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Rhesus and Kell Phenotyping of Voluntary Blood Donors: Foundation of a Donor Data Bank

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ABSTRACT

Objective: To assess the Rhesus (Rh) and Kell (K) phenotype of voluntary blood donors and lay foundation of a data bank of voluntary blood donors.

Study Design: Cross-sectional study.

Place and Duration of Study: Blood Bank, The Aga Khan University Hospital, Karachi, in the year 2014.

Methodology: Voluntary blood donors were inducted after taking written informed consent. Three -5cc of EDTA anticoagulated blood sample was taken to phenotype red cells for C, c, E, e, and Kell antigens using antisera. [DiaMed-Switzerland].

Results: Hundred blood donors were included in the study. ABO blood groups of the donors were: O [37%], B [31%], A [21%] and AB [11%]. Ninety-seven percent were Rh D positive while 3% were Rh D negative; 'e' antigen had the highest frequency [99%], while 'E' antigen was the least frequent [19%]. The most common probable Rh phenotype was R1R1 (DCe/DCe) in 44 [44%]. In the Kell system, all the donors [100%] had phenotype of K-k+.

Conclusion: The most common blood group was O +ve. The pattern of Rhesus antigen expression and phenotype found in this study was concordant to that reported previously from Asia. However, there was a much lower frequency of K antigen.

Key Words: Phenotype. Rhesus typing. Kell antigen. Pakistan. Blood donors.

INTRODUCTION

Since the discovery of the ABO system by Karl Landsteiner in the early twentieth century, many blood group antigens and their genes have been identified.¹ There are differences in the distribution of these red cell antigens between people of different ethnicity and races. Because of these differences when a person is exposed to red cell antigens of another person through transfusion or pregnancies, there is development of alloantibodies against those foreign antigens, a process known as alloimmunization.² Many of these blood group antibodies including Rhesus (Rh), Kell, Kidd and Duffy are clinically significant and may cause hemolytic transfusion reactions.³

Alloimmunization is commonly observed in multi-transfused patients. The beta-thalassemia carrier rate in Pakistan is around 5.5% and more than 6000 beta-thalassemia major children are born annually in Pakistan.^{4,5} The reported incidence of alloimmunization in thalassemia patients in Pakistan ranges between 4.97 - 9.2% and the most commonly reported antibodies belong to the Rh and the Kell blood group system.^{6,7}

Patients who have developed alloantibodies and require transfusion need to be transfused with extensive phenotyped matched red blood cells. The donor data bank may aid in quick provision of safe blood to the patient, reduce workload of the blood bank personnel and help in knowing the red cell antigen distribution pattern of the local population and assuming genotype frequencies. Keeping in mind the rate of alloimmunization and the commonly occurring alloantibodies, this study was conducted to assess the Rhesus and Kell phenotype of voluntary blood donors and lay foundation of a hospital based data bank of voluntary blood donors.

METHODOLOGY

This study was an observational cross-sectional study conducted at the Blood Bank of The Aga Khan University Hospital, Karachi. A total of 100 voluntary and regular blood donors of either gender were selected from three voluntary blood donation camps.

Detailed history was taken using a standard donor history questionnaire used for recruiting donors. Hemoglobin of donors was checked using copper sulphate method. Copper sulphate density method for hemoglobin estimation of blood donors was selected because of ease of use in blood camps. Written informed consent was taken from the donors.

All blood donations were screened serologically and by nucleic acid amplification testing (NAT) for HBV, HCV and HIV. In serology, antibodies were tested to HCV and

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HIV. Hepatitis B surface antigen, VDRL for syphilis and ICT malaria were also part of screening tests. Donors found to be negative for all the infectious disease markers were enrolled in the registry.

Donors' blood was collected in EDTA anticoagulant for blood grouping. ABO and Rh typing was done by gel technique using ID-Card [DiaMed-Switzerland]. Classification of blood groups was based on both forward and reverse grouping and a negative control result. For RhD, all D-negative donors were further tested for the presence of weak D-phenotype. Donors were classified as D-negative when testing for weak D was also negative.

Quality control of all reagents used for ABO and Rh typing was done to ensure that the reagents are functional. The quality control results were documented in worksheets.

Direct antiglobulin test was performed by gel technique using ID-Card [DiaMed-Switzerland] for all samples. Only negative samples were further used for red cell phenotyping. The blood was typed for C, c, E, e, K and k antigens using antisera [DiaMed-Switzerland] by conventional tube method as per manufacturer's instruction. A negative control tube was included with each specimen. The test was considered valid only if negative control was negative. Quality control of each antiserum was performed before the actual test by testing it against known antigen positive and antigen negative reagent red cells for reactivity and specificity. The anti-sera were used once the quality control was valid i.e. if reactions were positive with positive control reagent and negative with negative control reagent.

All the data was analysed using SPSS version 19.0 [SPSS Inc., Chicago,IL,USA]. Frequency was calculated for ABO, Rhesus and Kell blood group systems. Results were reported as percentage. Allele frequencies were calculated using Hardy-Weinberg equilibrium.⁸

The study was approved by the Ethical Review Committee of The Aga Khan University Hospital. (ERC approval Number: 2560-Pat-ERC-13)

RESULTS

Hundred voluntary blood donors consented to enroll in the donor data registry. The median age of the donors was 35 years (ranging from 19 to 60 years). Bulk of the donors i.e. 98 [98%] comprised of males while only 2 [2%] were females.

Blood group O was the most common blood group with a frequency of 37 [37%]. This was closely followed by blood group B that was seen in 31 [31%] of the donors. Group A was identified in 21 [21%] while AB blood group was the least common with a frequency of 11 [11%].

