Prevalence of ABO, RhD and other clinically significant Blood Group Antigens among blood donors at tertiary care center, Gwalior

Shelendra Sharma, Dharmesh Chandra Sharma, Sunita Rai, Anita Arya, Reena Jain, Dilpreet Kaur, Bharat Jain

ABSTRACT

Background: Among all blood group systems and antigens, ABO and D antigens of the Rh blood group system are of primary importance, hence included in routine blood grouping and transfusion. Other blood group systems and antigens that are important in multiparous women, multi-transfused patients and hemolytic disease of newborn are Kell, Rh, Duffy, and Kidd.

Aims and Objectives: To know the pattern of ABO, RhD and other clinically significant major antigens of Rh, Kell, Duffy and Kidd blood group systems as well as evaluation of phenotypes, genotypes and gene frequency of related antigens among blood donors in Gwalior region.

Materials and methods: A prospective study was carried out at the Blood Bank from July 2017 to June 2019. During the study period 48500 blood donors were included for ABO and RhD grouping. Out of them 1000 randomly selected donor samples were processed for complete Rh, Kell, Duffy and Kidd blood grouping.

Result: ABO group pattern was; A - 22.56%, B - 36.52%, AB - 9.8% and O - 31.12%, while RhD status was RhD+ 90.99% and RhD- 9.01%. Prevalence of Rh phenotypes was Dccee 43%, Dccee 33%, Dccee 10%, Dccee 6.5%, Dccee 4.5%, Dccee 1%, Dccee 0.3% and dccee 0.2%. Kell phenotype was K+k+ 95.5%, K+k+ 4.5%, while K+k- and K-k- phenotypes were not encountered in this study. Duffy phenotypes were Fy+a+ Fy+b+ 47.5%, Fy+a+ Fy+b- 35%, Fy-a+ Fy-b+ 17% and Fy-a- Fy-b- 0.5%. Kidd phenotype was Jk+a+ Jk+b- 44.5 %, Jk+a- Jk+b- 26.5%, Jk+a+ Jk+b+ 18% and Jk+a- Jk+b+ 11%.

Conclusion: Present study is helpful in the transfusion management of multiparous women and multi-transfused patients. Extended blood grouping is the need of future to ensure safe blood transfusion practices.

Key words: Blood Groups, Rare blood group, Multiparous women, Multi-transfused Patients.


INTRODUCTION

Till date, 36 blood group systems and more than 420 blood group antigens have been discovered.6 As far as blood transfusion is concerned; the ABO blood group system has prime importance because of the presence of reciprocal antibody which is of IgM nature in the serum of patients. Apart from ABO grouping, antigen D of Rh blood group system has also been included in routine blood grouping and transfusion services because of its highly immunogenic nature. Other blood group systems and antigens that are important in cases with prior multiple blood transfusion and hemolytic disease of the newborn are Kell (K), Other Rh blood group antigens (C, c, E, e), Duffy (Fy+a, Fy-b) and Kidd (Jk+a, Jk+b).

International Society of Blood Transfusion (ISBT) recognized 9 blood group systems known as ABO, Rhesus, Kell, Duffy, Kidd, MNS, P, Lewis, and Lutheran, that are clinically significant and related to hemolytic transfusion reactions (HTR) and hemolytic disease of the fetus and newborn (HDFN).2,5 Out of these, antigens of the ABO, Rh and Kell blood group systems are highly immunogenic and active in body temperature, therefore, these three blood group systems are important in clinical transfusion practices.6

