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Original Article

Proportion of Rh phenotypes in voluntary blood donors

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ABSTRACT

Background: The Rh system is the major blood group system besides ABO system. Even after proper blood grouping and cross matching there is a possibility of alloimmunization and antibody production in the recipients against the Rh or minor blood group antigens like Kell, MNSs, Duffy etc. 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 and adverse events in multitransfusion cases. To determine the proportion of Rh phenotypes in voluntary blood donors with a view to generate blood bank data for constitution of panel of blood donors for multipurpose utilities.

Method: Identification of Rhesus factors (Rh) was done by the antigen antibody agglutination test by the test tube method on 10,133 healthy voluntary donors.

Results: The phenotypic frequencies of Rh blood groups in the studied population were D-92.25%, C-87.55%, E-26.55%, c-51.06% and e-98.42%. Thus 'e' was the most common and E was the least common of all the Rh types. Phenotypically DCCee group was the most common phenotype and dccee was least common type.

Conclusion: Determination of Rh phenotypes can play a major role in preventing alloimmunization and avoiding adverse events in multitransfusion cases.

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Introduction

The true genetic make-up of an individual is termed as genotype and effect of the genes can be clinically observed by the apparent outcome, known as the phenotype. The ABO and RhD grouping along with cross matching by Anti Human Globulin (AHG) technique is a mandatory requirement for safe transfusions. The Rh system currently is composed of 49 antigens expressed by the genes on chromosome 1. The

important antigens of Rh system are: RhD, RhC, RhE, Rhc and Rhe. Even after proper blood grouping and cross matching there is a possibility of alloimmunization and antibody production in the recipients against the Rh or minor blood group antigens like Kell, MNSs, Duffy etc. 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

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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. Significantly the incidence of Kell is not so high in India as in the western countries.¹ Keeping in view the heavy financial burden of complete phenotyping the determination of only Rh phenotypes can therefore play a major role in preventing alloimmunization and avoiding adverse events in subsequent transfusions in multitransfusion cases. The present study was done to record the phenotypic frequency of various Rh antigens in blood donor population of Central Maharashtra. To determine the proportion of Rh phenotypes in voluntary blood donors of central Maharashtra with a view to generate data with multipurpose future utilities for health and prevention of alloimmunization. This study will also contribute to development of blood bank data for constitution of panel of blood donors, particularly, for multitransfused, alloimmunized patients.

Materials and methods

This study was carried out over a period of one and a half years from May 2010 to October 2011 on 10,133 Healthy Voluntary Donors reporting to the Blood Bank, Armed Forces Medical College, Pune. Determination of Rhesus factors (Rh) was done by the antigen antibody agglutination test by the test tube method. 5–10 cm segments of tubes were taken from blood bags by using tube sealers and stored at 2–4 °C. Five clean tubes were arranged and marked C, D, E, c and e & one drop of corresponding antisera and one drop of 2–5% of cell suspension was added, mixed and centrifuged at 1000 rpm for 01 min and the reaction was interpreted as agglutination +ve or –ve. Weak reaction or –ve reaction was confirmed by ID-Card “DiaClon Rh-subgroups” from Diamed AG, Switzerland by Gel method by antiglobulin test. Statistical analysis was done using Microsoft office Excel software.

Results

The Blood Bank of Armed Forces Medical College, Pune has an annual blood donation by 10,000 donors. A total of 10,133 (ten thousand one hundred and thirty-three) blood donor samples of all groups were typed for the presence of Rh (D, C, E, c, e) antigens. There were 9743 (96%) male donors and 390 (4%) female donors. The donors ranged from 18 to 60 years of age. Most of the donors were in the age group of 18–27 years. The donors were from all the districts of Maharashtra and majority of them were from Pune and army background.

ABO grouping in this study showed that “O” was the most common blood group (33%) and “AB” was the least common (9%) type. The trend was similar in male and female donor population. In Rh positive and negative males group “O” continued to be the most common type. While in Rh-positive females the result was similar to Rh-positive males, the expression of “O” and “A” groups was equal in the Rh-negative females. RhD typing along with other major Rh antigens was

done on all the donors and out of the 10,133 donors 9348 (92.25%) were D+ and 785 (7.74%) were D–. The percentage of Rh – females was 0.42% whereas in males it was 7.32%.

