Original Article

The Pattern of Rh Phenotype among Voluntary Blood Donors – A Single-Center Observation from North India

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Abstract

Background: Human blood is precious and its availability and requirement are important elements of a comprehensive health-care delivery. Healthy voluntary donors are key sources to maintain the supply of human blood, and due care is warranted to ensure that the right blood reaches the right patient. Blood donor screening is standard practice, and the knowledge of ABO and Rh phenotype study is important. Prevalence and pattern of Rh phenotypes in a particular area may be important for various functioning of any blood bank. **Materials and Method:** Various centers frequently showcase prevalence pattern of Rh phenotype at a specified centre or region. A similar study was undertaken from collected data of voluntary blood donors in our tertiary care center. **Results:** Out of 2000 donor samples, B, A, O, and AB were in the decreasing order of frequency with 33.3%, 29.6%, 25.7%, and 11.4%, respectively. The most common Rh antigens observed were e (97.1%), followed by D (93.8%), C (88.45%), c (51.05%), and E (18.45%). Out of eight phenotypes in D-positive donors, R1R1 (DCCee), R1r (DCcee), and R1R2 (DCcEe) were most frequently observed. In D-negative donors, rr (ddccee), r'r (ddCcee), and r'r' (CCddee) were the most common. **Conclusion:** The Rh phenotype pattern in our centre had diverse pattern and knowledge of this would guide further studies.

Keywords: Blood group systems, phenotype frequency, red cell antigens

Introduction

Blood banking is an integral part of the health-care system, and voluntary donations are the sole source for blood or its various components. Human blood, therefore, is a precious commodity and henceforth voluntary donations are frequently required and encouraged. ABO blood group system is the principal blood group system which occasionally is complemented by various other uncommon blood group systems.[1] While A, B, O, and Rh D antigens are commonly tested, uncommon blood group systems and Rh phenotypes are usually not tested routinely in blood banks. Rh phenotypes are other determinants of the blood group system, and the Rh blood group system is considered one of the most polymorphic and immunogenic systems with common Rh antigens being D, C or c, and E or e.[2] Rh phenotype knowledge, thus, is important as transfusion of ABO-compatible but unknown phenotype blood may produce alloimmunization and extended screening is of paramount importance to reduce posttransfusion complications.[3] Understanding of Rh phenotypes in the blood is important to determine their distribution in any particular region for creating a database for that geographical region and also for categorization of blood or blood products within a specific blood bank.

Materials and Methods

This is a cross-sectional study done in the blood bank of a tertiary care hospital in Uttarakhand from 2018 to 2019, comprising a record of 2000 voluntary donors from the records. ABO and Rh blood grouping were done in all cases with Matrix Gel Card ABO/Rh (D) forward grouping confirmation card (Tulip diagnostics Ltd., India). All Rh-negative samples were subjected to the Du test for weak D testing. Detection of other Rh antigens was done by Anti-C, Anti-c, Anti-E, and

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Access this article online

Quick Response Code:

Website: https://journals.lww.com/mtsm

DOI: 10.4103/mtsm.mtsm_3_23

How to cite this article: Pangtey T, Satyavali V, Jangpangi D. The pattern of Rh phenotype among voluntary blood donors – A single-center observation from North India. Matrix Sci Med 2023;7:62-5.

Received: 11-01-2023, **Accepted:** 26-01-2023, **Published:** 25-08-2023

Anti-e (ERYCLONE-monoclonal Rh/hr typing antibodies, Tulip Diagnostics [P] Ltd., India) by tube method, and Matrix Rh phenotype gel card (Tulip Diagnostics [P] Ltd, India) with Anti-D as per the instructions by the specific manufacturer.

Rh phenotyping method

The Rh phenotype was determined by testing the patient red blood cells (RBCs) with the five standard antisera: Anti-D, Anti-C, Anti-c, Anti-E, and Anti-e. If the antigen was found to be present in the red blood cells, agglutination was observed and if the antigen was absent, no agglutination was seen.

Tube method for Rh phenotyping

ERYCLONE monoclonal monospecific antisera of Anti-C, Anti-c, Anti-E, and Anti-e was used and used as per the standard method. A 5% suspension of red cells to be tested was prepared. Four test tubes with donor bag numbers were labeled. One drop of ERYCLONE Anti-C, Anti-c, Anti-E, and Anti-e were added, respectively, in each test tube and then one drop of 5% RBC suspension of the donor bag was added in all the test tubes. Test tubes were incubated at 37°C for 5 min and centrifuged for 1 min at 1000 rpm (revolution per min) or 20 s at 3400 rpm. The cell button was gently resuspended and looked for the agglutination and results were recorded for all test tubes. Negative reactions were re-centrifuged after 5 min and results are reread so that to not overlook any weak antigen.

