhibit notable variations across diverse racial and ethnic groups. Accurate knowledge of these variations not only ensures the efficient matching of blood products to recipients but also plays a pivotal role in enhancing the overall safety and efficacy of transfusion practices<sup>9</sup>.

Effectively managing cases that involve patients undergoing numerous transfusions, such as individuals with thalassemia, sickle cell anemia, cancer patients and individuals undergoing dialysis, is a complex task. These patients are susceptible to develop antibodies against blood group antigens and matching all the antigens before transfusion is often impractical. The antigens found within major blood group systems are crucial in influencing the effect of transfusions for recipients of blood and its components.

When antibodies of clinical significance are detected in a patient, it becomes imperative to provide that patient with a blood that lacks the corresponding antigen. This task is streamlined through the

utilization of donor information stored in blood banks, which allows for the selection of a compatible unit based on the identified blood groups<sup>10</sup>.

Previous studies in various geographic regions have highlighted the importance of understanding the ethnic distribution of RBC antigen phenotype frequencies. This knowledge is essential for creating a comprehensive donor database, developing indigenous cell panels, and ensuring the availability of antigen-negative blood compatible with patients who have multiple alloantibodies<sup>4,5,6</sup>.

Therefore, this study aims to assess the prevalence of ABO, Rh, and Kell antigens among both voluntary and replacement blood donors representing diverse ethnic groups within the Union Territory of Jammu & Kashmir.

## **Material and Methods:**

### **Study design**

This prospective observational cross-sectional study spanned one year, from November 2022 to October 2023 at a tertiary care hospital in northern india.

### **Study subjects**

Healthy voluntary and replacement blood donors, representing diverse ethnic groups, including Dogra's, Kashmiri Pandits(KPs), Gujjar Muslims (GMs), Christians, Non-Gujjar Muslims (NGMs) and Sikhs were screened and chosen based on the specified inclusion and exclusion criteria outlined in the Transfusion Medicine Technical Manual (DGHS) 3<sup>rd</sup> ed. 2023, National Standards for Blood Centres & Blood transfusion Services (DGHS) 2<sup>nd</sup> ed. 2022 and Standard Operating Procedures (SOP) of the department

Written informed consent was taken from each donor. Approval from the Institutional Ethics Committee was taken for this research work.

All guidelines as per declaration of Helsinki and good clinical practice guidelines were followed.

Samples were selected through a process of simple random sampling, ensuring an unbiased selection where every individual had an equal opportunity to be included. Convenience sampling method was used and out of all those who came to the blood centre to donate blood during one year of study duration, those who agreed to participate in the study were included in the study.

500 such healthy replacement and voluntary blood donors were included in the study. The donors who refused to be part of this study were excluded from the study.

Phlebotomy procedure for blood collection was executed under aseptic conditions in accordance with the guidelines outlined in Transfusion Medicine Technical Manual (DGHS) 3<sup>rd</sup> ed. 2023, National Standards for Blood Centres & Blood transfusion Services (DGHS) 2<sup>nd</sup> ed. 2022 and Standard Operating Procedures (SOP) of the department.

As a part of our routine practice, approximately 2ml of blood sample was obtained from each blood donor during the blood collection process in EDTA vacutainer. This sample was used for blood grouping and screening for infections. Furthermore, that same sample was later utilized for antigen typing within a time frame of 24 hours from its initial collection, ensuring timely assessments.

All blood samples were phenotyped for A,B,D antigens using Anti-A/Anti-B/Anti-D/Control/Reverse Diluent ORTHO BioVue® System column agglutination technology gel based cassettes and Rh (C, E, c, e) and Kell (K) antigens using Anti-C/Anti-E/Anti-c/Anti-e/Anti-K/Control ORTHO BioVue® System column agglutination technology glass bead based cassettes. The cassettes were centrifuged using Ortho™ Workstation<sup>7</sup>.

The data was compiled into Microsoft Excel and was then analyzed through IBM SPSS Statistics 27.0.1.0.

Frequencies (n) and percentages (%) were utilized for data presentation. The point prevalence was presented as a percentage along with a 95% confidence interval and the Chi-Square ( $\chi^2$ ) test was utilized to evaluate the significance of relationships.

**Results:**

This study involved phenotyping 500 samples collected from donors of diverse ethnic groups, including Dogras, Non-Gujjar Muslims (NGM), Gujjar Muslims (GM), Sikhs, Kashmiri Pandits (KPs), and Christians. [Table 1]

Table 1: Distribution of different ethnic groups within the study population.

