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Incidence of Rh Antigens, Phenotype & Probable Genotype in the Population of Gwalior and Chambal Region, Central India

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Authors' contributions

This work was carried out in collaboration between all authors. Author DCS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors SR and SI managed the literature searches, analyses of the study performed the spectroscopy analysis. Author SS managed the experimental process and authors BJ and SSao supervised the research work. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Introduction: Rhesus (Rh) antigen was discovered in 1940 by Karl Landsteiner and Wiener. In later years, because of its immunogenecity along with ABO grouping, RhD antigen testing was made mandatory before issuing a compatible blood. Presently there are five major antigens i.e. D, C, E, c and e in Rh blood group system.
Aims: To know the distribution of major Rh antigens, its phenotype and most probable genotype in the population of Gwalior region i.e. Central India.
Place and Duration of Study: This study was carried out at Blood Bank, Department of Pathology, Gajra Raja Medical College, Gwalior, India from 1st October 2008 to 30th September 2010.
Methodology: The distribution of Rh antigens, its phenotype and most probable genotype was studied in 1000 samples collected from blood donors, blood recipients and other patients. Samples were tested for ABO blood group and five major antigens of Rh system by tube agglutination method /or by gel technology.
Results: Out of 1000 samples studied, the incidence of RhD was 91.6% and only 8.4% samples were negative for D antigen (*p=.000005)*. The Incidence of other Rh antigens i.e.

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C, E, c and e was 84%, 25.6%, 58.3% & 78.5% respectively (*p*=.000005) Most common phenotype in RhD positive samples were DCCee (41%) and in RhD negative it was dccee (5.6%) (*p= .000005)*. Eleven samples (1.1%) were negative for antithetical antigens E & e. Most probable genotype in order of frequency was DCe/DCe (R_1R_1)-41%, DCe/Dce (R_1R_0)-25.5% & dce/dce (rr)-5.6% (*p= .000005)*.

Conclusion: Like previous studies, our study also concluded that there is a wide range of racial and geographical variation in the distribution of Rh phenotype and genotype. The Rh blood group system has vital role in population genetic study, in resolving medico legal issues and more importantly in transfusion practice.

Keywords: Rhesus blood group; phenotype; genotype; antigenicity.

1. INTRODUCTION

Almost 32 blood group systems [1] and over 600 different blood group antigens [2] are discovered so far, ABO and Rh blood group systems are most important blood group systems in the field of transfusion practice. '**Antigens'** are inherited substance on the surface of red cells. These antigens may be proteins, carbohydrates, glycoproteins or glycolipids depending upon the blood group system. Several of these red cells surface antigens can stem from one allele (or very closely linked genes) and collectively form a '**Blood group system'** [3]. Importance of ABO blood group system is due to the fact that it is the only blood group system in which antibodies are constantly, predictably and naturally present in the serum of the people who lack the antigen. These ABO antibodies are by and large IgM in nature and cause the intravascular hemolysis in ABO mismatch transfusions while importance of Rh blood group system is because of immunogenicity, polymorphism and complexity of its antigens.

Presently the term Rh refers not only to a specific red cell antigen i.e. **RhD** but also to complex blood group system that is currently comprised of more than 50 different antigenic specificities. In 1939, Levine & Stetson [4] first described the case of a mother, after giving birth to a stillborn child, having a hemolytic transfusion reaction following transfusion of her husband's blood. Her serum agglutinated her husband's red cells and those of 80% of ABO-compatible donors. In 1940, Landsteiner & Wiener [5] injected Rhesus-monkey red blood cells into rabbits. The rabbit serum agglutinated Rhesus-monkey red cells and also 85% of human red cells. At that time it was thought that both antibodies have the same pattern of reactivity but in 1963 Levine et al. [6] finally proved that human and rabbit anti-Rh did not react with the same antigen. However, lastly the name Rh was retained for the human produced antibody. The Anti-rhesus antibody formed by the animals was renamed anti-LW in honor of Landsteiner & Wiener [7].

