Original Article

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Determination of C,c,E and e Antigens Prevalence in Rh D Negative Individuals: Is it Good Exercise with Utilities in Clinical Blood Transfusion Practices?

Transfusion Medicine Section

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ABSTRACT

Introduction: Besides D antigen, C,c,E and e antigens of Rh blood group system are the most important antigens involved in alloimmunisation. The prevalence of C,c,E and e antigens in Rh D negative individuals in local population has many utilities in transfusion medicine practice.

Aim: To determine the prevalence of C,c,E and e antigens in Rh D negative individuals.

Materials and Methods: This prospective observational study was performed at Bhanumati clinical laboratory, Navsari, Gujarat, India, from January 2020 to January 2022. A total of 270 Rh D negative samples irrespective of ABO blood group status were typed for C,c,E and e antigen using monoclonal reagents from two different manufacturers using conventional tube technique. The most likely phenotype and genotype were determined using reference textbooks. Possibility of weak D in these 270 samples was ruled out using polyspecific Antihuman Globulin Reagent performing test for weak D antigens. Results were analysed using Excel spread sheet.

Results: Prevalence of C,c,E, and e antigen in the 270 Rh D negative samples were, C (17, 6.29%), c (267, 98.88%), E (1, 0.37%) and e (270, 100%). Most probable genotypes were dce/dce(rr), dCe/dce(r'r), dCe/dCe(r'r') and dcE/dce(r"r).

Conclusion: Data obtained of C,c,E and e antigen prevalence in Rh D negative individuals is having varied utilities in blood transfusion services such as formulating rare blood group registry, preparing in house screening and panel red cells, and maintaining inventory of rare blood group units using freezing technology, if available.

Keywords: Alloimmunisation, Haplotypes, Red cell panel, Rh antigen

INTRODUCTION

The Rh blood group system is the second most important blood group system in terms of transfusion, as the Rh system antigens are very immunogenic [1]. With 56 antigens so far describe, Rh system is largest of all the blood group system [2]. The unusually large number of Rhesus antigens (56 antigens) is attributable to complex genetic basis.Two adjacent genes RhD (carrying the D antigen) and RhCE (carrying the C or c antigen and the E or e antigen) found at the RH locus (assigned to chromosome 1), are responsible for formation of Rh blood group antigens [3]. Out of the 56 antigens belonging to Rh system, the five most important Rh antigens D, C, c, E and e are the causes of most alloimmunisation following blood transfusion or pregnancy [4-7].

Most of the studies on red cell alloimmunisation done in India have found that antibodies to Rh blood group system antigens were the most common one to be detected ranging from 67-95% [8-15].

The knowledge of antigen frequency in the local 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 such alloantibodies. With the above primary objective, the present study was performed to determine antigen prevalence of C, c, E and e antigen in Rh D negative individuals of Navsari district and to create a data base for its further use as and when needed.

MATERIALS AND METHODS

This prospective observational study was performed at Bhanumati clinical laboratory, Navsari, Gujarat, India, from January 2020 to January 2022.

Sample collection: A 5 mL of blood was collected from 270 known Rh D negative blood group individuals of Navsari District, irrespective of ABO blood group status in K3 EDTA (tripotassium Ethylenediaminetetraacetic acid) (Labtech Disposable blood collection tube). Rh D negative status in these sample was first established by testing with two different IgM Monoclonal anti-D reagents (Tulip Diagnostics private Ltd. and J. Mitra and Company Private Ltd.) by conventional tube technique as per the guidelines by British Committee for Standards in Haematology for pretransfusion compatibility procedures in blood transfusion laboratories [16]. Test for weak D Antigen wasalso performed on all these 270 samples using polyspecific Antihuman Globulin Reagent (Tulip Diagnosis Private Ltd) to rule out possibilities of weak D by conventional tube technique.

Inclusion and Exclusion criteria: Sample showing clear cut Rh negative samples were included in the study. EDTA samples containing small fibrin clots and haemolysis were excluded from the study.

All these 270 sample were typed for the presence of C,c,E and e antigens using the Eryclone Monoclonal Rh/hr Typing reagents (Tulip Diagnostics private limited) by conventional tube technique. The results were reconfirmed using a second set of (monoclonal antisera Anti-C, Anti-c, Anti-E and Anti-e) Sera clone (Bio-Rad Laboratories).

Sample size: Most studies published from India determining the antigen frequency of C,c,E and e antigen in Rh D Negative individuals have a sample size of less than 100 [17-19]. However the current study has a larger sample size of 270 samples. Sample size of 270 samples in the current study provides a confidence level of 90% with a margin error of 5% [20].

The quality of these blood grouping reagents were verified as published in literature giving a 2+reaction in undiluted reagent [21,22]. Appropriate negative and weak-positive controls were used while performing the antigen typing for C,c,E and e antigen in these 270 sample. These controls were selected from Red cell panel (ID-Diapanel) for antibody detection procured from Bio-Rad laboratories.