Ethnic Group	No. Of Donors (n)	Prevalence (%)	95 % CI for Prevalence
Dogras	361	72.2%	(68.12 – 75.95)
Non-Gujjar Muslims	52	10.4%	(8.02 – 13.52)
Gujjar Muslims	43	8.6%	(6.45 – 11.38)
Sikhs	30	6%	(4.24 – 8.44)
Kashmiri Pandits	12	2.4%	(1.38 – 4.15)
Christians	2	0.4%	(0.11 – 1.45)
Total	500	100 %	

Nearly 35.4% (n= 177) donors were B group followed by O = 28.2% (n= 141), A = 25.6% (n= 128), and AB = 10.8% (n= 54) [Table 2].

Table 2: Distribution of ABO Groups in study population

Blood Group	No. Of Donors (n)	Prevalence (%)	95 % CI for Prevalence
A	128	25.6	(21.97 – 29.6)
B	177	35.4	(31.33 – 39.69)
O	141	28.2	(24.43 – 32.3)
AB	54	10.8	(8.37 – 13.83)

On extended phenotyping for Rh antigens, frequency of ‘e’ antigen was 97.6% (n=488) followed by ‘D’ antigen 94.6% (n=473), ‘C’ antigen 86.6% (n=433), ‘c’ antigen 62% (n=310) and ‘E’ antigen 19.4% (n=97). [Table 3]

**Table 3: Distribution of Rh antigens among different ethnic groups.**

Antigen Present	Dogras (N= 361)	Non- Gujar Muslims (N= 52)	Gujjar Muslims (N= 43)	Sikhs (N= 30)	Kashmiri Pandits (N= 12)	Christians (N= 2)	Total (500)
D	343 (95.01%)	48 (92.3%)	40 (93.02%)	28 (93.3%)	12 (100%)	2 (100%)	473 (94.6%)
C	319 (88.3%)	45 (86.5%)	34 (79.06%)	25 (83.3%)	8 (66.6%)	2 (100%)	433 (86.6%)
E	69 (19.1%)	9 (17.3%)	11 (25.5%)	5 (16.6%)	3 (25%)	0 (0%)	97 (19.4%)
c	219 (60.6%)	33 (63.4%)	33 (76.7%)	17 (56.6%)	7 (58.3%)	1 (50%)	310 (62%)
e	355 (98.3%)	51 (98.07%)	42 (97.6%)	27 (90%)	11 (91.6%)	2 (100%)	488 (97.6%)

Eight distinct phenotypes within the Rh blood group system were identified.

R1R1 had the highest incidence (35.4% n=177) followed by R1r (35.2% n=176), R1R2 (14% n=70), rr (4.8% n=24), R2r (4.2% n= 21), R2R2 (3% n=15), R0r (3% n=15) and least common phenotype among Rh antigens was dCe/dce (r'r) i.e., 0.4%(n=2) in this study.

It was observed that R1R1 phenotype was highest among Sikhs (43.33%), R1r among Christians (50%), R1R2 among Gujjar Muslims (20.93%), R2r among Kashmiri Pandits (16.66%), R2R2 among Christians (50%), R0r among Gujjar Muslims (4.65%), rr among Gujjar Muslims (11.62%) and r'r (0.55%) among Dogra population. [Table 4]

**Table 4: Distribution of Rh phenotypes among different ethnic groups.**

Ethnic groups	R1R1 n (%)	R1r n (%)	R1R2 n (%)	R2r n (%)	R2R2 n (%)	R0r n (%)	rr n (%)	r'r n (%)	Total N
Dogras	135 (37.3)	127 (35.1)	52 (14.4)	14 (3.87)	7 (1.93)	10 (2.77)	14 (3.87)	2 (0.55)	361
NGMs	18 (34.6)	17 (32.6)	7 (13.4)	2 (3.84)	1 (1.92)	2 (3.84)	5 (9.61)	0	52
GMs	8 (18.6)	15 (34.88)	9 (20.93)	2 (4.65)	2 (4.65)	2 (4.65)	5 (11.62)	0	43
Sikhs	13 (43.33)	11 (36.66)	2 (6.66)	1 (3.33)	2 (6.66)	1 (3.33)	0	0	30
KPs	3 (25)	5 (41.66)	0	2 (16.66)	2 (16.66)	0	0	0	12
Christians	0	1 (50)	0	0	1 (50)	0	0	0	2
Total n (%)	177 (35.4)	176 (35.2)	70 (14)	21 (4.2)	15 (3)	15 (3)	24 (4.8)	2 (0.4)	500

**NGM – Non Gujjar Muslim**

**GM – Gujjar Muslim**

**KP- Kashmiri Pandit**

The overall frequency of the Kell (K) antigen was 2.6% as indicated in Table 5. Notably, the highest percentage of Kell (K) positive antigen was observed among Gujjar Muslims (4.65%). [Table 5] The chi square test demonstrated no association of K antigen with the ethnic groups.