ABO and Rh antigens are hereditary characters and are useful in the population genetics study, in resolving medico legal issues and more importantly in compatibility issues in transfusion practice [8]. Presently there are 50 antigens in the Rh system but **D, C, c, E** and **e** are the most commonly identified and the most significant antigens in blood transfusion. In routine protocol of grouping only RhD antigen is tested and person's Rh blood group is reported as Rh positive or negative. The RhD antigen can vary in both the quantity of antigen expressed and the qualitative nature of the antigen, so RhD antigen has multiple antigenicity and has variants like incomplete D, D partial, D mosaic; weak D, etc. [9,10].

Four theories have been postulated to explain the inheritance and to classify the complex Rh system.

1. Fisher and Race 1940 [11]
2. Weiner 1939 [12]
3. Rosenfield 1960 [13, 14, 15]
4. International Society of Blood Transfusion (ISBT) [16]

Fisher and Race [11] postulated that antigen of the Rh system is produced by three closely linked sets of allele genes i.e. D/d, C/c and E/e and each gene is responsible for producing the antigen D, C, c, E and e on the surface of RBC. No'**d**' antigen has been found on RBC, so d gene is considered an amorphous gene (silent allele) or the absence of D antigen. There are eight possible haplotype arrangements of Rh genes on the short arm of chromosome 1 i.e. ***Dce, DCe, DcE, DCE, dec, dCe, dcE and dCE*** results to 36 possible genotypes. Weiner [12] terminology is complex and less widely used. He proposed the single locus and eight allele genes theory. Weiner's allele genes are ***Rh, Rh^1, Rh^2, Rh^z, rh, rh^1, rh^2 and rh^y.*** He labeled five major antigens as D-Rh_0, C- rh′, E- rh″, c- hr′, e- hr″. Finally Tippett [9] in 1986 explained that the Rh system is controlled by two closely linked loci, RHD and RHCE. The RHD locus carries the gene for the RHD polypeptide, which expresses all the D antigen epitopes. The RHCE locus carries the genes for the RHCE polypeptide, which expresses both the C/c and E/e antigens. Alfa numerical terminology is given by Rosenfield [13,14,15] in 1960 and this system simply demonstrates the presence or absence of antigen on red cells. For five major antigens symbols are assigned as D –RH 1, C-RH 2, E-RH 3, c-RH 4 and e-RH 5. I**nternational Society of Blood Transfusion (ISBT)** [16] committee assigned a numerical terminology. Six digit numbers has been adopted for specific antigen; first three numbers represent the blood group system (for RH system it is 004) while last three represent the antigen specificity (for D antigen it is 001), so D antigen is marked as 004001. Symbols of Five major antigens in different nomenclatures are summarized in the Table 1.

Table 1. Symbols of five major Rh antigens in different nomenclatures.

Fisher & Race	Weiner	Rosenfield	ISBT
D	Ro	RH-1	004001
C	rh'	RH-2	004002
E	rh''	RH-3	004003
C	hr'	RH-4	004004
e	hr''	RH-5	004005

The blood group Rhesus (Rh) antigens of human red cells have recently been characterized as integral membrane proteins of apparent Mr, -30000 [17,18]. The antigens are located on two proteins. [19,20] RhD carries the D (Rh1) antigen, and RhCE carries the C, c, E, and e (Rh2 to Rh5) antigens. Both D and CE have 10 exons [21] and proteins are composed of 417 amino acids [19,22]. Current structural models predict 6 extracellular loops and 12 transmembranous and 7 intracellular protein segments. [23,24] Both C- and N-terminal protein ends are intracellular (Fig. 1). Depending on the *RHCE* allele considered, RhD and RhCE differ in 34 to 37 amino acids. These differences are dispersed throughout the amino acid sequence of the protein. Only a limited number of these differences are located exofacially; such exofacial differences are restricted to loop 3 encoded by exon 4, loop 4

encoded by exon 5, and loop 6 encoded by exon 7. In loop 2 encoded by exon 2, the *c* allele but not the *C* allele of *RHCE* differs from RhD (Fig. 1).