Table 1 shows gender-wise distribution of Rh (D, C, E, c, e) antigens in the study population. The e antigen was found to have the highest frequency (98.42%), followed by D and C antigen (92.25% and 87.55%, respectively). Since genotyping was not done, the presumed Rh phenotype frequencies in our population are shown in Table 2. Nine probable phenotypes were found to be present in our population, the most common being DCe/DCe (R1R1; 35.2%). dce/dce (rr⁺) (0.07%) was the most rare phenotype observed. The apparent homozygosity for CC, cc, EE and ee genes was found in 45.71%, 9.21%, 0.93% and 72.79% of donors tested, respectively. Table 3 shows antigen frequency (AF) of other Rh antigens (CcEe) in Rh (D) positive and Rh (D) negative blood donors in the study population. The maximum antigen frequency in Rh (D) positive donors was e (94.6%) followed by C (90%) and in Rh (D) negative donors e (99.5%) followed by c (97.8%).

Discussion

The aims of the study were to phenotype the 5 major types of Rh antigens in voluntary donors, to determine the Rh composition of the population in central Maharashtra to generate a database of donors for all future activities.

Prevalence of Rh (D) positive and Rh (D) negative blood donors in India

A total of 10,133 blood donors were tested for Rh (D) antigen by commercially available antisera where 92.25% of the donors were found to be Rh (D) positive and the remaining 7.74% Rh (D) negative. Comparison of the results of our study with the previous studies from India is shown in Table 4.

Our result of 92.25% Rh (D) positive blood donors is in agreement with earlier study from South India (92.25% vs. 94.53%).² Comparing our results with studies from different

Table 1 – Gender-wise distribution of Rh antigens in the study population.

Rh antigen type	Male	Female	Total	Percentage
Rh ‘D’ +ve	9001	347	9348	92.25%
Rh ‘D’ –ve	742	43	785	7.74%
Total	9743	390	10133	
Rh ‘C’ +ve	8534	338	8872	87.55%
Rh ‘C’ –ve	1209	52	1261	12.45%
Total	9743	390	10133	
Rh ‘c’ +ve	4965	209	5174	51.06%
Rh ‘c’ –ve	4778	181	4959	48.94%
Total	9743	390	10133	
Rh ‘E’ +ve	2577	114	2691	26.55%
Rh ‘E’ –ve	7166	276	7442	73.45%
Total	9743	390	10133	
Rh ‘e’ +ve	9587	386	9973	98.42%
Rh ‘e’ –ve	156	04	160	1.58%
Total	9743	390	10133	

Table 2 – Rh phenotype frequencies in blood donors in this study.

S no	Rh phenotypes ^a	Frequency in the study population	Percentage (%)
1.	DCe/DCe (R ₁ R ₁)	3563	35.2
2.	DCe/dce (R ₁ r)	3110	30.7
3.	DcE/DcE (R ₂ R ₂)	823	8.1
4.	DcE/DcE (R ₂ R ₂)	74	0.7
5.	Dce/dce (R ₀ r)	225	2.2
6.	DcE/dce (R ₂ r)	600	5.9
7.	dce/dce (rr)	27	0.3
8.	dCe/dce (r'r)	252	2.5
9.	dce/dce (r'r')	7	Rare

^a The Rh phenotypes are “presumed” as genotype frequency is unknown in our population.

parts of the world, it can be observed that there is tremendous variation in the prevalence of Rh (D) antigens in blood donors worldwide (Table 5). The prevalence of Rh (D) antigen in different population in the world ranges from 60% to 99%, the highest prevalence is seen in the Japanese and Burmese population (100–99%) while the minimum being in the Basque people of Southern France and Northern Spain (60–80%). Rh (D) is a high frequency antigen in the Far East regions reaching 100% in some population (Table 5).³ This major difference in the distribution of Rh (D) antigen in the different population groups has a clinical significance as D is the most important red cell antigen after A and B, and Rh antibodies are considered potential agents of hemolytic disease of newborn and of hemolytic transfusion reaction. The confirmation of negative or weak result is a standard practice in blood banking. The significance of this confirmation lies in the fact that different methods of testing have different sensitivities. A negative test by a less sensitive method such as tube method may actually be a weak or moderately positive test by gel method which is much more sensitive method than tube method. The clinical significance of doing this test is that weak positives are considered as Rh positives when they are donors and as Rh negatives when they are recipients.

