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Original Article

A Two-Year Study of The Frequency of Rhesus Phenotype and Probable Genotype in a Tertiary Care Hospital at Dhaka, Bangladesh

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Abstract:

Background: *The Rhesus system is a major blood group system like the ABO system. Despite proper blood grouping and cross-matching, recipients can develop Rhesus antibodies, leading to Hemolytic Disease of the Newborn (HDN) or Delayed Hemolytic Transfusion Reactions (DHTR). Rhesus-negative individuals are particularly at risk if exposed to Rhesus-positive red cells through transfusion or pregnancy. Identifying Rh phenotypes and potential genotypes can significantly help in preventing alloimmunization and adverse reactions in patients receiving multiple transfusions.*

Objective: *To assess the frequency of Rh antigens phenotype and most probable genotype in the Bangladeshi population.*

Method: *This cross-sectional study was conducted with 325 randomized samples from both patients and donors at the Transfusion Medicine Department of BIRDEM General Hospital from January 2020 to December 2021. Samples were tested for Rh antigens phenotype and most probable genotype by using the tube agglutination method. The Anti-Human Globulin Test (AHGT) was performed as needed or in specific situations.*

Results: *Out of the 325 blood samples, 167 (51.2%) were from males and 158 (48.8%) were from females. Incidence of Rh D positive were 94.12% (306 samples) and 5.88% (19 samples) belong to Rh D negative. The most common genotypes among Rh D positive samples were DCe/DCe (R1R1)- 41.84%, DCe/dce (R1r) -28.61%, DCe/DcE (R1R2)- 19.07%, and dce/dce (rr)- 3.38%. Rare genotypes of Rh D positive individuals detected in our study included DcE/dce (R2r)-1.84%, DCe/DCE (R1Rz) -1.23%, Dce/dce (R0r)- 0.92%, and DcE/DcE (R2R2) - 0.61%. Among Rh D negative samples, the detected rare genotypes were dCe/dce (r'r) - 1.89% and dcE/dce (r''r) - 0.61%.*

Conclusion: *There is significant racial and geographical variation in the frequency of Rh phenotypes and genotypes. The Rh blood group system is essential for developing strategies to prevent Rh sensitization in clinical transfusion practices. Additionally, it can be valuable for addressing medico-legal issues and conducting genetic studies in Bangladesh.*

Key Words: *Antigens, Genotypes, Phenotypes, Rhesus.*

Introduction

Determining the distribution of ABO and Rh blood group systems is crucial for effectively managing patients who require regular blood transfusions. Ensuring the compatibility and improving the transfusion practice is ensured by meticulous determination of blood group distribution.[1] There are currently around 32 recognized blood group systems and more than 600 unique blood group antigens. These blood group antigens typically result from a single allele or closely associated genes, which together make up a blood group system.[2] The Rh blood group system consists of multiple immunogenic antigens located on various forms of RhD and RhCE proteins. The genes responsible for encoding these Rh proteins are located on the short arm of chromosome 1.[3,4,5] The Rh system comprises 50 antigens, which are encoded by genes on chromosome 1. However, the most important ones for blood transfusions are D, C, c, E, and e. In standard blood typing, only the Rh D antigen is assessed, and a person's Rh status is classified as either Rh D positive or Rh D negative. The Rh D antigen can differ in both amount and structure, resulting in various forms such as weak D, partial D, and mosaic D.[6,7] The Rh D antibody is produced in a patient following exposure to D-positive red blood cells, either through stimulation or transfusion.[8] Fisher and Race[9] proposed that the Rh system antigens are produced by three closely linked sets of allele genes: D/d, C/c, and E/e, each responsible for producing the D, C, c, E, and e antigens on red blood cells. The'd' gene is considered an amorphous gene, representing the absence of the D antigen. There are eight possible Rh gene haplotypes (Dce, DCe, DcE, DCE, dec, dCe, dcE, and dCE), resulting in 36 possible genotypes. Weiner's [7]systems, though more complex and less widely used, involves a single locus with eight allele genes: Rh, Rh1, Rh2, Rhz, rh, rh1, rh2, and rhy, and labels five major antigens as D-Rh0, C-rh′, E-rh′′, c-hr′, and e-hr′′. Rhesus genotype consists of any two of the possible chromosome arrangements.Fisher-Race and Weiner nomenclature is often used interchangeably. An individual's true genetic makeup is termed the genotype, while the observable traits resulting from these genes are known as the phenotype. For safe blood transfusions, ABO and Rh D blood grouping, along with cross-matching using the Anti-Human

