Section: Pathology

Original Research Article

STUDY OF MINOR BLOOD GROUP ANTIGENS (RHESUS AND KELL ANTIGENS) IN VOLUNTARY BLOOD DONORS IN KOLHAPUR REGION

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ABSTRACT

Background: The notion of confirming the safety of blood began with the identification of the ABO blood group (BG) in 1901 followed by the discovery of the Rh blood group around 1939 and 1940. The Rh blood group system (BGs)" encompasses a total of 50 antigens (Ag) with the five most significant being "D, C, c, E, and e. Among these, D is the most immunogenic and the presence of its corresponding antibody (Ab) (anti-D) can result in haemolytic disease of the foetus and new-born (HDFN). **Materials and Methods**: The above study was carried out at a tertiary care hospital for a period of 18 months. A total of 250 donors satisfying the inclusion and exclusion criteria were involved in the study. Blood was collected from voluntary donors and later phenotyped for ABO and Rh (C, c, E, e,) and Kell Ags using Matrix Rh phenotype Card with Anti- K. The data was collected and compiled on an excel sheet and used to analyse statistically by using "SPSS version 26.0 software. **Result:** 97.6% of the donors were RhD-positive, while 2.4% were Rh D-negative. The C Ag was positive in 92% of the donors and negative in 8%. The c Ag showed a nearly equal distribution with 49.2% positive and 50.8% negative. The E Ag was present in 20.4% of the donors and absent in 79.6%. The e Ag was highly prevalent, being positive in 98.8% and negative in only 1.2%. The Kell Ag was found to be positive in just 1.2% of donors and negative in 98.8%. **Conclusion:** Most frequently observed minor BG Ag in Rhesus BGS is 'e' Ag followed by 'D' then 'C', 'c', and 'E' Ags. Conversely, the Kell Ag was observed to be the least common among voluntary blood donors. The study helped to prepare a partial database of "minor BG Ags (Rhesus & Kell Ag) of voluntary blood donors for future safe Blood Transfusion services in Hospital.

INTRODUCTION

The notion of confirming the safety of blood began with the identification of the "ABO blood group (BG) in 1901 followed by the discovery of the Rh blood group between 1939 and 1940.[1]" The "Rh blood group system (BGs)" encompasses a total of 50 antigens (Ag) with the five most significant ones being D, C, c, E, and e. Among these, D is the most immunogenic and the presence of its corresponding antibody (Ab) (anti-D) can result in haemolytic disease of the foetus and new-born (HDFN)." Following the discovery of the antiglobulin test, the Kell BGs was recognized in 1946 and is considered the third most immunogenic BGs.[2] The Kell BGs comprises a total of 25 Ag with in the existence of anti-K being associated with HDFN. It is significant to note that antibodies from all BGs have the potential to be harmful and can lead to "alloimmunization, HDFN, and haemolytic transfusion reactions (HTR)". When red blood cells (RBCs) containing a specific Ag are transfused into a recipient who is deficient in that particular Ag, there is a likelihood of the recipient developing antibodies against it. The probability of Ab production relies on the immunogenicity of the Ag."

The prevalence and antigenicity of different Ag play a vital role in defining the frequency of confirming Ab. Abs in contradiction of high-frequency Ag are comparatively rare whereas alloantibodies contrary to low or intermediate-frequency Ag with greater antigenicity are more common. As a result, "blood grouping, cross-matching, Ab screening, Ab identification, and red cell phenotyping are essential for ensuring safe transfusions." However, these events contribute to the overall cost of blood banking. Considering the economic burden associated with phenotyping all clinically substantial Ag, focusing on

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phenotyping “Rh and Kell Ag” can significantly contribute to preventing “alloimmunization” and decreasing complications in succeeding blood transfusion blood particularly in instances involving multiple transfusions.[3,4]

Patients who have established “alloantibodies and need a blood transfusion must receive extensively phenotyped matched RBCs can reduce the frequency of blood transfusions which further decreases risk of alloimmunisation and post blood transfusion reactions. It also reduces iron overload and iron chelation due to reduced number of blood transfusions as a result of better survival of transfused RBCs.

The donor database aids in quickly providing safe blood to these patients, reducing the workload of blood centre personnel and understanding the red cell antigen distribution patterns in the local population which helps estimate genotype frequencies.[5] Normally RBCs are not routinely tested for other minor BG antigens unless the recipient has previously been immunized.” Multitransfused patients (multipara, thalassemia patients, sickle cell anaemia patients, cancer patients, and dialysis patients) develop alloantibodies. “The presence of RBC alloantibodies can lead to serological incompatibility complicating the selection of suitable blood bag units for future transfusions, delaying potentially lifesaving treatments and increasing the risk of HTR and delayed HTR. Using phenotyped matched blood bag units for transfusion benefits patients with alloantibodies and helps prevent further alloimmunization.”