Knowledge of the distribution of Rh (D) antigen in blood donor population also helps in formulating policies regarding pre transfusion testing. For instance, the frequency of Rh (D) negative person among the Taiwanese population is 0.3% hence the incidence of anti-D in Taiwanese population is very uncommon i.e. 1 in 295,000 blood donors. As a result of these findings routine Rh (D) typing in Taiwanese hospital patients requiring blood transfusion has been discontinued.⁴ However,

Table 3 – Antigen frequency (AF) of other Rh antigens (CcEe) in Rh (D) positive and Rh(D) negative blood donors in the study population.

Total no. of donors	C	c	E	e
	AF (%)	AF (%)	AF (%)	AF (%)
Rh (D) positive (9348)	87.7	51.75	25.35	98.55
Rh (D) negative (785)	85.73	42.8	40.89	96.81

Table 4 – Comparison of prevalence of Rh D antigens in blood donors in India.

Author	Year	Region	No. of donors	% Of Rh (D) positive donors	% Of Rh (D) negative donors
Kumar et al ¹⁵	2002	North India	34,942	93.7	6.3
Roy et al ¹⁶	2004	East India	556,214	97.8	2.2
Das et al ²	2001	South India	150,536	94.5	5.5
Vasanth et al ¹⁷	–	West India	–	–	5
Verma et al ⁵	2006	Lucknow (North India)	21,135	95.3	4.7
Thakral et al ⁶	2010	Chandigarh (North India)	1240	93.4	6.6
Present Study	2011	Pune (South India)	10,133	92.25	7.74

due to relatively high prevalence of D negative (5–10%) in our population, D typing is a must for blood donors as well as for patients requiring blood transfusion in Indian scenario.

Comparison of Rh phenotypes within different population

Out of 9348 Rh (D) positive randomly selected blood donors in the present study the most common phenotype was found to be R₁R₁ (35.2%) followed by R₁r (30%). Nanu and Thapliyal (42.64%) and Thakral et al (43.8%) and Verma et al (39.8%) also found similar results.^{5–7} However, the above phenotype frequency is present in only 17.6% of white and 2.9% of black population.^{8–10} The comparative account of the reported frequency in different population is shown in Table 6.

The Dce/dce (R₀r) phenotype frequency in our population is approximately 2% (2.2%) which is in contrast to the commonest phenotype in African Blacks (22.9%). It can be seen from Table 6, that there is lot of variation in the common Rh genotypes in different world populations. Since blood donors and patient population is genetically homogenous in our country, it can be surmised that same types of Rh genotypes that are observed in blood donors, would be prevalent in patients also. It is clinically important to know the common genotypes as it helps in serological management of patients forming alloantibodies because of transfusions. For instance, R₁R₁ is the most

Table 5 – Percentage of Rh (D) blood donors in different population in the world.

Region	Rh (D) positive blood donors (%)	Rh (D) negative blood donors (%)
Southern France and Northern Spain ³	80–60	20–40
Europeans and Americans ³	83–85	15–17
India		
North India ¹⁵	93.7	6.3
South India ²	94.53	5.47
Lucknow (North India) ⁵	95.3	4.7
Present Study	92.25	7.74
China ³	99	1
Japanese, Burma, Malaysian, American Indian and Eskimos ³	99–100	0–1

Table 6 – Comparison of Rh phenotype frequencies in South Indian, North Indian, Whites and African blood donors.

S no	Phenotypes	Present study n = 10133 (%)	Thakral et al ⁶ n = 1240 (%)	Verma et al (%) ⁵	Whites (%) ^{8–10}	African Blacks (%) ^{8–10}
a)	DCe/DCe (R1R1)	3563 (35.2)	543 (43.8)	39.8	17.6	2.9
b)	DCe/dce (R1r)	3110 (30.7)	372 (30)	35.2	31.1	8.8
c)	DcE/DcE (R1R2)	823 (8.1)	102 (8.22)	11.4	11.8	3.7
d)	DcE/DcE (R2R2)	74 (0.7)	18 (1.45)	2.9	2	1.3
e)	Dce/dce (R ₀ r)	225 (2.2)	12 (0.97)	–	3	22.9
f)	DcE/dce (R2r)	600 (5.9)	111 (8.95)	5.4	10.4	5.7
g)	dce/dce (rr)	27 (0.3)	72 (5.81)	–	15	7
h)	dCe/dce (r'r)	252 (2.5)	7 (0.56)	–	1	1
i)	dce/dcE (rr'')	7 (Rare)	3 (0.24)	–	1	Rare