STATISTICAL ANALYSIS

Results were analysed using excel spread sheet.

RESULTS

Prevalence of C,c,E, and e antigen in the 270 Rh D negative samples in current study were C (17/270,6.29%), c (267/270,98.88%), E (1/270,0.37%) and e (270/270,100%). The highest antigen prevalence was observed for e antigen (100%) and least was observed for E antigen (0.37%). The result obtained for C,c,E and e antigen prevalence are represented in [Table/Fig-1].

Sample	C	c	E	e
Number of positive samples	17	267	1	270
Percentage positivity	6.29%	98.88%	0.37%	100%

[Table/Fig-1]: Prevalence of C,c,E, and e antigens in 270 Rh negative individuals as follows.

Based on the reaction pattern observed with five antisera (anti-D, anti-C, anti-c, anti-E and anti-e), the most probable phenotypes described in current study were dce/dce(rr), dCe/dce(r'r), dCe/dCe(r'r') and dcE/dce(r"r) [3]. The frequency of these probable phenotypes are depicted in [Table/Fig-2].

Probable phenotype	Number of positive results (%)	Total
dce/dce(rr)	252 (93.3%)	N=270
dCe/dce(r'r)	14 (5.1%)	
dCe/dCe(r'r')	3 (1.1%)	
dcE/dce(r''r)	1 (0.3%)	

[Table/Fig-2]: Rh phenotype frequencies detected in 270 Rh D negative samples. The phenotypes were derived using guidance from reference [3]

DISCUSSION

Rh blood group system is one of the most important protein based blood group system. The two adjacent genes at the *RH* locus that are responsible for expression of Rh protein, *RHD* and *RHCE* are closely linked near the 3' end of chromosome 1p36.11 [21]. Rh D Negative persons inherit only a single *RHCE* gene from each parent. The Rh genes behave as autosomal co dominant allele. There are eight possible haplotype arrangements of Rh genes on the short arms of chromosome 1, Viz Dce, DCE, DCe, DcE, dce, dCE, dCe and dcE [Table/Fig-3] [23].

As with other blood group genes, the offsprings inherit a set of genes from each parent. In the case of Rh, this is in the form of an Rh haplotype from each parent [Table/Fig-4].

Haplotype combination		RHCE			
RHD		ce	CE	Ce	cE
	D	Dce	DCE	DCe	DcE
	d (no RHD gene)	dce	dCE	dCe	dcE

[Table/Fig-3]: Eight possible Rh haplotype combination.

Using the five readily available Rh antisera (anti-D, anti-C, anti-c,anti-E and anti-e), one is able to determine the Rh phenotype of the red cell being tested. Based on the phenotype and the gene frequencies for the population under study, one is able to estimate the most probable Rh genotype [3]. Determining probable genotypes is useful for parentage studies are well as for population studies. Probable genotype also may be useful in predicting the potential for Hemolytic disease of new born in offspring of Rh-negative women with an Rh antibody [Table/Fig-5].

Paternal haplotype	Maternal haplotype				
		dce	dCe	dcE	dCE
		r	r'	r"	r^y
dce	r	rr	rr'	rr"	r r^y
dCe	r'	rr'	r'r'	r'r"	r' r^y
dcE	r''	rr"	r'r"	r"r"	r" r^y
dCE	r^y	r r^y	r' r^y	r" r^y	r^yr^y

[Table/Fig-4]: Rh haplotypes combination possible in offsprings of Rh D negative individuals.

Reaction with anti					Rh Antigensh	Most likely phenotype	Most likely Genotyoe	Other possible phenotype
D	C	E	c	e				
0	0	0	+	+	c,e	r r	*RHce/RHce*	None
0	+	0	+	+	C,c,e	r'r	*RHCe/RHce*	None
0	0	+	+	+	c,E,e	r''r	*RHcE/RHce*	None
0	+	0	0	+	C,e	r'r'	*RHCe/RHCe*	None
0	0	+	+	0	c,E	r''r''	*RHcE/RHcE*	None
0	+	+	+	+	C,c,E,e	r'r''	*RHCe/RHcE*	r"r
0	+	+	0	0	C,E	r^yr^y	*RHCE/RHCE*	None
0	+	+	0	+	C,E,e	r^yr'	*RHCE/RHCe*	None
0	+	+	+	0	C,c,E	r^yr''	*RHCE/RHcE*	None

[Table/Fig-5]: Rh haplotypes combination possible in offsprings of Rh D negative individuals.

Out of the C,c,E and e antigens of Rh blood group system, typed in the current study, e and c antigen were found with a higher frequency of 100% and 98.9% respectively, which is comparable with similar studies published from India [17-19].