This study assists in creating a partial database of minor antigens (Rhesus and Kell antigens) from voluntary blood donors for future safe blood transfusion.

MATERIALS AND METHODS

The prospective observational study was carried out after institutional ethical clearance was obtained. The healthy voluntary blood donors from outdoor blood donation camps and in-house camps at Dr. D.Y. Patil Medical College Hospital and Research Institute, Kolhapur were selected during the study period over 18 months. A total of 250 donors satisfying the below inclusion and exclusion criteria were involved in the study.

Inclusion Criteria

Donors with “O” blood group only, age group 18 yrs. -50 years, who are fit to donate blood as per the DGHS Manual of transfusion medicine 3rd edition.

Exclusion Criteria

Donors other than “O” blood groups and unfit donors. After informed consent of voluntary blood donors, blood donations were taken in triple blood bags. It was done from the anti-cubital vein following all aseptic actions. All blood samples were “phenotyped for ABO and Rh (C, c, E, e,) and Kell Ags” using Matrix Rh Phenotype Card with Anti- K. It was Gel column agglutination technology. It consists of a plastic card (gel card) with six inbuilt microtubes. The microtube contained Monoclonal anti-c, anti-c, anti-E, anti-e, anti-K, and neutral gel in appropriate microtubes (following FDA guidelines).” 5% Red cell suspension was prepared by adding 25 microlitre of packed RBCs from donor blood sample with 500 microlitre of LISS (Low Ionic Strength Solution). 10 microlitres of 5% donor Red cell suspension was added to each micro tubes, gel card was incubated in a gel card incubator at 37 degrees Celsius for 15 min. Then centrifuged gel card was at 1000 rpm for 10 min. in a specially devised card centrifuge and read the result with Rh view box. The D Ag was also tested by gel card method.

In negative reactions, the antibodies did not bind to the red cells allowing the red cells to pass freely through the gel and settle at the bottom of the micro tube indicated the absence of the corresponding antigen.

In positive reactions, the agglutinates were trapped in the gel to varying extents, depending on the degree of agglutination.

The data was collected and compiled on an excel sheet and used to analyse statistically by using “SPSS version 26.0 software.” Categorical variables were expressed in terms of percentage.

RESULTS

In a study of 250 blood donors, the majority (48.4%) were aged 18–30 years, while 12.8% were aged 41–50 years. Most participants were male (97.2%, n=243). Rh D antigen was present in 97.6% (n=244) of individuals.

Antigen distribution by gender showed a consistent predominance of the e antigen in both males (98.77%) and females (100%), while the K antigen was detected only in males (1.23%). Age-wise analysis revealed the highest e antigen prevalence across all groups, with 99.17% in 18–30 years, 97.94% in 31–40 years, and 100% in 41–50 years. The C antigen also showed high prevalence across age groups (91.73%–93.75%). The c antigen varied from 43.3% to 53.72%, and the E antigen ranged from 17.36% to 24.74%. The K antigen was absent in both the youngest and oldest age groups and was detected only in 3.09% of individuals aged 31–40 years.

The ‘e ’Ag was the most predominant, with 247 out of 250 individuals (98.8%) testing positive. The Rh D antigen was the second most common with a frequency of 244 representing 97.6% of sample population. The C antigen was the third most common, with a frequency of 230, representing 92% of the sample population. The c Ag was present in 123 individuals, accounting for 49.2% of the sample. A smaller proportion, 51individuals (20.4%), tested positive for the E Ag. The K Ag was the least common, found in only 3 individuals, corresponding to 1.2% of the sample population.

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Table 1: Distribution of minor blood group antigens

Minor blood group antigens	Positive (n=250)	
	Frequency (n)	Percentage (%)
e	247	98.8
D	244	97.6
C	230	92
c	123	49.2
E	51	20.4
K	3	1.2

DISCUSSION

In the above study, the gender distribution of the study participants was significantly skewed, with 97.2% (n=243) being male and only 2.8% (n=7) female. Similar findings were also reported by Singh A. et al.[6] This disparity could be attributed to cultural, social, or logistical factors influencing the willingness or ability of women to donate blood in the region.[7] Further research could investigate these barriers to improve female participation in blood donation drives.

