

Study of Rhesus Genotype and Phenotype in Bangladeshi Population Attended in a Tertiary Care Hospital Transfusion Medicine

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ABSTRACT

Background: The Rhesus Blood Group is one of the most important Blood Group like ABO. Exposure of Rhesus Negative individuals to Rhesus Positive Red Cells by Transfusion and/or Pregnancy is most likely to stimulate the production of Rhesus antibodies. These Antibodies may cause Haemolytic Diseases of Newborn (HDN) and Delayed Haemolytic Transfusion Reaction (DHTR.) Rh-antigens, mainly D, C, E, e and small c are well developed before birth. d is not present or used hypothetically. The frequency of these antigens can be determined by using five different antisera; anti-D, -C, small c, -E and small e. Based on these Phenotype and Gene frequencies in a particular Population, the most probable Rh Genotype can be determined.

Methodology: This cross sectional comparative study was carried out with 1,000 randomized samples of both patient and donors in the department of Transfusion Medicine of Bangabandhu Sheikh Mujib Medical University (BSMMU). The eventual objective of the study was to determine the actual Rhesus phenotype-genotype frequency of Bangladeshi Native population. Sample was collected from both donor and patient. For each Test Saline and Anti Human Globulin Test (AHGT) were employed. Result: The most common genotype, we found, CDe/CDe i.e. R1R1 (38.60%) which is the most vulnerable genotype to be immunized, the next group in our study was CDe/cde R1r (29.20%) and next category was CDe/Cde i.e. R1R2 (22.20%). Very rare genotype in our study detected was cDE/cde i.e. R2R (1.90%), cDE/cDE i.e. R2R2 (1.50%) CDE/cDE i.e. R1Rz (0.60%), cde/cde i.e. R0R0 (0.60%). Among Rh (D) Negative samples genotypes found is cde/cde i.e. rr (4.50%), and cde/cde i.e. r'r (0.8%) and Cde/cdE i.e. rr" (0.10%). Frequency distribution of genotype among male and female was also determined.

Conclusion: The findings may be used in formulating Clinical Transfusion Practice and to formulate strategies to prevent Rh Sensitization, may thus also be useful in disputed paternity and genetic study in Bangladesh.

Key Words: Rhesus Antigens, Genotype, Phenotype, HDN, DHTR, Bangladeshi.

Introduction

Rhesus blood group system is highly immunogenic like ABO system. Most Rh (D) negative persons form anti D in any way specially either by transfusion or by transplacental hemorrhages. Not only D antigen but also other antigens of Rh system specially C & E are responsible. for the production of antibody is multi-transfused patient. More than 80% of Rh(D) women form anti D after single exposure, further transfusion of Rh(D) positive blood may cause delayed Hemolytic transfused reaction. Moreover one in six cases of pregnant women with Rh(D) positive child may cause Hemolytic disease of newborn by inducing transplacental haemorrhage & lysis of infant red cell. Rh antigens are

also involved in the auto-immunization specifically of some of the warm antibodies of auto immune hemolytic anaemia. Outside D other antigens of Rh system may also immunize negative persons and may also cause HTR & HDN but in small proportion.

In 1943, RA Fisher proposed that there were three closely linked genes Cc, Dd & Ee which determine corresponding antithetical antigens¹. He later proposed that the order of the genes was DCE². Experience soon showed that such not occur. It was presumed that d was an amorphic allele or is not present at all. There are five antigens at present, D, C, c, E & e. Molecular biology

technique has shown that there are only two genes. D which has no allele and a second gene Cc Ee which has many alleles. But it is convenient to use d to indicate the absence in absence of D³.

The importance of determination of genotype is that when a woman form anti D in her serum it is important to know whether her partner is Dd or DD. If he is DD he can only be father of D positive offspring but if he is Dd, will be 50% chance of being D positive child. One with D negative parents can not be DD. It is now recognized that there are three alternative antigens in set in Rh system. Every person inherits one set of these alternative antigens from each parent.

Each chromosome carries three of these genes situated closely together on the chromosome & genes inherited as a unit & not independent of each other⁴. The eight combinations of six Rh genes are such that each Rh chromosome has one C, one D, one E locus. The genotypes are expressed as pair of three genes inherited from each parents. Haplotypes are CDe, (R1), cDE (R2), cDe (R0), CDE (Rz), cde (r), cdE (r') and Cde (r'')⁵. Haplotype frequencies among British Population are: 0.421, 0.141, 0.026, 0.002, 0.389, 0.010 and 0.012 respectively.