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Globulin (AHG) technique, are essential.[10] Even with proper blood grouping and cross-matching, recipients may still develop alloimmunization and produce antibodies against Rh or minor blood group antigens such as Kell, MNSs, and Duffy. Some countries require extensive phenotyping and complete cross-matching for patients likely to need multiple transfusions. Due to the high expense of comprehensive phenotyping, concentrating on Rh phenotyping alone can be highly effective in preventing alloimmunization and minimizing complications in patients receiving multiple transfusions.[11] The present study aimed to assess the frequency of Rh antigen phenotypes and the most probable genotypes in the Bangladeshi population.

Materials and methods:

This retrospective cross-sectional observational study was conducted with 325 randomized samples from both patients and donors at the Transfusion Medicine Department of BIRDEM General Hospital at Dhaka, over a period of two years, from January 2020 to December 2021. Samples from both patients and donors were kept in 5 cm test tubes, stored at 2 to 4°C, and tested as fresh as possible in EDTA solution. Rhesus phenotyping and probable genotyping were performed using an antigen-antibody agglutination test by the test tube method. According to the manufacturer's instructions, five clean test tubes were arranged and marked C, D, E, c, and e. One drop of the corresponding monoclonal antisera (Tulip Diagnostics Pvt. Ltd.) and one drop of a 2- 5% cell suspension were added, mixed, and centrifuged at 1000 rpm for one minute. The reaction was interpreted as agglutination positive or negative. Weak or negative reactions were confirmed microscopically. Results were recorded, and phenotypes, probable genotypes, and their frequencies were determined as percentages.

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Operational Definition:

Phenotype: The outward expression of genes (e.g., a blood type). On blood cells, serologically demonstrable antigens constitute the phenotype. Rh blood group phenotypes are based on whether or not Rh surface antigens are present on red blood cells. When The D antigen is found on the red blood cell it is called Rh D positive and if the D antigen is absent from the red blood cell is called Rh D negative. **Genotype:** It is the genetic expression of a blood group antigen. The Rhesus genotype consists of two alleles for the Rhesus factor, a protein present on red blood cells that is inherited. These alleles are dominant (R) and recessive (r). An individual's genotype can either be Rh+ (RR or Rr) or Rh− (rr).

Blood group antigens: These antigens are inherited substances found on the surface of red blood cells, which can be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the system. These antigens typically result from a single allele or closely associated genes, which together make up a blood group system

Results:

Out of the 325 blood samples, 167 (51.2%) were from males and 158 (48.8%) were from females, as shown in Figure 1. Incidence of Rh D positive were 94.12% (306 samples) and 5.88% (19 samples) belong to Rh D negative as shown in Figure 2. Ten types of phenotypes were observed the our studied sample,the most common being DCCee (41.84%) . Both DcE/DcE (R2R2) and dcE/dce (r"r) phenotypes were observed at a frequency of 0.61%, with the rare phenotype appearing in Rh D-positive and Rh D-negative individuals, respectively.

In our study the most common probable genotype was DCe/DCe (R1R1) 41.84% followed byDCe/dce (R1r) 28.61%and Ce/DcE (R1R2)19.07%. The most common genotype was dce/dce (rr) 3.38 %belong to RhD negative group. Next probable genotypes in order of descending frequency in Rh D positive individuals were DcE/dce (R2r)1.84%, DCe/DCE (R1Rz)1.23%, Dce/dce (R0r)- 0.92%, and DcE/DcE (R2R2) 0.61%. While in Rh D negative samples it was Cde/cde (r'r) at 1.89% and cdE/cde (r"r) at 0.61%, as shown in Table I.

