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Frequency of Rh Phenotypes in Voluntary Blood Donors

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Abstract
Background: *Very few reports are available regarding Rh phenotypes prevalence in India and no reports are available from Rajasthan. There is a possibility of alloimmunization and antibody production in the recipients, even after proper grouping and cross matching. Due to the heavy financial burden of complete phenotyping; the knowledge of Rh phenotypes can play a major role in preventing alloimmunization. The aim of this study to prevalence of principal Rh blood group antigens like D, C, E, c & e in the voluntary blood donors with a view to generate blood bank data for constitution of panel of blood donors for multipurpose utilities.*
Materials and Methods: *A prospective study was carried out on 3014 healthy blood donors from April 2016 to Nov 2016 at our blood bank. Donors were grouped and typed for ABO and Rh major antigens. Statistical analysis was carried out using Microsoft excel software. Incidence was given in proportion with 95% confidence interval. Allele frequencies were also calculated using Hardy-Weinberg principal.*
Results: *Among Rh antigens, e was the most common antigen (98.71%), followed by D-91.94%, C-81.85%, c-62.77% and E-17.25% with DCe/DCe (R1R1) (36.43%) being the most common phenotype and the least common phenotype is r''r', r"r", r'r" (0.03%).*
Conclusion: *Determination of Rh phenotypes can play a major role in preventing alloimmunization in multi-transfusion cases. Database for antigen frequency to at least Rh blood group system in local donor population helps to provide antigen negative blood unit to patients with multiple alloantibodies, minimize alloimmunization rate, there by improve blood safety.*
Keywords: *Rh blood group antigens, Rh phenotypes, Allele, Prevalence, Alloimmunization*

Introduction

The primary goal of any blood transfusion is to provide the patient, donor red blood cells with optimal intravascular survival after transfusion and serve their function. The criteria for selection of donor cells focuses on absence of antigens on donor cells against that antibodies are detected in the patient's serum in need of transfusion.[1]

India is a vast country with several distinct population groups, so there is an obvious need for

phenotype frequencies to be determined in different parts of India. The data on incidence of antigens of various blood groups in the local donor population helps in routine blood transfusion practices of a blood transfusion centre.[2] In situations where clinically significant antibodies are identified in patient's serum, antigen-negative donor units for such cases can be easily retrieved from the donor database of various blood groups available with a blood transfusion centre. For this particular reason, all blood banks should have the donor database on antigen frequency of other blood group systems in their local donor population.

Very few studies regarding the incidence of various blood groups in the blood donor population are published from India.[3,4] Blood transfusion can cause immediate or delayed immunological reactions, out of these most serious is the hemolytic transfusion reaction by antibody incompatibility.

A total of 308 RBC antigens are recognized till now by the International Society of Blood Transfusion (ISBT), 270 of which are clustered in 30 blood group systems.[5] The Rh blood group system (including the Rh factor) is one of thirty-five current human blood group systems. It is the second most important blood group system, after ABO and consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e are the most important. The main antigens are D, C, E, c and e, which are encoded by two adjacent gene loci, the RHD gene which encodes the RhD protein with the D antigen (and variants)[6] and the RHCE gene which encodes the RhCE protein with the C, E, c and e antigens (and variants).[7] There is no d antigen. Lowercase "d" indicates the absence of the D antigen (the gene is usually deleted or otherwise nonfunctional).

Retrospective studies in the general population reported antibody frequencies after transfusion in less than 1 to 3 percent. However, in multi-transfused patients, alloimmunizaton occurs in up to 70% of patients.[8,9] In countries where phenotyping is mandatory (eg. in France since 2002) in all donated blood and in recipients, post-transfusion alloimmunization have become rare.[10] Some developed countries have already made revolutionary changes in their cross match protocols and have started complete genotyping of their donors to make a huge database of donors for future usage and references. Some other countries have made extensive phenotyping and complete cross matching compulsory for the category of patients who may require multiple transfusions in future. These procedures have added massively to the cost of blood banking in developed nations and thus its implementation in developing countries like India is way behind.[11]Keeping in view the heavy financial burden of complete phenotyping of blood; the determination of only Rh phenotypes can play a major role in preventing alloimmunization in multi-transfusion cases. The current practice of providing compatible blood to patients in such cases in India is still reliant upon random cross matching of available units in the inventory. There is wide variation in distribution and frequency of Rh antigens throughout the world and lack of study especially from west part of India i.e. in the population of Rajasthan, impelled us to identify the frequency of five major Rh antigens and its phenotype. This study was carried out to determine the phenotypic frequency of various Rh antigens (D, C, E, c, e) in healthy blood donors and to generate blood bank data for constitution of panel of blood donors for multipurpose utilities.