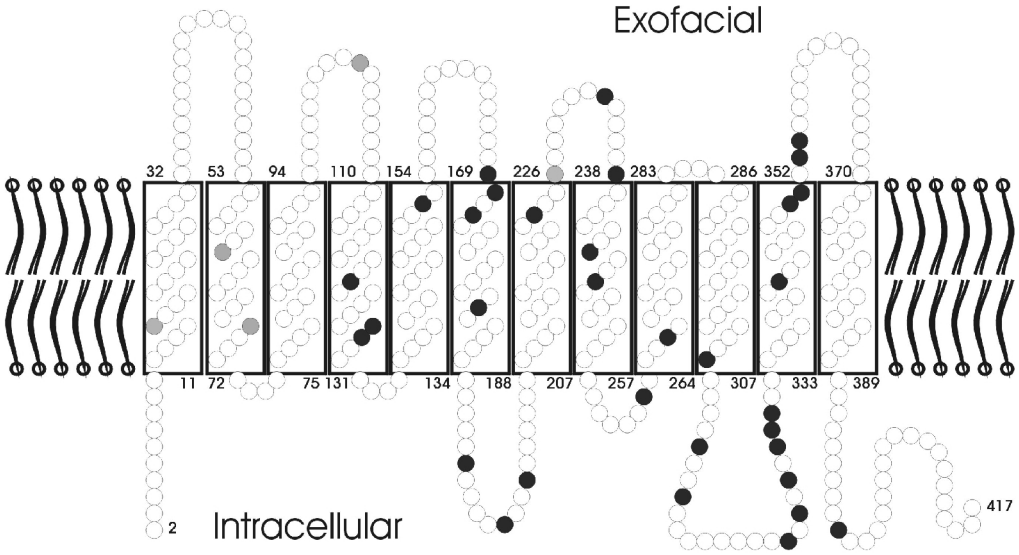

Fig. 1. Rh topology in the RBC membrane. The protein is assumed to possess 12 transmembranous segments, 6 extracellular loops, and 5 intracellular loops. Both the C- and N-terminal ends of the protein are intracellular. Each amino acid is depicted by a circle; black circles indicate positions that differ between RhD and RhCE in all frequent alleles, grey circles indicate positions that differ between RhD and RhCE only in some alleles

In the RBC membrane, the Rh proteins form a complex with Rh-associated glycoprotein (RhAG), previously known as RH50 [25]. This "Rh complex" is tightly linked to the cytoskeleton [26]. Several additional proteins, such as CD47, LW, and the Duffy glycoprotein, are associated with the Rh complex (Supra-molecular complex) but not necessary for Rh expression. The membrane expression of Rh depends on functional RhAG: mutations in RhAG could be shown to underlie the "**regulator form**" of the Rh_{null} phenotype characterized by lack of all Rh antigens [27]. The "**amorphous**" type of Rh_{null} was shown to lack any functional *RHCE* and any functional *RHD* genes. Generally, they are caused by nonsense mutations in *RHCE* in an *RHD* negative background [28,29]. The Deletion of Various Rh antigen was reported by different workers from time to time and reported the genotypes as D--/D--, DC-/Dc- and many more [30,31,32].

The initial confusion of Rh and LW antigens was not unlikely, because the level of LW antigen expression is greater in RhD positive than negative RBCs, and LW antigens are altogether lacking in the Rh negative phenotype. On the basis of these phenotypic relationships, it has been speculated that Rh might be the precursor of LW [33]. Purified LW glycoprotein conclusively demonstrated that Rh and LW are distinctly different proteins [34]. The LW glycoprotein is an intercellular adhesion molecule (ICAM-4) and a ligand for integrins. LW has 30% sequence identity with other ICAMs. ICAM-4 binds to CD11/CD18 ($\alpha_1\beta_2$) integrin and LFA-1 leukocyte integrins $\alpha_4\beta_1$, $\alpha v\beta_1$, $\alpha v\beta_5$ and maybe $\alpha v\beta_3$ [35,36]; possible marker for lymphocyte maturation or differentiation.

There is wide variation in distribution and frequency of Rh antigens throughout the world and lack of study from India especially central part of India i.e. in the population of Gwalior and Chambal region of Madhya Pradesh propelled us to know the frequency of five major Rh antigens, its phenotype and most probable genotype from the possible genotypes.