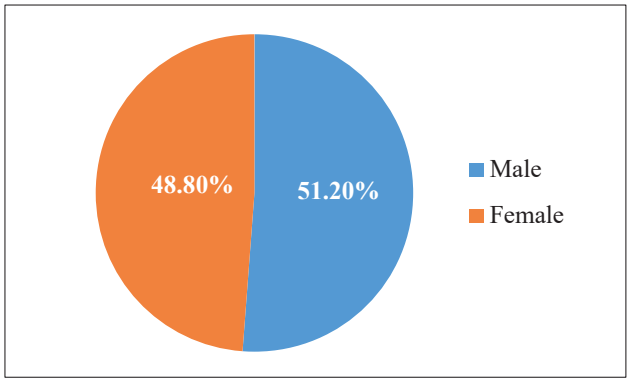

Figure 1: Gender wise distribution of the studied sample (N=325)

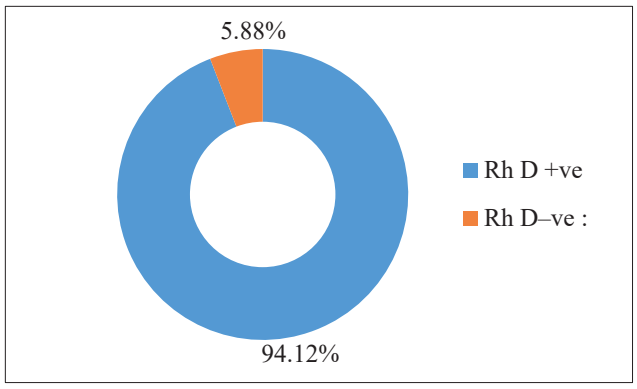

Figure 2: Distribution of Rh D status in the study population (N=325)

Table I: The frequency and percentage of Rhesus genotype in present study (N=325)

Fisher and Race	Wiener	Frequency	Percentage (%)
DCe/DCe	R1R1	136	41.84
DCe/dce	R1 r	93	28.61
DCe/DcE	R1R2	62	19.07
DcE/dce	R2r	6	1.84
DCe/DCE	R1RZ	4	1.23
Dce/dce	R0r	3	0.92
DcE/DcE	R2R2	2	0.61
Dce/dce	rr	11	3.38
dCe/dce	r'r	6	1.89
dcE/dce	r"r	2	0.61

Table II: Percentage of Rh D in different population in the world

Country	Rh D positive in percentage (%)	Rh D negative in percentage (%)
Southern France & Northern Spain [13]	80-60	20-40
Europeans and Americans[13]	83-85	15-17
India[11,13,14,15]	92.25- 95.3	4.7-7.74
West Africa[15]	97	3
China[13]	99	1
Japan[15]	99.7	0.3