The Rhesus Ag system, particularly the Rh D Ag, is one of the most critical in transfusion medicine due to its high immunogenicity. In this study, a substantial majority (97.6%, n=244) of donors were Rh D positive, whereas, 2.4% (n=6) of donors were Rh D negative. A systematic review by Patidar GK and Dhiman Y of the Indian population found that 94.13% of donors were RhD-positive. When assessed by geographical regions, the positivity of Rh D was 94.88% in the western regions of India, including Maharashtra, Karnataka, and Gujarat. In the northern, eastern, central, and southern regions, the incidence of Rh D positive was 92.88%, 97.42%, 94.27%, and 93.68%, respectively.[8] Moreover, various other studies from the north, south, and east regions of India showed Rh D positive in 94.1%, 85.6%, 95.77%, and 99.05% of donors respectively.[9-12] The difference in Rh D positive rate in the Indian subcontinent suggests possible genetic or demographic variations in the regions. In the above study, C Ag was found positive in 92% (n=230) of donors, the high prevalence of the C Ag suggests its dominant presence in the studied population, which is higher compared to other populations. For instance, in the study of Kulkarni S. et al., 84.8% of donors had positive C Ag.[13] In another study conducted by Ranjan S et al. C Ag was found positive in 90.47% of donors.[11] Similarly, C Ag positivity was observed to be 87% in study conducted by Makroo R et al,[14] 88 % in study conducted by Gundrajukuppam DK et al,[9] and 84.7% by Thakral B et al,[15] of donors.

Comparison of distribution of Rh c positive antigen between different studies, c Ag was positive in 49.2% (n=123) of donors, the c Ag shows an almost equal distribution with a slight inclination towards negativity (50.8%, n=127). According to the "American Association of Blood Banks", 47% of donors in the Asian population were c Ag positive.[17] In the Indian subcontinent, the incidence of c Ag positivity among donors ranged from 52.82% to 58%,[14-15] in the northern region, and from 50.5% to 55.89% in the western region.[18,16] In the southern and eastern regions, the incidence was reported to be 54.9% and 47.9%, respectively.[9,16] These findings highlight the genetic diversity within Indian populations. In contrast, 80% of white donors and 96% of black donors are c Ag-positive.[17] Comparison of distribution of Rh E positive antigen between different studies, The E Ag was positive in 20.4% (n=51) of donors. This prevalence is within the typical range found in other populations, where E Ag frequency varies widely but is often less common than C or c Ags.[14,9,15-16] The lower prevalence underscores the importance of screening for E Ag to prevent alloimmunization in RhE-negative patients. Similarly, a remarkably high prevalence of 98.8% (n=247) was observed for the e Ag, indicating it is almost ubiquitous in the donor population. This finding is consistent with global patterns, as the e Ag is usually the most predominant of the Rhesus Ags.

Among non-"D" Ags, "K" is the most immunogenic, followed by c and E.[20] These Ags are also major culprits of alloimmunization in multi-transfused patients.[21] "In the present study, the K Ag was positive in 1.2% of donors. Similarly, Pahuja S et al. and Gajjar et al. have reported the prevalence of the "K" Ag to be 1.81% and 1.78% respectively. However, these rates are lower than those reported from North India, in which it has been reported as 5.56% and 3.5%. [14-15]"

The data from this study underscore the importance of detailed Ag typing in blood banks, especially for regions with diverse genetic backgrounds. The high prevalence of Rh D and e Ags suggests that mismatches in these Ags are less likely. However, the relatively lower frequencies of c and E Ags, and the presence of K Ag, highlight the need for vigilant screening practices to prevent alloimmunization.

CONCLUSION

The findings reveal most frequently observed minor BG Ag in Rhesus BGS is 'e' Ag followed by 'D' then 'C', 'c', and 'E' Ags. Conversely, the Kell Ag was observed to be the least common among voluntary blood donors. The frequency of minor BG Ags was seen in the order "e > D > C > c > E > K". Understanding these patterns is crucial for improving blood transfusion services in hospitals and enhancing the management of blood bank resources. So, for patients who have developed alloantibodies and multi-transfused patients (multipara, thalassemia

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patients, sickle cell anemia patients, cancer patients, dialysis patients), if the blood donor data bank is available beforehand concerning minor BG Ag status of voluntary blood donors then, it will be easy for rapid establishment of safe blood to the subject, decrease the load of blood center personnel and also this will aid in knowing the red cell Ag distribution form of the local population & assuming genotype incidences Thus, this study helps us to prepare a partial database of "minor BG Ags (Rhesus & Kell Ag) of voluntary blood donors for future safe Blood Transfusion services in Hospital.

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