Materials and Methods

A total of 3014 samples from random voluntary blood donors coming in blood donation camp organized by the Department of IHBT, S.P. Medical College and Associated group of Hospital, Bikaner, were collected for extended antigen typing during April 2016 to November 2016 after obtaining approval from the Institutional ethical committee. Written consent was taken at the time of donor screening. Collected blood samples were from VBD camps organized in different areas so that we have

representative samples in the study from urban and rural areas of different zones of Rajasthan, India.

The D, C, c, E, e antigens were typed using monoclonal antisera from Immucor derived from clones D175-2/TH28, MS24, MS33, MS258+MS80 and MS16+MS21 respectively. Donors typed as D negative were confirmed using an antiglobulin weak D test by tube method using commercially available antisera [Novaclone anti-D (Immucor Rodermark, Germany) which contains IgG clone D415 in addition to IgM clone D175-2.]

Before proceeding to extended Rh phenotyping, the donor's ABO grouping was done. For Rh antigens (D, C, c, E, and e), red blood cells were tested against specific antisera to observe antigen-antibody reactions (haemagglutination) by the microplate haemagglutination method with IgM monoclonal antiserum on a fully automated system (Galileo, Neo, Immucor Inc., Norcross, GA, USA) as per instructions provided in the instrument operator manual. Conventional tube method was also done using 1 drop of specific antisera with 1 drop of 3-4% suspension of red blood cells to be tested. No agglutination indicated its absence. Agglutination reactions in positive test results were recorded and graded as 1+ to 4+. All samples that showed a negative agglutination with anti-D were tested again in the antihuman globulin phase with monoclonal antisera (Blend IgG + IgM) by tube technique for the presence of weak "D" as per the manufacturer's instructions.

Statistical analysis was done by Calculation of red cell antigen and phenotype frequencies of the various blood group systems by totalling the number of donors positive for a particular antigen phenotype divided by the total number of donors screened. Results were expressed as a percentage. Statistical analysis was done using Microsoft office Excel software. Allele frequencies were calculated under the standard assumption of Hardy-Weinberg equilibrium. Incidence was given in proportion with 95% confidence interval.

Results

During the study period, antigen typing was done on 3014 voluntary blood donors. ABO grouping in this study showed that "B" was the most common blood group (37.92%) followed by "O" (32.25%), "A" (20.97%) and "AB" was the least common (8.86%) type. RhD typing along with other major Rh antigens was done on all the donors and out of the 3,014 donors 2,771 (91.94%) were D positive and 243 (8.06%) were D negative (Table 1). shows the distribution of Rh (C, E, c, e) antigens in the study population. Among the five major antigens, "e" antigen was found to be the most common antigen (98.71%) followed by "C" (81.85%), "c" (62.77%) and "E" being least common antigen (17.25%). Blood group wise distribution of Rh principal antigens is shown in (Table 2). There was no difference in distribution of Rh antigens irrespective of their blood group. Allele frequency is shown in the Figure 1. Antigen frequency (AF) of other Rh antigens in Rh (D) positive and Rh (D) negative blood donors in the study population is shown in (Table 3). The maximum antigen frequency in Rh (D) positive donors was e (98.63%) followed by C (88.42%) and in Rh (D) negative donors e (99.59%) followed by c (97.53%). Since genotyping was not done, the presumed Rh phenotype frequencies in our population are shown in (Table 4). The most common phenotype observed was R_1R_1 (DCCee) followed by R_1r (DCcee)> R_1R_2 (DCcEe)> rr(dce)> R_2r (DccEe) and the least common being r''r, r"r" and r'r" among the total study population. Most common phenotype in Rh positives was R_1R_1 and among Rh negatives was rr.

Table 1. Prevalence of other Rh blood group antigens in the study population

Antigen	No. (n=3014)	Percentage (%)	95% CI
C	2467	81.85	80.47-83.23
c	1892	62.77	61.04-64.5
E	520	17.25	15.9-18.6
e	2975	98.71	98.31-99.11

Table 2. Prevalence of Rh blood group Antigens in ABO Blood group system.in the study population

Blood Group	Rh antigens				
	D%	C%	c%	E %	e%
A (n=632)	92.25%	83.23 %	64.40%	18.20%	99.05%
B (n=1143)	91.60%	80.66%	61.94%	17.41 %	98.51%
O (n=972)	92.18%	82.30%	63.68%	17.28 %	98.77%
AB (n=267)	91.76%	82.02%	59.18%	14.23%	98.50%

Table 3. Prevalence of other Rh blood group Antigens in Rh (D) positive and Rh (D) negative blood donors in the study population