An Rh genotype therefore consists of any two of these chromosome arrangements. The Fisher Race & the Weiner nomenclature are used interchangeably. The Fisher Race terminology assumes inheritance of three closely linked genes & Weiner terminology assumes inheritance of single gene from each parent which is a mosaic structure⁶, Another importance of genotype determination is that antibody develops when immunized in absence of the corresponding antigen i.e. gene⁷.

The completeness with which the Rh genotype can be determined depends on five antibodies: Anti-D, Anti-C, Anti-c, Anti-E & Anti-e antisera are used for genotype study. Standardization of antisera is important. Day by day antisera is standardized⁸.

In the present study five standard antisera were used, probable genotype & phenotype determined with their frequencies in Bangladeshi native population attending in a Tertiary Care Hospital, BSMMU, and compared with previous studies.

Aim Of The Study

The aim of the study is to identify actual Rh genotype-phenotype in Bangladesh and thus help to formulate transfusion protocol to prevent alloimmunization & strategy of transfusion in-patient with antibody. The study may also help in disputed paternity & genetic study in Bangladesh.

Materials & Methods

1000 samples (50.7% male & 49.3% female, 94.6% Rh D Positive, Sample mean age 36.36 ± 0.077) were randomized out of 1500 samples using calculator method & included in the present cross sectional study. Samples were of both patient and donor. The study was conducted in the dept. of Transfusion medicine within the period from 01.10.2000 to 30.10.2013. Samples were tested fresh as possible in CPD solution with five standard monoclonal anti sera Anti-D, Anti E, Anti e (small), Anti C, Anti c (small) of Lorne Lab Uk. Tests were done according to manufacturers instruction. The results were recorded, Phenotypes, probable genotypes and their frequencies in percent determined. The result was compared with the results of previous three studies. The data was analyzed using SPSS version-16 windows. P value was set at <0.05 to show the test results significant.

Results

In the present study, out of 1000 samples (50.70 Percent male and 49.30 percent female of both donor and patient having mean age 36.36 ± 0.077 most samples were Rh (D) Positive (94.60 percent). Most genotypes found were R1R1, R1r, R1R2 and rr with R1R1 in first position (38.6%) and among Rh (D) negative rr (4.50%) highest. This result was compared with previous three results by Haque (2000), Rahman (1992) and Das (1980). It was found that the result of present study is about same as that of Haque (2000) and the (1980). The result of Rahman et al (1992) seems to be misleading. Among Rh (D) Negative we found rr (4.5%) but the result of all previous studies are about half or below of the present study. There are some controversies within the said samples.

Table 1: Samples on Rh (D) Positive Negative and on gender (n-1000) P < 0.001

Donor	Number	Percentage	P
Rh D +ve	946	94.6 %	P < 0.04
Rh D - ve	54	5.4 %	P < 0.05
Male	507	50.70%	P < 0.001
Female	493	49.30%	P < 0.001

* 94.60% of samples are Rh D+ve. Male & female are almost equal.

Table 2: Rhesus Phenotype and most probable Genotype found in study.

AntiC	AntiD	AntiE	AntiC	AntiE	Phenotype	Most Probable Genotype
+	+	-	-	+	CCDee	R ₁ R ₁
+	+	-	+	+	CcDee	R ₁ r
+	+	+	+	+	CcDEe	R ₁ R ₂
-	+	+	+	+	ccDEee	R ₂ r
-	+	+	+	-	ccDEE	R ₁ R ₂
+	+	+	-	+	CCDEe	R ₁ R _z
-	+	-	+	+	ccDee	R ₀ R ₀
+	-	-	+	+	ccee	r r
-	-	-	+	+	Ccee	r'r
-	-	+	+	+	ccEe	rr''

* Most genotypes are R₁R₁ , R₁r, R₁R₂ and rr.

Table 3: Genotype frequency found in study (n=1000) P<0.005

Fisher-Race	Weiner	No of sample	Frequency
CDe/CDe	R ₁ R ₁	386	38.60
CDe/cde	R ₁ r	292	29.20
CDe/cDE	R ₁ R ₂	222	22.20
E	R ₂ r	19	1.9
cDE/cDE	R ₂ R ₂	15	1.5
CDe/CDE	R ₁ R ₂	06	0.6
cDe/cDe	R ₀ R ₀	06	0.6
cde/cde	rr	45	4.5
Cde/cde	r'r	08	0.8
Cde/cdE	r r''	01	0.1

* R₁R₁ is 38.60 percent & R₁r is 29.20 Percent.