India is a developing country with limited resources and heavy patient load, thus making it impossible to do extensive blood grouping for every donation, so we rely upon ABO and RhD blood grouping in routine. In specific groups of population like females of childbearing age, newborn, pregnant women and patients requiring multiple blood transfusions (hemolytic diseases), it is mandatory to determine the phenotype of clinically significant blood group antigens on the donor red blood cell (RBC) since alloimmunization is particularly undesirable in such cases. To prevent alloimmunization we have to select a particular blood and blood products lacking corresponding antigens, in case of emergency.7
ABO was the first human blood group system discovered and it plays a significant role in transfusion medicine, hematopoietic stem cell transplantation, and solid organ transplantation. ABO blood groups are not fully developed at birth until the age of 2-4 years, and after that it remains unchanged throughout the life.\textsuperscript{3} The ABO blood group system is classified on the basis of the presence of one, both, or neither of the A and B antigens on the surface of red blood cells. Anti-A and Anti-B antibodies or agglutinins are usually of IgM type and naturally occur in the human body, these are produced in the first year of life by sensitization to environmental factors such as food, bacteria and viruses.\textsuperscript{7}

Unlike ABO blood groups, the formation of anti D antibodies is not natural; it is due to immunization by fetal RhD positive RBCs in Rh negative mothers or transfusion of RhD positive RBCs in RhD negative person. These antibodies persist for many years. Antibodies against the D antigen are responsible for clinically significant HDN and transfusion reaction.\textsuperscript{10} ABO and RhD blood group systems carry the hereditary character which is useful in blood transfusion practice, in genetic study of specific population and medico-legal cases.\textsuperscript{14} Blood group systems are genetically determined and most of them are inherited in simple Mendelian fashion and have stable characteristics, which also have importance in paternity testing.\textsuperscript{12}

Antibodies against antigens related to Kell, Duffy and Kidd blood group systems are responsible for HTR and HDFN, and are regarded as clinically significant if they react with indirect Antiglobulin at 37\textdegree{}C temperature. In antenatal patients and patients who require repeated blood transfusion, testing of these antigens is of clinical significance and should be done.

Ethnic differences of various blood group antigens are common and it shows various striking and interesting findings. There are very few studies that were conducted on minor blood system in our country.\textsuperscript{13}

Blood group systems and antigens were included in the present study, are summarized in table 1.

**MATERIALS AND METHODS**

The present prospective study was done at Blood Bank in the Department of Pathology at Gajra Raja Medical College and J.A. group of Hospitals, Gwalior from July 2017 to June 2019. During the study period, 48500 blood donors/ units were included for ABO and RhD grouping. Out of 48500 blood donors (voluntary/replacement, male/ female), randomly selected 1000 donors were processed for extended blood grouping; complete Rh, Kell, Duffy and Kidd blood grouping.

The inclusion criterion for selection of blood donors was: donor fitness, according to the donor’s questionnaire, their physical examination, hemoglobin (Hb %) above 12.5 GMs/dl and aged between 18-60 years.

The exclusion criterion for selection of blood donors was: unfit donor, according to the donor’s questionnaire, their physical examination, hemoglobin (Hb %) less than 12.5 GMs/dl and age <18 year and >60 year.

Aseptically 5 ml blood was collected from the donor/ blood unit and 3ml was transferred into plain disposable vials and 2ml in EDTA/ CPD anti-coagulant. After centrifugation of the plain vial sample, serum was obtained and labeled appropriately and immediately stored at 20-80\textdegree{}C if tests were not performed simultaneously. It was then used for ABO reverse grouping and agglutinin reaction study to know the irregular antibody. With whole blood sample in EDTA/ CPD, three washings with normal saline was done and button was used to prepare 5% saline suspension of RBCs by adding 95% of saline to the 5% of RBCs. This cell suspension was used for forward blood grouping in the study.

ABO and RhD grouping was done by ABD Gel Card Make Tulip. Test for the rest major Rh antigens i.e. C, c, E, e was done by the Complete Rh Gel Card. For Kell, Duffy and Kidd blood group antigens AHG (Coombs) test card and the neutral gel card was used. For antigens K, J\textsuperscript{k}a and J\textsuperscript{k}b neutral gel card was used in the study because Antisera anti-K, anti-J\textsuperscript{k}, and anti-J\textsuperscript{k} used was saline agglutinating while for antigen k, Fy\textsuperscript{a} and Fy\textsuperscript{b} coombs Gel card was used because anti-k, anti-Fy\textsuperscript{a}, anti-Fy\textsuperscript{b} used in the study was AHG (Anti Human Globulin) agglutinating.