Ethnic Group	Kell (K) antigen			Statistical significance	
	K Positive n (R%)	K Negative n (R%)	Total n(R%)	$\chi^2$ -value	p-value
Dogras	8 (2.21)	353 (97.79)	361(100)	1.681	0.891
NGM	2 (3.84)	50 (96.16)	52(100)		
GM	2 (4.65)	41 (95.35)	43(100)		
Sikhs	1 (3.33)	29 (96.67)	30(100)		
KPS	0(0)	12 (100)	12(100)		
Christians	0(0)	2 (100)	2(100)		
Total	13 (2.6)	487 (97.4)	500(100)		

The prevalence of Kell (K) antigen was notably higher among 'A' blood group donors (3.9%), followed by the 'AB' group (3.7%), 'O' group (2.83%), and 'B' group (1.12%). [Table 6] The chi square test demonstrated no association of K antigen with the ABO groups.

Blood group	Kell (K) antigen			Statistical significance	
	K Positive n (R%)	K Negative n (R%)	Total n(C%)	$\chi^2$ -value	p-value
A	5 (3.9)	123 (96.1)	128 (25.6)	2.664	0.446
B	2 (1.12)	175 (98.88)	177(35.4)		
O	4 (2.83)	137 (97.17)	141(28.2)		
AB	2 (3.7)	52 (96.3)	54(10.8)		
Total	13 (2.6)	487 (97.4)	500(100)		

## Discussion:

In this study, beyond the conventional ABO blood grouping, phenotypic analysis of major antigens within the Rh (D, C, E, c, e) and Kell (K) systems was done. Our main goal was to ascertain the frequency of clinically relevant antigenic phenotypes among donors representing diverse ethnic groups in this region. All the findings obtained in this study were juxtaposed with data gathered from published articles, encompassing both domestic and international sources.

The prevalence of ABO blood groups exhibits notable variations across different races, making it a key consideration in clinical settings. This blood group system holds particular clinical significance due to the frequent development of antibodies within it. The capacity of these antibodies to initiate HTR (Hemolytic Transfusion Reaction) and HDFN (Hemolytic Disease of the Newborn) highlights the necessity of comprehending and handling ABO blood group compatibility<sup>11</sup>.

In addition to ABO, the Rh and Kell blood group system emerges as highly polymorphic. They pose challenges and necessitate meticulous attention to their antigenic compatibility in transfusion practices. The emphasis on ABO, Rh and KELL systems underscores their paramount importance in ensuring safe and effective transfusions.

The prevalence of the D positive phenotype in our study was found to be 94.6%, while the D negative phenotype accounted for 5.4%. This aligns with the findings of several other investigations conducted in the Jammu region. Kotwal U et al's study on healthy blood donors in Jammu reported

Rh (D) antigen positivity of 94.52% and negativity of 5.48%<sup>12</sup>. Similarly, Yasmeen I et al observed Rh (D) antigen positivity of 94.4% and negativity of 5.6% in the same region<sup>13</sup>.

A study conducted by Garg N et al yielded similar results, indicating a prevalence of D positive antigen as 93.8% and 6.2% for the D negative antigen<sup>14</sup>. Similarly, a study by Agarwal N et al., India, exhibited a Rh (D) antigen positivity rate of 94.36% and negativity rate of 5.64%<sup>4</sup>. Nanu A et al. reported a prevalence of 95.37% for D positive and 4.63% for D negative in their research<sup>15</sup>. In a separate study, Prinja N et al. investigated donors from north-western India and identified a prevalence of D positive antigen as 93.8% and D negative as 6.2%<sup>9</sup>.

In our study, prevalence of rest of Rh antigens was observed as follows: 'C' antigen was positive in 433 donors (86.6%), 'E' positive in 97 donors (19.4%), 'c' positive in 310 donors (62%), and 'e' was positive in 488 donors (97.6%). [Table 7]

Antigens	Present Study	Yasmeen I et al <sup>13</sup>	Prinja N et al <sup>9</sup>	Thakral B et al <sup>16</sup>
<b>D</b>	94.6%	94.4%	93.8%	93.39%
<b>C</b>	86.6%	85.2%	85.4%	84.76%
<b>E</b>	19.4%	20.6%	17.5%	17.9%
<b>c</b>	62%	64.0%	60.1%	52.82%
<b>e</b>	97.6%	97.2%	99.3%	98.3%

**Table 7: Comparison of distribution of Rh antigens (D, C, E, c, e) among blood donors.**

Significantly, the occurrence rates of 'C' and 'e' antigens in our investigation aligned with the discoveries of Yasmeen I et al's study based in the Jammu region, where the 'e' antigen was seen in 97.2%, and the 'C' antigen was seen in 85.2% donors<sup>13</sup>. These findings correlate with another study conducted by Thakral B et al. which reported 'e' antigen frequency at 98.3% and 'C' antigen at 84.76%<sup>16</sup>.