Gel card method for Rh phenotyping

Matrix Rh phenotype gel cards with Anti-D were labeled with donor names or identification numbers. Ten microliters of 5% donor red cell suspension was added to all the microtubes. The gel cards were centrifuged for 10 min in the gel card centrifuge. The results were then recorded for all samples.

Results

A total of 2000 donors were typed during the study population, 1876 donors were Rh positive and 124 donors were Rh negative. Donor distribution noted in the study was as the following – blood group A, B, O, and AB constituted 514 (25.7%), 666 (33.3%), 592 (29.6%), and 228 (11.4%) of the total donor pool.

The most common Rh antigen observed in the study population was e (97.1%), followed by D (93.8%), C (88.45%), c (51.05%), and E (18.45%) [Table 1]. A total of eight Rh phenotypes were observed among the D-positive donors. The R1R1 (DCCee) phenotype was the most frequent at 47.95%, followed by the R1r (DCcee) at 25.9% and R1R2 (DCcEe) at 12.2% [Figures 1 and 2]. A rare phenotype R1Rz (DCCEe) was also documented in the current study in 10 donors, with a frequency of 0.5%. The most common Rh phenotype in D-negative donors observed was rr (ddccee) which was 4.6%, followed by r'r (ddCcee) which was 0.95%, and r'r' (CCddee) which was 0.5% [Table 2].

Discussion

Rh group system was discovered by Karl Landsteiner and A. S. Weiner in 1940–1941.[4,5] Blood group phenotype studies

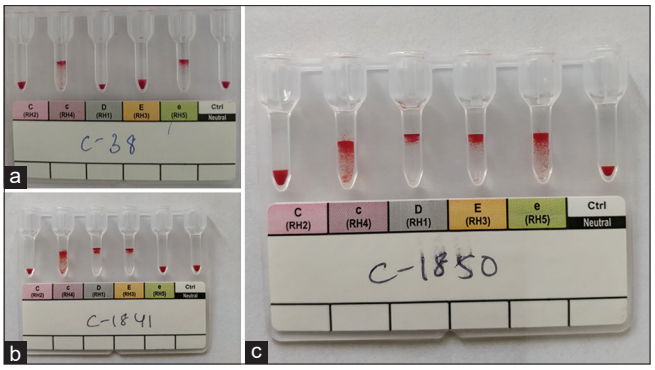

Figure 1: The image (a) shows the card test positive for the phenotype - ce (3 + agglutination observed in c and e antigen). Phenotype - DcE (3 + agglutination observed in c, 4 + agglutination observed in D and E) in image (b). Image (c) showing the phenotype - DcEe (3 + agglutination observed in c and e, 4 + agglutination observed in D and E)

Table 1: The distribution of various Rh phenotypes in the samples

Total	D	C	E	C	E
2000	1876	1769	369	1021	1942
Percentage	93.8	88.45	18.45	51.05	97.1

Table 2: Rh phenotype in current study

Weiner	Fisher-race	Number of cases, *n* (%)
R1R1	DCCee	959 (47.95)
R1r	DCcee	518 (25.9)
R1R2	DCcEe	244 (12.2)
R2r	DccEe	54 (2.7)
R0r	Dccee	33 (1.65)
R2R2	DccEE	50 (2.5)
R1Rz	DCCEe	10 (0.5)
R2Rz	DCcEE	8 (0.4)
RzRz	CCDEE	0
rr	ddccee	92 (4.6)
r'r	ddCcee	19 (0.95)
r'r'	ddCCee	10 (0.5)
r''r	ddccEe	2 (0.1)
r'r''	ddCcEe	1 (0.05)
r''r''	ddccEE	0
r''ry	ddCcEE	0

are commonly reported from various geographic locations and underline its complex pattern and prevalence. Major blood groups that are studied frequently are Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran among others.[6] Fifty Rh antigens have been discovered so far, but only five main Rh antigens are D, C, E, c, and e. Rh D is the most important since it is the most immunogenic and causes severe transfusion-related reactions. The Rh antigen poses a danger if it is transfused to Rh-negative individuals. After the first transfusion, there are no immune reactions but there is the formation of anti-Rh antibodies. On subsequent transfusion, it attacks the RBCs of the host causing