Rare Antiser were used in the study were from Immucor India Pvt. Ltd. Figure 2(A). All agglutination tests were done as per standard operating procedures for the test and in accordance with the manufacturer’s instructions. Grading of agglutination is shown in Figure 2(B).

In the present study gene frequency was calculated by the Hardy-Weinberg equation. The data has been collected, tabulated. Summarized and compared statistically by distribution and percentage proportion. Chi square (X2) test was applied to understand the many (p value) ratio of difference statistically.

**RESULTS**

During the study period 48500 blood donors were included for ABO and RhD study. The ABO group pattern was; A- 22.56% (n= 10942), B - 36.52% (n= 17721), AB - 9.8% (n= 4753) and O - 31.12 % (n=...
15093), (Figure 3) statistically significant ($p: 0.000991$) while RhD status was; RhD positive cases were 90.99% ($n = 44130$) and RhD negative were 9.01% ($n = 4370$), statistically significant ($p: 0.000001$).

Total number of donors from July 2018 to June 2019 was 25840. Out of 25840 donors, male versus Female Donor was 95.76%: 4.24%, where 87.92% were male and 4.0% female voluntary donors. The percentage of male and female replacement donors were 7.84% % and 0.24%, respectively, statistically significant ($p: 0.000001$).

In the study most common age group among the donors was 21-30 year (32%), followed by 31-40 years (26%), 41-50 years (20%), 51-60 years (12%) and least common age group was 18-20 years (10%) which is shown in table 2, statistically significant ($p: 0.001768$).

In the present study gene Frequency of $A$, $B$ and $O$ gene was calculated by the Hardy-Weinberg equation. Gene frequency of $A= .1756$, $B= .2666$ and $O= .5578$. In this study prevalence of RhD positive blood donors were 90.99% and prevalence of RhD negative blood donors was 9.01%. Gene frequency of D and d gene was .6999 and .3001 respectively.

Out of 48500 blood donors (voluntary/ replacement, male/female), randomly selected 1000 donors were processed for extended blood grouping; complete Rh, Kell, Duffy and Kidd.

In the present study, most common Rh phenotypes phenotype was DCCee - 43% ($n = 430$) followed by DCcee - 33% ($330$), DCCeE - 10% ($n = 100$), dccee - 6.5% ($n=65$), DccEe - 4.5% ($n = 45$), Dccee - 1% ($n = 10$), DCCeE - 0.3% ($n = 03$) while least common was dccee - 0.2% ($n = 02$).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Blood group system and antigens studied</th>
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<tbody>
<tr>
<td>ISBT No.</td>
<td>System Name</td>
</tr>
<tr>
<td>001</td>
<td>ABO</td>
</tr>
<tr>
<td>004</td>
<td>Rh</td>
</tr>
<tr>
<td>006</td>
<td>Kell</td>
</tr>
<tr>
<td>008</td>
<td>Duffy</td>
</tr>
<tr>
<td>009</td>
<td>Kid</td>
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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distribution of blood donors in respect of age</th>
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<tr>
<td>Age group</td>
<td>No. of blood donors</td>
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<tr>
<td>18-20</td>
<td>4850</td>
</tr>
<tr>
<td>21-30</td>
<td>15520</td>
</tr>
<tr>
<td>31-40</td>
<td>12610</td>
</tr>
<tr>
<td>41-50</td>
<td>9700</td>
</tr>
<tr>
<td>51-60</td>
<td>5820</td>
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<thead>
<tr>
<th>Table 3</th>
<th>The prevalence of Rh phenotypes and Genotypes</th>
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<tr>
<td>Rh phenotype</td>
<td>Number</td>
</tr>
<tr>
<td>DCCee</td>
<td>430</td>
</tr>
<tr>
<td>DCcee</td>
<td>330</td>
</tr>
<tr>
<td>DccEe</td>
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<tr>
<td>DccEe</td>
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<td>Dccee</td>
<td>15</td>
</tr>
<tr>
<td>dCcee</td>
<td>10</td>
</tr>
<tr>
<td>DCCEe</td>
<td>03</td>
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<tr>
<td>dccEe</td>
<td>02</td>
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</tbody>
</table>
Phenotypes, possible genotypes and most provable genotypes in the study is summarized in the Table 3. Gene frequency of C and c gene is 0.658 and 0.342 respectively, while the gene frequency of e and E gene was 0.921 and 0.079 respectively. In the present study, most common Kell phenotype was K- k+ 95.5% (n = 955) followed by K+ k+ phenotype 4.5% (n = 45) while K+ k- and K- k- phenotypes were not encountered in the study. Gene frequency of k and K gene was .9772 and .0228 respectively. (Figure 4(B))