common genotype in Indian population followed by R1r, therefore, it can be assumed that the most common alloantibodies among Rh blood group system in patients receiving blood transfusion, would be anti-E and anti-c. This is in accordance with the studies on multitransfused patients at a center in north India in which the most common alloantibodies were anti-E and anti-c in thalassemics¹¹ and chronic renal failure patients.¹² In contrast to this, in another study on prevalence on alloimmunization in 531 multitransfused patients, it was found that anti-c was the most common antibody with specificity of 38.8% and all of them were present in D+ recipients.¹³ Table 7 shows comparison of Antigen frequency (AF) for other Rh antigens among RhD positive African blacks, whites and Indian¹⁴ blood donors. Among RhD positive blood donors, the AF of the e antigen is maximum (98.42%) followed by C (87.55%). This is in contrast to Rh D negative donors in whom e antigens (96.81%) had the maximum AF.

The knowledge of AF in the given population is clinically important as one can predict the common alloantibodies that could be formed in patients receiving transfusions and also helps in selection of antigen negative blood units for patients with presence of alloantibodies. For instance, AF of E antigen in our population is quite low compared to Whites and African Blacks, and therefore, it is not uncommon to find alloantibodies against E antigen in patients receiving transfusions. This is in agreement with the studies published from a center in north India conducted on multitransfused thalassemics¹¹ and patients on hemodialysis.¹² Another advantage of knowing AF is that it helps in selection of antigen negative units. For instance, in a north Indian patient with alloantibody

against C and requiring two units of blood, a minimum of 30–40 units of ABO matched blood will need to be tested for C antigen to find 2 units of antigen negative blood (since C negative donors form 15–20% of all donors in India).

Conclusion

The conventional tube technique for complete antigen phenotyping is time consuming and labor intensive. The gel technology is however simple, user friendly, less time consuming and with reproducible results. Moreover, there is less subjective bias and the reaction readings being stable for 24 h, can be preserved as digital images for future reference. Therefore this test is practical for antigen typing in population studies. Larger studies from various parts of India need to be carried out to see for regional differences in Rh antigen phenotype frequencies, keeping in view the ethnic heterogeneity in our population.

This study concludes that the prevalence of Rh (D) negative donors is sufficiently high to warrant maintaining an adequate inventory of Rh (D) negative units to support all D negative patients. About 10,000 blood donations occur per year at this blood bank with 7.74% of Rh (D) negative donations, implying that almost 700–800 Rh (D) negative blood bags are donated per year. This data will help the department in maintaining adequate stock and plan future studies on RhD negative subjects. The knowledge of antigen frequency of Rh antigens would be helpful in serological investigations and transfusion support to multitransfused patients with presence of alloantibodies. Among the Rh (D) positives, the AF of E antigen is around 26.55%. In Maharashtra, donor as well as patient population is quite homogenous; therefore, recipients of blood transfusion would be having the same AF for E antigens. It is therefore not uncommon to find alloanti-E antibodies in the thalassemics and patients on hemodialysis.

The study has helped us in establishing a database of donors for future multipurpose utilities for creating a donor data bank for preparation of indigenous cell panels, and providing antigen negative compatible blood to multitransfused patients with problems of alloimmunization.

The gene frequency of Rh antigens and their constitution is not clearly known in the Indian population. Therefore, further

Table 7 – Comparison of antigen frequency for other Rh antigens among Rh D positive African Blacks, Whites and Indians.

Antigen	Whites	African Blacks	India Thakral et al	India Verma et al	Present study
C	70	34	84.76	90	87.55
c	30	21	52.82	58.7	51.06
E	80	97	17.9	23.3	26.55
e	97	99	98.3	94.6	98.42

studies are required on this subject using large sample size to establish the genetic make-up of Rh system in the Indian population. Finally, we have performed only serological investigations, while molecular investigations are more informative. The present study would be further extended in future to include molecular investigations.

Intellectual contribution of authors

Study concept: Brig R.S. Sarkar.

Drafting and manuscript revision: Brig R.S. Sarkar, Col J Philip, Surg Cdr R.S. Mallhi, Dy Comdt Pramod Yadav.

Study supervision: Brig R.S. Sarkar, Col J Philip.

Conflicts of interest

All authors have none to declare.

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