2. MATERIALS AND METHODS

The study was conducted in blood bank, Department of Pathology, G. R. Medical College, Gwalior from 1st October 2008 to 30th September 2010 (Duration 2 yeas). Blood Samples from 1000 individuals were selected for the study. Blood samples were collected from blood donors who came to donate blood in blood bank, outdoor patients who came to blood bank for routine blood grouping and indoor patients who were admitted in the different wards of the J. A. Group of hospitals affiliated to G. R. Medical College, Gwalior. Out of five milliliter (ml) blood drawn from individual, 3.0 ml was collected in plain sterilized vial, while 2.0 ml in citrated vial. Following tests were performed on collected samples.

1. Forward and reverse ABO grouping was performed by conventional tube method and by Gel technology. For forward ABO grouping, commercially available monoclonal blood group antisera i.e. Anti A, Anti B, Anti AB, Anti H and Anti A_1 (Make- J. Mitra & Co. Pvt. Ltd.) were used while for reverse grouping 5% pooled cell suspension of A, B and O cells prepared in own blood bank were used. Gel technology (Make - Tulip Diagnostics Pvt. Ltd.) was also used as & when required.
2. RhD typing was done by tube method as well as by Gel technology using monoclonal/ polyclonal Anti-D (Rh0 & Rh_1) and by using gel card (Make-Tulip Diagnostics Pvt. Ltd.) respectively.
3. For detection of status of rest of the major antigens of Rh system apart from Antigen D i.e. Antigen C, c, E & e specific monoclonal antisera (Make- Tulip Diagnostics Pvt. Ltd.) were used and test was performed by tube method.
4. For screening of antibodies in the serum was done by indirect coomb's test (ICT) exclusively by Gel technology.

False positive and false negative results were strictly avoided by taking quality control measures at each step. By testing the red cells for five major antigens of Rh group using antisera D, C, E, c, and e, phenotype of the patient is reflected in the results. Determination of exact genotype is not possible without testing parents and other family members or by RNA testing. For this reason most probable genotype is determined from gene frequency estimates (Table 2) [37].

Table 2. Gene frequency in United States [37]

Gene combination	Frequency in percentage			
	White	Black	Native Americans	Asians
DCe (R_1)	43	17	44	70
Dce (R_0)	37	26	11	3
DcE(R_2)	14	11	34	21
dce(r)	4	44	2	3
dCe(r′)	2	2	2	2
dcE(r″)	1	0	6	0
DCE(Rz)	0	0	6	1
dCE(r^y)	0	0	0	0

Knowledge of ethnicity is important when determining the most probable genotype. Following steps are taken to determining the possible genotypes from the individual's phenotype [38]: (1). If D is positive, the number of possible genotypes is one less than the number of positive reactions (except if all 5 are positive, in which case there are 6 possible genotypes). (2). If D is negative, the number of possible genotypes is two less than the number of positive reactions (unless there are only 2 positive reactions, in which case there is 1 possible genotype). (3). Remember that one haplotype is inherited from each parent (for example, DCe/dce is one genotype having both the DCe and the dce haplotypes). Determine the most probable haplotypes: These rules are based on probability so the least likely genotypes will involve R^z or R^y. Most probable genotype from the possible genotypes was calculated as shown in the following example. If patient's phenotype is D+, C+, E - , c+, e+, then we have 4 positive reactions and the D is positive, we should have 3 possible genotypes. When D is positive, we don't know if the patient inherited D from 1 parent or both parents. So we immediately have 2 possibilities: A. One genotype where D was inherited from both parents: D??/D?? B. One genotype where D was inherited from only 1 parent: D??/d?? Next look at alleles C and c. If only one is present, it must be in both haplotypes. If both are present assign one allele to one haplotype, and the other allele to the remaining haplotype. From our example, we have both C and c present, so C will be assigned to one haplotype and c to the other. A. DC?/Dc?; B. DC? /dc? Repeat this process for E and e. From our example, only e is present, so it must be in both haplotypes. A. DCe/Dce = R_1R_o; B. DCe/dce = R_1r. These are only 2 of the 3 possibilities. Remember in genotype "B", we randomly assigned C to the haplotype with D and c to the haplotype without D. To get our third genotype, reverse the positions of C and c. C. Dce/dCe = R_or' .To determine which of the 3 choices is most likely, look at the haplotype frequencies for the patient's race. In this example, if the patient is White, then "B" R_1r is the most likely; if the patient is Black, then "A" R_1R_o is more likely [22]. Terminology, of all the Rh nomenclatures was used in the present study to explain the subject matter.