In the present study, most common Duffy phenotype encountered was Fya+ Fyb+ - 47.5% (n = 475) followed by phenotype Fya+ Fyb- - 35% (n = 350), Fya- Fyb+ - 17% (n = 170) and least common phenotype was Fya- Fyb- - 0.5% (n = 05). The possible genotype of Duffy antigens are Fya/ Fya, Fyb/ Fyb and Fya/ Fyb. Gene frequency of fy a and fy b gene was: 0.5865 and 0.4135 respectively (Figure 4(D)).

In present most common Kidd phenotype encountered was Jka+ Jkb- - 44.5 % (n = 445) followed by phenotype Jka- Jkb+ (Jk null) - 26.5% (n = 265), Jka+ Jkb+ - 18% (n = 180) and least common phenotypes was Jka- Jkb+ - 11% (n = 110). The possible genotype of Kidd antigens is Jka/Jka, Jkb/ Jkb and Jka/Jkb. Gene frequency of Jka and Jkb gene was: 0.6281 and 0.3719 respectively. (Figure 4(C)).

DISCUSSION

Extended blood grouping, i.e. apart from ABO and RhD which include complete Rh, Kell, Duffy and Kidd antigens are equally important in the cases of multiparous women, in massive transfusions and transfusion dependent patients like thalassemia, aplastic anemia, immune thrombocytopenia, sickle cell anemia etc.

In our study prevalence of ABO group was; A- 22.56% (n= 10942), B - 36.52% (n= 17721), O - 31.12 % (n= 15093) and AB - 9.8% (n= 4753) i.e. B> O> A> AB similarly reported by Chandra T et al, Kaur H et al and Wadhwa et al while from north India and Pakistan most common blood group was B.17,18

There is a lot of variation in the prevalence of ABO blood group worldwide. Aboriginal people (O=63%, A=39, B=0% and AB=0%), Bororo, Brazil, Shompen Nicobarese and Peruvian Indian (O=100%), Mayans (O=98%), Hawaiians (A=61%), Indians (B=40%), Ainu, Japan (AB=18%), etc.19

In the present study, predominance of male over female blood donors was noted which is similar to other studies conducted in India.20,21,22 In a developing country like India, because of social taboo, cultural practice, lack of incentive and panic of blood donation, female donors are very less. In present study most common age group encountered in blood donation was 18-40 years, which is similar to the study of Patel Piyush A et al.21

In this study prevalence of RhD positive and Negative blood donors was 90.99% and 9.01%, respectively similarly reported by Periyavan et al23 and Falusi et al.24 In the present study, the gene frequency of D and d was .6999 and .3001 respectively.
respectively, which is almost similar to the results of the study by Periyavan et al. In present study gene frequency of C gene, c gene, E gene and e gene were .658, .342, .079 and .921 respectively which were similar to the study of Lejla Lasić et al.

