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Original Research Article

Prevalence of Rh Antigens among voluntary blood donors in Chennai, Tamil Nadu, India

Chitra M.[1]*, Jagannathan S. Y.[2], Arumugam P.[2], Ravishankar J.[3]

[1]Department of Transfusion Medicine, Government Royapettah Hospital, Chennai, Tamil Nadu, India
[2]Department of Transfusion Medicine, The Tamil Nadu Dr. MGR Medical University, Chennai, Tamil Nadu, India
[3]Department of Transfusion Medicine, Government Villupuram Medical College, Villupuram, Tamil Nadu, India

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***Correspondence:**
Dr. Chitra M.,
E-mail: drchitra81@gmail.com

ABSTRACT

Background: The Rh blood group system is the most polymorphic of the human blood groups and is the most clinically significant in transfusion medicine next to ABO system. The aim of the study was to find out the prevalence of 5 major types of Rh antigens in voluntary blood donors, to determine the Rh composition of the population in Chennai and to generate a database of donors for all future activities.
Methods: This study was carried out over a period of 3 months from August 2015 to October 2015 on 100 healthy voluntary blood donors who attended the Department of Transfusion Medicine, The Tamil Nadu Dr. MGR Medical University, Guindy, Chennai Tamil Nadu, India. Determination of Rhesus antigens (Rh) was done by the Hemagglutination test using the conventional tube technique.
Results: Our study on prevalence of Rh antigens among voluntary blood donors showed D-91%, C-84%, E-25%, c-67%, e-98% and 'e' (98%) was the most common antigen, followed by D (91%).Regarding predicted Rh phenotypes, DCe/DCe (R_1R_1) 35% was the most common predicted phenotype and dce/dce (rr) 7% was the most common predicted phenotype among Rh negatives. DcE/DcE (R_2R_2) 2% and dCe/dce (r'r) 2% were the rare predicted phenotypes observed in our study.
Conclusions: This study helped us in establishing a database of donors for future preparation of indigenous cell panels and to provide antigen negative compatible blood to multi transfused patients with problems of alloimmunization.

Keywords: Alloimmunization, Hemagglutination test, Rh blood group, Rh phenotype

INTRODUCTION

The Rh blood group system is the most polymorphic of the human blood groups. The Rh blood group system currently comprises of more than 50 different antigenic specificities and is the most clinically significant intransfusion medicine next to ABO system.[1]

In 1986, Tippet proposed that Rh system is controlled by two closely linked loci RHD and RHCE.[2] These Rh genes are located on chromosome 1. RHD locus carries the gene which encodes the D antigen. RHCE locus carries the gene which encodes Cc and Ee antigens. Both genes have 10 exons and share about 97% sequence identity throughout all introns and exons. RhD and RhCE are non-glycosylated proteins containing 417 amino acids and they differ by 32 to 35 amino acids. Rh proteins cross the membrane 12 times, providing six extracellular loops, the potential sites for expression of Rh antigens. N- and C-termini are inside the cytosol.[3] The Rh antigens are expressed on the erythrocyte surface only if Rh Associated Glycoprotein (RhAG) is also present. RhAG

shares 39.2% and 38.5% amino acid sequence identity with RhCE and RhD proteins respectively. This amino acid sequence homology between Rh and RhAG proteins indicates an ancestral relationship and are collectively referred to as the Rh protein family.[4,5]

The Rh antigens are well developed before birth.The most clinically significant Rh antigens are: D, C, E, c, and e. Rh D antigen is the most immunogenic of all protein antigens followed by c. Anti-D is the most common antibody, which reacts optimally at 37°C and is best detected by indirect antiglobulin test or enzyme-treated red cells.[2] Rh antigens have clinical significance in the haemolytic disease of the newborn (HDFN), haemolytic transfusion reaction (HTR), autoimmune haemolytic anaemia (AIHA) and in forensic medicine

The common Rh antigens D, C or c, and E or e, were originally written in alphabetical order CDE but later the order was changed to DCE, when it was recognized that C and E antigens are inherited en bloc. The d antigen which was initially thought to be antithetical to D, does not exist, so the letter ''d'' is used to indicate the D-negative phenotype. Rh- positivity indicates that an individual's red blood cells possess 'D' antigen and Rh-negativity indicates that the red blood cells lack the 'D' antigen.