Total no. of donors	Antigen frequency (%)			
	C	c	E	e
D positive (2771)	88.42	59.73	18.62	98.63
D negative (243)	7.00	97.53	1.65	99.59

Table 4. Frequency of various Rh Phenotypes in study population

Rh Phenotypes	Frequency	Percentage	95% CI
CCDee (R_1R_1)	1098	36.43	34.71-38.15
ccDEE (R_2R_2)	36	1.19	0.8-1.58
CcDee (R_1r)	1056	35.04	33.34-36.74
ccDEe (R_2r)	186	6.17	5.31-7.03
ccDee (R_0r)	98	3.25	2.62-3.88
CCDEE (R_ZR_Z)	-	-	-
CCDEe (R_1R_Z)	15	0.50	0.25-0.75
CcDEE (R_2R_Z)	2	0.07	*
CcDEe (R_1R_Z)	279	9.26	8.23-10.29
Ccdee (r'r)	11	0.36	0.15-0.57
CCdee (r'r')	5	0.17	0.02-0.32
ccdEe (r"r)	1	0.03	*
ccdEE (r"r")	1	0.03	*
ccdee (rr)	223	7.40	6.47-8.33
CcdEe (r'r")	1	0.03	*
D--ee	1	0.03	*
d--Ee	1	0.03	*
*(proportions are too small to calculate confidence interval)			

Table 5. Comparison of prevalence of other Rh antigens with other Indian and worldwide studies

Indian Studies	C	c	E	E
Thakral et al.[3]	84.8	52.8	17.9	98.3
Sarkar et al.[11]	87.55	51.06	26.55	98.42
Sharma et al.[12]	84	58.3	25.6	78.5
Makroo et al.[13]	87	58	20	98
D. Lamba et al.[14]	85.1	62.3	21.5	99.0
World Population				
Caucasians[2,15,16]	68	80	29	98
Blacks[2,15,16]	27	96	22	98
Whites[2,15,16]	68	80	29	98
Chinese[17]	93	47	39	96
Present Study	81.85	62.77	17.25	98.71

Discussion

In Rhesus system our study shows frequency of Rh positive was 91.94%, while only 8.06% was Rh negative. These figures are similar to the other studies carried out in different parts of India.[3,11] The prevalence of other Rh Antigens (C, c, E, e) among the study population was compared with that of other studies carried out in India at different regions[3,11-14] and with other populations[2,15-17]as presented in (Table 5).

Out of 3014 voluntary blood donors in present study the most common phenotype was found to be R_1R_1 (36.43%) followed by R_1r (35.03%). In our study most common phenotype among Rh positives (n=2771) is R_1R_1 with 39.62% and the least common phenotype is R_2R_Z with 0.07%. Among Rh negatives (n=243), most common phenotype is rr with a frequency of 91.77% and the least common is r''r, r''r'' and r'r'' with 0.41%. No sample of Rh_{null} was reported in present study while in 2 samples (0.07%) deletion of antithetical antigen C/c was found i.e. 1 case of D--ee and 1 case of d--Ee). Rare blood is defined, on the basis of the blood group characteristics, as being found at a frequency of $\leq$ 1:1000 random samples in a given population.[18,19] The Rh phenotype R_2R_Z, r''r, r''r'' and r'r'' were found to be rare in our population with percentage prevalence of 0.07, 0.03, 0.03 and 0.03, respectively. Weak D phenotype was not observed in our study.

On comparing the Rh phenotype distributions among international studies, significant differences were found. In blacks, most common Rh phenotype is R_0r (45.8%) and the least common is R_2R_2 with 0.2%. In Caucasians most and least common Rh Phenotype is R_1r & R_ZR_Z with 35.6% and 0.0004% respectively.[2,15,16]

Conclusion

There is wide range of distinctions in phenotypes and probable genotypes among different races and religion. Our study concluded that most frequent antigen amongst five major antigens of Rh system was Rh "e" (98.71%) whereas the least common was antigen "E" (17.25%). DCCee was the most common phenotype. The most frequent probable genotype was DCe/DCe (R_1R_1) among Rh positives whereas among Rh negatives it was dce/dce (rr).

Outcomes of such studies can be used to formulate a Rare Blood group Donor registry (donors lacking high frequency antigens) at national level, and patients with antibodies against high frequency antigens can be directed to such Rare Blood Group donor registry.

Though the sample size of this study was relatively small compared to the huge population of the country, it still gives an estimate of the frequencies of Rh blood group antigens The gene pool of people from other parts like South/ East/ North India may be somewhat different from that of the West Indians and thus a multi-centric study in hospitals located in different regions, would be valuable to provide information regarding the frequencies of the various antigens in different regions of India.

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