Kell blood group antigen follows RhD antigen in importance because of its high immunogenicity. Out of 25 antigens in the Kell blood group system, major antigens were K and k which were included in the study. In the present study the most common Kell phenotype was K- k+ 95.5% (n = 475) followed by K+ k- phenotype, which was 4.5% (n = 45). K+ k- and K+ k+ phenotypes were not encountered in this study. The results obtained in the present study were very similar to those derived in another study on Indian blood donors by Thakral et al. and R.N. Makroo et al. In present study gene frequency of k and K gene was .9772 and .0228 respectively. Similar results was found in study of Guelsin G.A et al.

In the present study the prevalence of major Duffy antigens phenotypes were; Fya+ Fyb+; 47.5% (n = 475), being the most common phenotype, followed by Fya+ Fyb-; 35% (n = 350), Fya- Fyb+; 17% (n = 170) and Fya- Fyb-; 0.5% (n = 5), being the least common phenotype. Similar results were reported by Thakral et al. and Nanu & Thapliyal. In the present study gene frequency of Fya and Fyb gene was; .5865 and .4135 respectively, while in study of Guelsin G. A et al. gene frequency of Fya+ and Fyb+ gene was .365 and .635 respectively. These may be because of population variation.

In the present study the prevalence of Kidd phenotype was; Jka- Jkb-; 44.5 % (n = 445) being the most common phenotype followed by a Jka+ Jkb- (Jk null); 26.5% (n = 265), Jka+ Jkb+; 18% (n = 180) and the least common phenotypes was Jka- Jkb+; 11% (n = 110). Variable results as compared to our results were reported in the study by Thakral et al. In his study, the prevalence of Kidd phenotype were Jka+ Jkb+; 33.44%, Jka+ Jkb-; 49.21%, Jka- Jkb+; 17.35% and Jka- Jkb- (Jk null); 0.0 % These may be because of population variation. In present study gene frequency of Jka+ and Jkb+ gene was; .6281 and .3719 as similarly reported in study of Guelsin G. A et al.

The knowledge of the prevalence of different blood group antigens in any given population has been always helpful in preventing alloimmunization as well as in managing cases of alloimmunization. Patients with prior multiple transfusions and multiparous women are likely to develop antibodies against these minor blood group antigens as it is not practically feasible to match all these minor antigens before transfusion so as to avoid immunization. Finding compatible units for such patients without having any knowledge of the prevalence of the implicated antigens in the local population is a difficult task, more so if the patient has developed more than one antibody. With the intention of improving blood safety by performing antibody screening in all prospective patients is a great step towards reducing adverse reactions caused by transfusion.

**CONCLUSION**

Blood bank and blood transfusion are a newborn baby in the field of medicine, only approximately one century old while the existence of human (Homo sapiens) and allopathic medicine is thousand centuries old. In a last century lot had been done in the field of transfusion medicine and much more has to be done in future. The present study...
is conducted with the same aim, to add a little bit in the field of blood grouping and transfusion medicine. The present study is helpful in the transfusion management of multiparous and multi-transfused patients. Sooner or later extended blood grouping is the need of future to provide the safe blood and its components to the needy patient. So our work is genuine and helpful in the field of transfusion.

ACKNOWLEDGMENT

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CONSENT

The authors state that written informed consent was taken from the patients before being recruited for this work.

ETHICAL APPROVAL

All authors hereby declare that each one procedure are examined and approved by the acceptable ethical committee of Gajra Raja Medical College, Gwalior, India (Ethical Clearance D. No. 491/ Bio/ MC/ Ethical, dated 24-03-2018) and research have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

AUTHOR CONTRIBUTION

This work was administered together between all authors. The authors DCS and SR designed the study, perform the statistical analysis, wrote the protocol and wrote the primary draft of the manuscript. Authors AA and RJ manage, the analysis of the study. Authors SS and DK managed the literature search and clinical aspect of the study. Author BJ supervise the work. All authors read and approved the ultimate manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this text.

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