The most frequently occurring haplotypes of RHCE and RHD are Dce (R_0), dce(r), DCe(R_1), dCe(r'), DcE(R_2), dcE(r"), DCE(R_Z), and dCE(r_y). The uppercase ''R'' is used when the D antigen is expressed, lowercase ''r'' when it is not. This notation is used to communicate the Rh phenotype of a patient or donor. Rarely deletion phenotypes use dashes in the notation to indicate a lack of antithetical antigens; eg. Dc- (RBCs lack C, E and e antigens), and D-(RBCs lack C, c, E and e antigens). RBCs with the Rhnull phenotype do not express any of the Rh antigens.[1] It may of importance to know the frequency of different Rh antigens in the population which will help to provide antigen negative blood to recipients with alloantibody. It will also help in preparation of indigenous screening and identification cell panels.

Aim

The aim of the study was to find out the prevalence of 5 major types of Rh antigens in voluntary blood donors, to determine the Rh composition of the population in Chennai and to generate a database of donors for all future activities.

METHODS

This study was carried out over a period of 3 months from August 2015 to October 2015 on 100 healthy voluntary blood donors who attended the Department of Transfusion Medicine, The Tamil Nadu Dr. M.G.R Medical University, Guindy, Chennai, India. Determination of rhesus antigens (Rh) was done by the Hemagglutination test using the conventional tube technique. 1 or 2 segments were taken from blood bags by using tube sealers and stored at 2 to 4°C. Five clean tubes were arranged and marked D, C, E, c and e. One drop of corresponding anti-sera and one drop of 2 to 5% cell suspension were added, mixed and centrifuged at 1000 rpm for1 min and the presence of agglutination was noted. Weak D testing was done to confirm the D status by tube technique. Statistical analysis was done using Microsoft office Excel software.

RESULTS

A total of 100 blood donor samples were typed for the presence of Rh (D, C, E, c, e) antigens. There were 94 (94%) male donors and 6 (6%) female donors. The donors ranged from 18 to 52 years of age.

Table 1: Distribution of blood group.

Blood group	Number of donors	Percentage %
A	18	18
B	30	30
O	46	46
AB	6	6
Total	100	100

Blood group distribution among the 100 voluntary blood donors was (A-18%, B-30%, AB-6%, O-46%) as given in Table 1.

Table 2: Distribution of Rh Antigens.

Rh antigens	Number of donors
D	91
C	84
c	67
E	25
e	98

Table 3: Rh Phenotype.

Predicted phenotype	Predicted genotype	alternative genotype	Percentage (%)
DCe/DCe	R_1R_1	R_1r'	35
DCe/dce	R_1r	R_1R_0 R_0r'	30
DCe/DcE	R_1R_2	R_1r'' R_2r' R_Zr R_0R_Z	13
DcE/dce	R_2r	R_2R_0 R_0r''	8
DCe/DCE	R_1R_Z	R_Zr'	3
DcE/DcE	R_2R_2	R_2r''	2
dce/dce	rr	---	7
dCe/dce	r'r	---	2

In this study population, amongst the Rh antigens, e was the most common antigen (98%) followed by D (91%), C (84%), c (67%) and E (25%) as shown in the Table 2.

Rh D antigen was found positive in 91% and negative in 9% (Table 2). Since genotyping was not done in the samples, 8 predicted phenotypes were found to be present in our study population as shown in the Table 3.

The most common predicted phenotype was DCe/DCe (R_1R_1; 35%). DcE/DcE (R_2R_2), dCe/dce (r'r) were the rarely observed predicted phenotypes.

DISCUSSION

Antigens of the major blood group systems play a major role in outcomes of transfusion in recipients of blood and blood components. When clinically significant antibodies are identified in patient's serum, antigen negative donor units for such cases can be readily made available if donor database of various blood groups is available with a blood transfusion centre. In addition all blood banks need to have donor database for phenotype frequencies of major blood group systems in their local donor population.

Table 4: Distribution of Rh antigens (C, E, c and e) in blood donors of Chennai, India and its comparison with other published data.