The findings of our current study also aligned with those of a study by Kahar MA et al., where the 'e' antigen was reported at 100%, and the 'C' antigen exhibited a frequency of 81.74%<sup>17</sup>.

Also, a study by Gajjar M et al., revealed a significant prevalence of the 'e' antigen at 98.65%, followed by 'D' (94.76%), 'C' (88.82%), 'c' (58.47%), and 'E' (17.18%)<sup>18</sup>.

The distribution of various Rh phenotypes in our study is consistent with findings from diverse regions within India and abroad. Notably, the R1R1 phenotype emerged as the most prevalent (35.4%), followed closely by R1r (35.2%). This finding aligns with other studies such as Garg N et al., where R1R1 was the most prevalent (44.60%), followed by R1r (32.60%).<sup>(14)</sup> Similarly, Yasmeen I et al reported a prevalence of 36.0% for R1R1 and 34.6% for R1r<sup>13</sup>.

Kahar MA et al noted the highest prevalence of R1R1 (40.87%), second highest being R1r (23.48%), whereas Agarwal N et al reported frequencies of 42.93% for R1R1 and 35.60% for R1r. Thakral B et al identified maximum occurrence of R1R1 (43.8%) seconded by R1r (30%)<sup>4,16,17</sup>.

The r'r phenotype exhibited the lowest frequency (0.4%), consistent with the findings of studies conducted by Yasmeen I et al. (0.6%) and Thakral B et al. (0.56%)<sup>13,16</sup>.

In our study, 2.6% of blood donors showed presence of Kell (K) antigen within the Kell blood group system, while it was absent in 97.4% which is similar to the findings by Yasmeen I et al, which showed K positive in 2.6% and K negative in 97.4%<sup>13</sup>.

These findings also align with Agarwal N et al's study, which reported 1.97% donors were positive for K antigen and its absence was noted in 98.03% donors<sup>4</sup>.

Significantly, our findings revealed a lower prevalence than the study by Kahar MA et al., where the K antigen was present in 6.09% and was absent in 93.91%<sup>17</sup>. [Table 8]

**Table 8: Comparison of Kell (K) antigen among different studies.**

Antigen	Present study (%)	Prinja N et al <sup>9</sup> (%)	Yasmeen I et al <sup>13</sup> (%)	Kahar MA et al <sup>17</sup> (%)	Agarwal N et al <sup>4</sup> (%)	Thakral B et al <sup>16</sup> (%)	Nanu A et al <sup>15</sup> (%)
K+	2.6	2.7	2.6	6.09	1.97	5.68	4.04
K-	97.4	97.3	97.4	93.9	98.03	94.32	95.96

The noted distribution of blood groups in this study was as follows: ABO (A=25.6%, B=35.4%, O=28.2%, AB=10.8%), Rhesus (positive =94.6%, negative=5.4%) and Kell (K=2.6%). The B blood group emerged as the most prevalent, aligning with findings reported in previous studies conducted in this region and North India<sup>4,9,12,13</sup>.

Comprehending the prevalence of antigens within a population holds significant clinical relevance. This understanding enables the anticipation of prevalent alloantibodies that may arise in patients undergoing multiple transfusions. Furthermore, it aids in selecting blood units lacking specific antigens for patients who have produced alloantibodies.

Adopting this proactive strategy could alleviate reported occurrences of RBC antigen allo-immunization and reduce the risk of associated complications, including hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions (HTRs)<sup>19</sup>.

In Jammu, the demographic landscape reveals a predominantly Hindu population, constituting 66% of the total with Muslims comprising 30%. Sikhs account for 1.9%, Christians for 0.28%, and other minority groups for 0.20%. Notably, a significant portion of the Hindu populace identifies as Dogras, encompassing 47% of the Hindu population(as cited in February 2024)<sup>20</sup>.

**Conclusions:** Red cell antigen frequencies across diverse ethnic populations helps in establishing a robust donor database and development of in-house cell panels and fulfils the need of antigen negative compatible blood. Phenotyping aids in diminishing the likelihood of RBC antigen allo-immunization and the related complications. This can further contribute to the development of a rare blood group registry at both regional and national levels.

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