Discussion

In our study, male and female ratio was 51.2:48.8. A study conducted by Shil Net al[10] from Bangladesh reported almost similar distribution of male to female ratio which was 50.70: 49.30. Other studies conducted by Khattak et al[12] from Pakistan and Sharma et al[4] from Central India observed male-to-female ratios of 74.86:25.14 and 91.6:8.4, respectively. These ratios do not match the findings of the present study, which may vary from country to country. In our study, the incidence of Rh D positive was 94.12%. The incidence of Rh D antigen in different population in the world ranges from 60% to 99% are shown in Table II[11,13,14,15] and geographically higher incidence of Rh D positive was reported in Japanese population while it was lower in the Basque people of Southern France and Northern Spain. In our study, the incidence of Rh D-negative individuals was 5.88%. Shil et al[10] also reported a similar distribution of the Rhesus phenotype in Bangladesh. Lower frequencies of Rh D-negative individuals were found in studies by Haque et al[16] (3.2%), Das et al[14] (3.25%), Pramanik et al[17] (3.3%), Loua et al[18](4.1%), and Mwangni et al[19] (4.8%). The lowest frequency of Rh D-negative individuals was observed in the study by Rahman et al[20](1.8%).In contrast, higher percentages of Rh D-negative individuals were reported by Enosolease et al[1] (6.01%), Kumar et al[21](6.3%), Thakral et al[22] (6.6%), Bashwari et al[23] (7%), and Sarkar et al[11](7.74%). The frequency and percentage of Rh (D) status in any country population is so much crucial for developing policies related to pretransfusion 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.[24] Comparing our results with studies from different parts of the world, it can be observed that there is tremendous variation in the prevalence of Rh (D) antigens in blood donors worldwide which may be due to ethnic factors. Out of 306 Rh (D) positive sample in the present study the most common phenotype was found to be DCCee at 41.84% which is similar to the study reported by Nanu A et al [25] (42.64%) and Thakral et al[22] (43.8%), Sarkar et al[11] (35%), Haque et al[16] (37%), Shil et al[10] (38.6%) and Verma et al[26] (39.8 %). The least common Rhesus D positive phenotype was DccEE (0.61%), consistent with Sarker et al[11] (0.7%), Thakral et al[22] (1.45%), Shil et al[10](1.5%) and verma et al[26] (2.9%). In comparison, its frequency is 1.3% in African Blacks and 2% in White populations.[27, 28, 29] In our study most common probable genotype was DCe/DCe (R1R1) 41.84% followed by DCe/dce (R1r) 28.61%, DCe/DcE (R1R2) 19.07%,dce/dce (rr) 3.38%, dCe/dce (r'r) 1.89% , DcE/dce (R2r)1.84%, DCe/DCE (R1Rz)1.23% and Dce/dce (R0r) 0.92%. The least common genotypes were DcE/DcE (R2R2) and dcE/dce (r"r), each at 0.61% in Rh D positive and Rh D negative individuals, respectively. Hassan FM et al[30] reported the frequency of Rh genotypes in Saudi Arabia in decreasing order as follows: R1R2, R1r'', R2r', R0R0, R0r, RzR1, R1r'', R2r'', R1r, R1R0, R0r', R1R1, R1r', R0, R0r, and r'r'. Rahman M et al[20] from Bangladesh reported that the most prominent Rhesus genotype was CDe/cDE (R1R2), occurring in 39.75% of individuals, while the Rhesus genotype cde/cde (rr) was found in only 1.75% of cases. The most common genotype reported in whites was DCe/dce (R1r), accounting for 39.4%; in blacks, it was Dce/dce (R0r), at 45.8%; and in Asians, it was DCe/ DCe (R1R1), comprising 51.8%.[31] It is clinically important to know the common genotypes as it helps in

serological management of patients with presence of allo-antibodies. Detection of common allo-antibodies will be helpful for selection of antigen negative blood units in patients receiving blood transfusion. Knowledge of the distribution of Rhesus phenotypes and probable genotypes in population also helps in formulating policies regarding pre transfusion testing.

Limitation:

This retrospective cross-sectional observational study had a small sample size and was conducted at a single center, so the findings may not fully represent the broader population of the country. Furthermore, the sample could be analyzed using reagents from different manufacturers.

Recommendation:

A study involving a larger sample size and multiple centers could be conducted to obtain more comprehensive results for the current research.

Conclusion:

It is concluded through our study that there is significant racial and geographical variation in the frequency of Rh phenotypes and genotypes. Rhesus antigenic phenotyping along with probable genotyping would be helpful in serological investigations and transfusion support to multi-transfused patients. The Rhesus blood group system is crucial for developing strategies to prevent Rh sensitization in clinical transfusion practices. Additionally, it can be valuable for addressing medico-legal issues and conducting genetic studies in Bangladesh.

Ethical measures: The study protocol was approved by the BADAS Ethical Review Committee. Informed written consent was obtained from both patients and donors. Confidentiality was strictly maintained during data collection, storage, and analysis.

Conflict of interest: The authors have no conflicts of interest to declare. No part of the study has been published or is being considered for publication in any journal. We did not receive any funding for this study.

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