	Present study			Thakral et al[6]	Whites[2,7,8]	Blacks[2,7,8]
Antigen	**Af* in D+ donors (91%) n=85**	**Af* in D- donors (9%) n=15**	**Total no. of donors n =100**			
C		84%		84.76%	68%	27%
E		25%		17.9%	29%	22%
c		67%		52.82%	80%	96%
e		98%		93.8%	98%	98%

*Af: Antigen frequency

Regarding the incidence of various blood groups in the blood donor populations, very few studies have been published from India. This study reports the phenotype frequencies of Rh blood group system antigens in 100 randomly selected voluntary blood donors from Chennai, Tamil Nadu, India. The results of our study are compared with White and Black populations and also with a study in North India. The incidence of D antigen differs in different ethnic population, being 85% in whites and 92% in blacks. In the present study, the D Antigen was found to be present in 91% of donors which is comparable with other studies from India. The frequency of 'C' antigen in our study was 84% which was high when compared to 68% (in whites) and 27% (in blacks), while this was comparable with the findings of study by Thakral B et al (84.76). The antigen 'c' was found to be positive in 67% of our samples which was less when compared to prevalence of 80% in whites and 96% in blacks.[6] However it was comparable with study by Thakral B et al.[6]

Table 5: Comparison of predicted Rh phenotypic frequencies in north Indian blood donors, Whites and Blacks.

Predicted Phenotype	Present study	Thakral et al[6]	Sarkar et al[9]	Whites[2,7,8]	Blacks[2,7,8]
DCe/DCe(R_1R_1)	35%	43.80%	35.2%	17.6%	2.9%
DCe/dce(R_1r)	30%	30%	30.7%	31.1%	8.8%
DCe/DcE(R_1R_2)	13%	8.22%	8.10%	11.8%	3.7%
dce/dce (rr)	7%	5.81%	0.3%	15%	7%
DcE/dce(R_2r)	8%	8.95%	5.9%	10.4%	5.7%
DCe/DCE(R_1R_Z)	3%	-	-	-	-
Dce/dce(R0r)	-	0.97%	2.2%	3%	22.9%
DcE/DcE(R_2R_2)	2%	1.45%	0.7%	-	-
dCe/dcE(r'r")	-	-	-	-	-
dce/dcE(rr")	-	0.24%	-	1%	rare
dCe/dCe (r'r')	-	-	-	-	-
dCe/dce (r'r)	2%	0.56%	2.5%	-	-

'C' antigen was associated with 90.1% of D positive donors and it was present in only 22.2% of D negative donors, suggesting that C antigen was more prevalent on D positive red cells. And the antigen 'c' was found in 100 % of D negative donors, while it was detected in only 63.7% of D positive donors.

The frequency of 'e' antigen was very high among the Indian blood donors also found in present study with 98% prevalence and was similar to the prevalence reported by Thakral B et al (98.3%).[6] This high frequency of 'e' antigens in Indian donors limits the availability of R_2R_2 donors for formulating in-house screening and panel cells. Since genotyping was not done, the comparison of predicted Rh phenotype frequencies in our population with previous studies from India, Whites and Blacks population was done (Table 5). On the basis of expression of different antigens of Rh system, most common predicted phenotype was found to be of DCe/DCe (R_1R_1) (35%) and similar findings by Thakral et al (43.8%) and Sarkar et al (35.2%).[6,9] However, the above phenotype frequency is found in only 17.6% of white and 2.9% of black population. In negative subjects, the most commonly seen predicted Rh phenotype in this study was ce (rr) which is similar to that reported in study by Thakral et al.[6] In contrast ce/ce (rr) was the least common phenotype reported by Sarkar et al Thakral et al showed ce/cE as the rarest phenotype.[6,9] DCe/dce (R_1r) 31.1% and Dce/dce (R_0r) 22.9% were the most common phenotype reported in whites and blacks respectively.

CONCLUSION

Our study on frequency of Rh antigens among voluntary blood donors showed 'e' (98%) was the most common antigen, followed by D (91%). Regarding predicted Rh phenotypes, DCe/DCe (R_1R_1) 35% was the most common predicted phenotype and dce/dce (rr) 7% was the most common predicted phenotype among Rh negatives. DcE/DcE (R_2R_2) 2% and dCe/dce (r'r) 2% were the rare predicted phenotypes observed in present study.

This study helped us in establishing a database of donors for future preparation of indigenous cell panels and to provide antigen negative compatible blood to multi transfused patients with problems of alloimmunization. The gene frequency of Rh antigens and their constitution is not clearly known in the Indian population. Therefore, further 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 will be conclusive.

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