Table 3: Rh phenotype patterns among D+ donors of the study compared with a few other previous national studies

Phenotype	Present study (*n*=2000)	Rageswari subramaniyan *et al*.[8] (*n*=359)	Agarwal *et al*.[13] (*n*=368)	Nanu and Thapliyal *et al*.[14] (*n*=265)
R1R1	47.95	49.58	42.93	42.64
R1r	5.9	30.64	35.6	35.09
R1R2	12.2	10.59	13.04	12.08
R2r	2.7	4.18	5.98	5.66
R0r	1.65	2.5	0.54	2.64
R2R2	2.5	2.23	1.63	1.89
R1Rz	0.5	0.28	0	0
R2Rz	0.4	0	0	0
RzRz	0	0	0	0

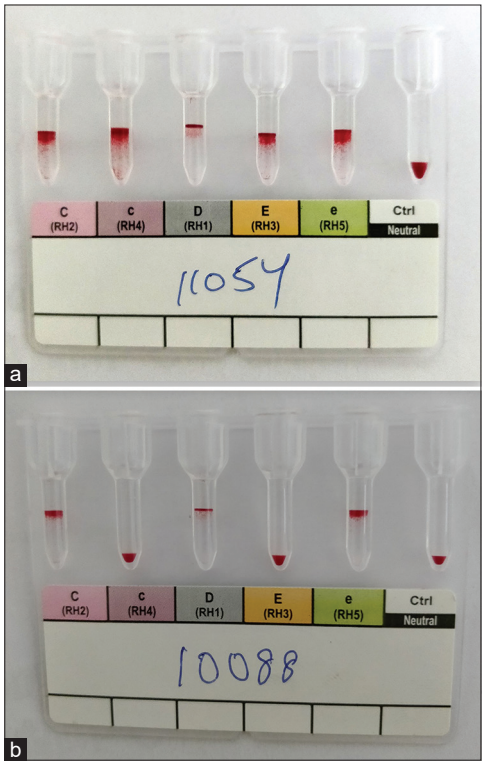

Figure 2: Image (a) shows phenotype - DCcEe (3 + agglutination observed in C, c, E, e and 4 + agglutination observed in D) and (b) shows phenotype - DCe (4 + agglutination observed in D, C, e)

it to agglutinate and forms clumps leading to severe hemolysis or even death.[7] A similar hazard occurs when Rh-negative mother gives birth to Rh-positive child. The first child does not manifest any symptoms but subsequent pregnancies can lead to severe hemolytic disease of the newborn which can be fatal to the child.[8] Rh phenotype patterns are important data for any health-care center so that basic prevalence and pattern are known for that region.

India

Very few studies of their pattern are available from India. One study involving 1000 blood donors regarding the prevalence of D, C, E, c, and e antigens from Andhra Pradesh, e was found to be the most common antigen (94.1%), followed by D, C, C, and E and DCe/DCe being most common phenotype.[9] The study from 1528 blood donors from eastern India the common antigens found were ABO, Rhesus, and Kell systems. The D antigen (RH1) was the most common and RH haplotype frequencies were noted in decreased order of frequency as CDe, cde, and CdE whereas the K antigen was found in 12 donors.[10] Another Indian study describes the prevalence of D, C, c, E, e, K, k Fy (a), Fy (b), Jk (a), Jk (b), M, N, S and s antigens in 3000 voluntary and replacement donors in New Delhi and adjoining area.[11] The frequencies of common antigens were found to be more similar to Caucasian than Chinese population studies. The evaluation of frequencies of Rh and Kell phenotype from 5670 donors was also reported from Gujarat, India. Antigen e (99.07%), D (95.40%), and c (88.77%) were common, followed by c and E.[12] The presence of antigen D was found to be higher than in Caucasians and lower than in Chinese. Nanu and Thapaliyal recorded the R1R1 being more than 42%, very similar to Agarwal *et al*.[13,14] Subramanian, only showed a higher percentage of more than 49%.[15] The salient comparative pattern in D + donors to these previous studies from the country is described in a table form [Table 3].

Conclusion

The importance of phenotype knowledge has been highlighted well as part of the safe transfusion practices, population genetic studies, or medicolegal issues. The Rh phenotype pattern in a hospital, serves background for blood management, references and future studies.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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