The C and E antigens had a lower frequency of with 6.3 and 0.3% in the present study. The frequency of C antigen in present study is comparable with two similar studies by Thakral B et al., and Lamba DS et al., [17,18]. However, in study by Makroo R et al., frequency of C antigen is reported as 33.7% [19]. The frequency of E antigen (0.3%) was the least in the present study between C,c,E and e antigens. Three studies used for comparison also had the E antigen as the least frequency in Rh D negative individuals [17-19]. However, the current study has a frequency of E antigen only 0.3% which is in contrast with reported frequencies of 1.8 to 4.3% reported in similar studies [17-19]. This could be due to some ethnic geographic variation as current study was performed in Western India. while all the other three studies used for comparisons were performed in North India. This discrepancy needs to be further evaluated by larger series from Western India determining frequency of E antigen in Rh D individuals [Table/Fig-6].

Author name	Study place	C	c	E	e
Thakral B et al., [17]	Chandigarh, India, 2010	8.5%	100%	3.6%	100%
Lamba D et al., [18]	Chandigarh, India, 2013	10%	98.6%	4.3%	100%
Makroo R et al., [19]	New Delhi, India, 2014	33.7%	99.2%	1.8%	99.8%
Present Study	Navsari, India, 2022	6.29%	98.88%	0.37%	100%

[Table/Fig-6]: Comparison of antigen frequency of C,c,E and e antigen in Rh D Negative individuals from current study with similar studies published in India.

The phenotypes found in the current study were rr, r'r,r'r', and r"r with highest incidence of rr (93.3%) and least incidence of r"r (0.3%). The other Rh phenotypes in Rh D individuals like r"r", r'r", r^yr^y, r^yr' and r^yr" were not found in the current study and they are also listed as rare in standard text books [3,21,24]. A rare blood is the one that on the series of the blood group characteristics,

is found in a frequency of 1:1000 random samples in a given population [25,26]. The phenotypes observed in Rh D individuals in our study were comparable with similar studies from Northern India with only exception of prevalence of C antigen in 33.7% in a study by Makroo R et al., [19]. The results of such antigen typed individuals may be useful in determining Antigen frequency in local population and preparing data base of C, c, E, e antigens in RhD negative individuals.

Use of such database is as follows:

1. **Providing Phenotype matched Antigen Negative blood Unit:** The relative difficulties in providing compatible blood products are determined by the frequency of the antigen in the population and by the clinical significance of the antibody. Knowing the frequency of the antigen(s) in the population is helpful when determining the number of units that should be antigen-typed to find a sufficient number to fulfil the crossmatch request. To determine the number of units to test, divide the number of units requested by the frequency of antigen negative individuals in the population [8,11,10,24,27].
2. **Preparation of Selected cell Panel in complex/multiple Antibody identification cases:** Selected cell preparation from the known database of such phenotyped Rh D negative red cells can be chosen for the specific antigen they carry or lack, to confirm or rule out the presence of antibodies [3,21,24].
3. **To assess the quality of commercial antisera anti-C, anti-c, anti-E and anti-e:** Well characterised cells from the database, may be used to check the quality of commercial antisera anti-C, anti-c, anti-E and anti-e, for their sensitivity and specificity. Cell having single dose of specific antigen (heterozygous) are preferred for quality check of the antisera [21,22].
4. **Use of rr cells for differential adsorption:** Differential adsorption can be performed when a patient's phenotype is unknown and performing antigen-typing is not an option (i.e., positive DAT or transfused within the last 3 months). For this method, group O cells with the following phenotypes are used: R1R1, R2R2, and rr [3,24,28].
5. **Preparing in house Screening and Panel cells respectively for antibody detection and identification:** Such specifically phenotyped red cells can be used in preparing in house screening and panel cells respectively for detection and identification of antibodies against red cell antigens [29,30].
6. **Freezing Rare phenotypedred cells and Autologous red cells for future use:** Rare blood group phenotyped red cells and autologous red cells can be stored in frozen state for use in future, if facilities are available at the blood centres [3,21,24].

Limitation(s)

The C, c, E and e antigens were determined by serological methods in the current study and none of the results was confirmed by molecular typing. Molecular typing would confirm the genotype for the blood group antigens.

CONCLUSION(S)

The current study provided institute a good database of prevalence of C, c, E and e antigens in Rh D negative individuals, such database has multiple utilities in immunohematology laboratory and clinical transfusion practices like, ease of providing blood unit with rare antigen phenotype, selective red cell panel preparation for use in complex antibody identification cases, assessing the quality control of commercial antisera anti-C, anti-c, anti-E, and anti-e, use of rr cells for differential adsorption, use of such phenotyped cells in preparing in house screening and panel cells for antibody detection and identification, freezing red cells with rare blood group antigens if facilities are available for future use. Multicentre studies in determining frequency of C, c, E and e antigens in Rh D negative individuals will create a large database throughout different geographic regions and at national level and can be made available to Transfusion Medicine specialists for further use.

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