In the Rhesus blood group system, majority of donors, n=97 [97%] were RhD positive and only 3 [3%] were RhD

Table I: Rhesus and Kell antigen frequency and comparison with other studies conducted in Asia.

Red cell antigen	Our study N=100 (%)	India (14) N=1240 (%)	Iran (17) N=522 (%)
D	97	93.3	90.2
C	87	84.7	75.9
c	57	52.8	73.9
E	19	17.9	29.5
e	99	98.3	97.9
K	0	5.56	8
k	100	100	97.7

Table II: Probable Rh phenotypes of blood donors.

Probable Rh phenotypes	(%)
R1R1 (DCe/DCe)	41
R1r (DCe/dce)	34
R1R2 (DCe/DcE)	10
R2r (DcE/dce)	8
R0r (Dce/dce)	3
R2R2 (DcE/DcE)	1
rr (dce/dce)	1
r'r' (dCe/dCe)	1
r'r'' (dCe/dcE)	1

negative. Weak D was not detected in any donor. Among the remaining Rhesus antigens, e-antigen had the highest frequency of 99 [99%]. Frequencies of all the Rhesus and Kell antigens and comparison of phenotype frequencies with other Asian populations is done in Table I.

In the Rh system, the most common probable phenotype was R1R1 (DCe/DCe) in 44 [44%]. Frequency of other phenotypes is given in Table II.

Calculated allele frequency of Rhesus and Kell antigens according to Hardy-Weinberg equilibrium were as follows: C= 0.645, c=0.355, E= 0.105, e=0.895, K=0 and k=1.

In the Kell system, all the donors [n=100, 100%] had the phenotype of K-k+.

DISCUSSION

In this study, the most common ABO group found was O (37%) followed by B (31%). The least common blood group was AB. A marginal difference in group O and B has been noticed in Pakistani population. Two other studies from Pakistan have reported blood group B to be most common (29.5% and 33.7%). Group O was reported as second most common (26% and 30.3%) and AB group as least common (9.7% and 8.9%) in both studies.^{9,10} A study from Asia has reported similar results to our study indicating group O to be most common and AB as least common in all ethnicities.¹¹

In the Rhesus system, the frequency of RhD antigen is reported as being around 90.9 - 92% in Pakistan.¹⁰ Similar rates of 92 - 93% RhD positive donors have been reported from India.¹² In this study, the rate of RhD

positive blood donors was higher and found to be 97%. Similar, rate of 97.5% RhD positive donors has been reported from a study conducted in Malaysia.¹¹

The frequency of e-antigen was found to be highest (99%) in this study. This high frequency of e-antigen is seen in all populations of the world including whites and blacks and is found to be around 98%.¹³ The least common Rhesus antigen worldwide is E-antigen occurring with a frequency of 17.9% in Indians, 29% in whites and 22% in blacks.¹⁴ Similarly, E-antigen was the least common antigen found in these donors (19%).

The frequency of C-antigen in this study was 87 [87%]. This is similar to that reported from India (89.5%) and China (93%).¹² However, C-antigen is less frequent in Europeans (68%) and Africans (27%).¹³ The expression of c antigen also varies among Asians, Europeans and Africans. Frequency of c-antigen was 57 [57%] in this study. It has been reported as 52.8% and 58.6% in two other studies from Asia.^{12,14} Whereas, c-antigen is commonly found in African and European people occurring with a frequency of 96% and 80%, respectively.¹³

The most common Rh phenotype detected in this study was R1R1 (CDe/CDe) found in 44% of donors. Similarly, in various other studies conducted in India, Thailand and Malaysia R1R1 has been reported as the most common phenotype.^{11,14,15} This shows that R1R1 is the most common phenotype in the Asian population.

In the Kell blood group system, it was interesting to see that none of the donors (0%) had K-antigen. K-antigen is known to be less frequent than 'k' antigen in all populations and has a frequency of about 5.6% in Asians, 8 - 9% in Europeans and about 1.5 - 2% in Africans.^{11,16} The very low frequency of K-antigen in Pakistani population is reflective of the high incidence of anti-K (33.3%) detected in the thalassemia patients as reported by Bilwani *et al.*⁷

Very few studies from Pakistan have reported on the complete Rhesus phenotype frequencies of blood donors. Knowledge about phenotype frequencies is required when finding compatible blood for patients with alloantibodies, in calculating the number of blood units that need to be cross-matched to find a compatible blood unit, in paternity testing and preparation of in-house panel cells for serological techniques. Knowledge of frequency will help in planning the set of primers and inclusion of phenotypes in case of future research in molecular methods of screening.

Foundation of a donor data bank have been laid. The next step could be establishment of a rare blood group donor Registry which is non-existent in Pakistan. This would be beneficial when blood for a patient with rare blood group is needed and is not available in blood bank.

The limitation of this study is the small sample size. Studies of similar nature must be conducted on a larger scale to know the actual red cell phenotype of our population. It is essential to know the prevalence and antigen distribution pattern of the local population because the pattern varies among different populations across the globe as was quite evident from this study. Furthermore, it is recommended that regular Rh and Kell typing of all voluntary blood donors and transfusing phenotype match blood to all patients requiring chronic transfusions. This intervention will not only reduce rate of alloimmunization in patients but will also save time and resources.

CONCLUSION

The most common blood group was O +ve. The pattern of Rhesus antigen expression and phenotype found in this study was concordant to that reported previously from Asia. However, there was a much lower frequency of K-antigen in this study.

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