Table 4: Genotype Frequencies among male and female (n=1000) P < 0.05

No of sample	Geno type	Genotype Frequency	Male Sample	Geno type Freq uency	Female Sample	Geno type Freq uency
386	R ₁ R ₁	38.60	200	39.44	186	37.73
292	R ₁ r	29.20	142	28.0	150	30.43
222	R ₁ R ₂	22.20	113	22.28	109	22.11
19	R ₂ r	1.9	11	2.16	8	1.63
15	R ₂ R ₂	1.5	8	1.57	7	1.42
06	R ₁ R _z	0.6	4	0.78	2	0.40
06	R ₀ R ₀	0.6	3	0.59	3	0.60
45	Rr	4.5	22	4.33	23	4.67
08	r'r	0.8	4	0.78	4	0.81
01	rr''	0.1	0	0	1	0.20
1000		507 (50.7%)	100	493 (49.3%)	100	

* R₁R₁ in male is 39.44% & 37.73% female.

Table 5: Genotype Frequency in different study:

Genotype	Das et al 1980 ⁹	Rahman et al ¹⁰ 1992	Haque et al ¹¹ 2000	Present study Shil 2014
Rh (D) Positive				
R ₁ r	30.89	26.25	33.20	29.20
R ₁ R ₁	46.75	24.06	37.00	38.60
R ₁ R ₂	11.99	39.75	18.60	22.20
R ₂ r	5.89	2.06	4.40	1.90
R ₂ R ₂	0	0.06	0.20	1.50
R ₁ R _z	0.2	0.50	0.80	0.60
RoRo	0	1.75	0	0.60
Rh (D) Negative				
r'r	2.85	1.75	2.80	4.50
r'r''	0.20	0.125	0.20	0.80
rr''	0.20	0	0.20	0.10

* In the present study R₁R₁ is 38.60 which match with Haque (2000) & is highest.

Discussion

The results of the study for Rhesus Phenotype, Genotype and their Frequencies shows the most frequent probable Genotype being R₁R₁ among Bangladeshi population and it is 386 (38.60%). Least frequency was found in rr'' and it is 0.1% among 1000 random blood samples examined. Within 1000 samples Rh D positive samples were 966 (96.6%) and negative 54 (5.4%). Most frequent Rh D positive genotype was maximum among male samples 200 (39.44%). Second frequent genotype was R₁r 292 (29.20 %) and was found maximum among female samples 150 (30.42 %). Third frequent genotype was found R₁R₂ and was 220 (22.20%) and it was maximum among male 113 (22.28%). Rh D Negative genotype rr is frequent and it is 4.5%. Other Two negative genotype found was r'r 0.8% and rr'' 0.1 %. Highest among female 4.53%. It may be pointed out that there are more than one probability of genotypes. Most probable option is taken as genotype & is expressed as phenotype frequency. Another fact is that no anti d is present & so no detection of r₀. The probability of genotype also depends upon the frequency of gene. So various phenotype can be guessed as first probable genotype. By using family studies in addition may be possible to determine genotype perfectly.

Various studies are available to compare the result of present study- one among Bengali Hindu population & two among Bangladeshi population. The question generally arise whether any genetic difference between Bengali Hindu & Bengali Muslim population exist. But

the result is different. Highest frequency found in present study R1R1 was also found among Bengali Hindu population. But R1R2 was highest in Bangladeshi population¹⁰. It should be realized that there are wide racial differences between frequencies of the Rh gene complex 7,8. In the present study genotype R1R1 was found highest and was also found highest in study done in 2000 by Haque et al¹¹. The present study was done with mixed Hindu & Muslim (Bengali) population of Bangladesh although most cases are Muslims. The study seems more accurate in racial consideration. Bengali population seems to be of same genetic origin but genetic origin of Bangladeshi may be different. The variation of genotype in previous study & present Bangladeshi Bengali study seems to be due to the difference of five antisera used. Studies were done in 1980 & 1992 or before no monoclonal antibody was available. Now a day's antisera have developed & standardized much. Test in the present study was done with monoclonal antibodies. No Du or D variant was identified.

Conclusion

All multi-Transfused should be genotyped and phenotyped along with ABO Grouping to prevent immunization. In Bangladesh genotype R1R1 is highest.

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Conflict of Interest: The authors have no conflict of interest to any body.

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