3. RESULTS

In the present study 1000 samples were selected randomly i.e. 365 (36.5%) - blood donors, 420 (42%) - indoor patients, 194 (19.4%) - outdoor patients and 21 (2.1%) samples were of cord blood/ neonatal samples (Table 3). Age of the patients/ Donors varied from 01 day to 72 years. Mean age of the patients/ Donors was 31.18 years. Out of 1000 samples, male were 949 (94.9%) while female were 51 (5.1 %) (Table 4). Incidence of RhD positive were 91.6% (916 samples) and 8.4% (84) samples belong to RhD negative (*p= .000005)*. 16 samples were reported as D^u variant. Statistically these samples were included in RhD positive group (Fig. 2).

Table 3. Group wise distribution of samples studied (1000 Samples)

Blood donors	OPD patients	Admitted patients	Pediatric patients
365 (36.5%)	194 (19.4%)	420 (42%)	21 (2.1%)

Table 4. Male and female ratio in the samples studied (1000 samples)

Male	Female
949 (94.9%)	51 (5.1%)

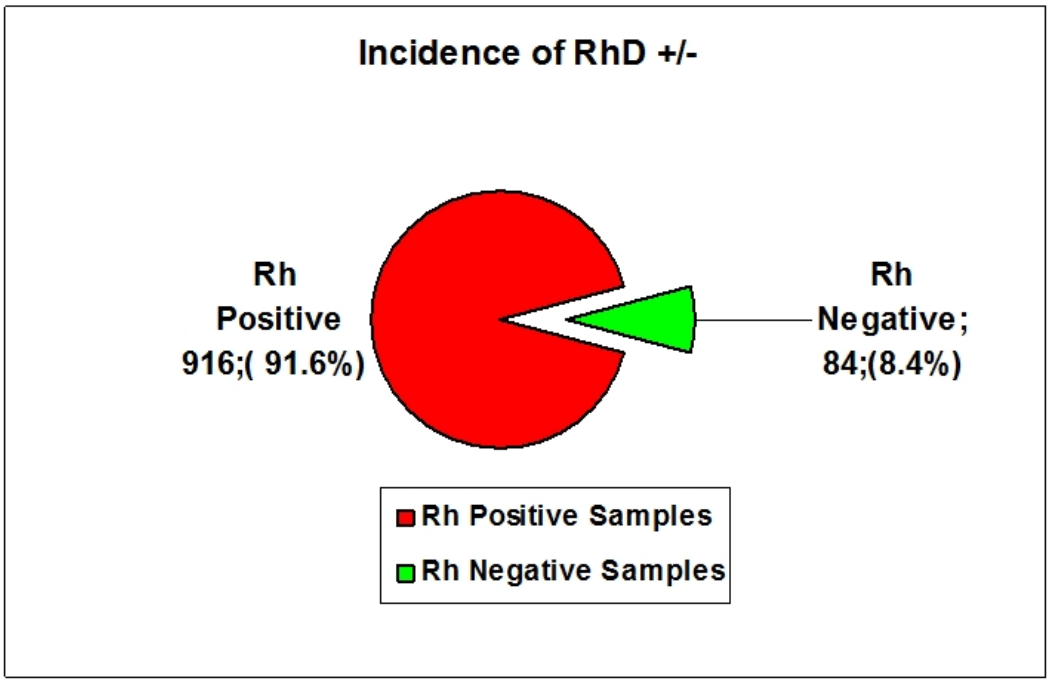

Fig. 2. Incidence of Rh positive/negative in the present study

Frequency of five major Rh antigens was D-91.6%, C-84%, E-25.6%, c-58.3% and e-78.5% (*p= .000005)* (Fig. 3). Out of 18 possible phenotype combinations (9 each belongs to Rh D positive and negative group), we could not detect any case of phenotype dCcEe and dCCEE. In order of descending frequency the most common phenotypes were DCCee - 41% , Dccee - 25.5%, dccee - 5.6%, DccEe - 5.5%, DccEE- 4.7%, DCcEE - 3.3%, DCcEe- 3.1% (*p=.000005).* The less common phenotypes were Dccee - 3.0%, DCCEe- 2.2%, DCCEE - 1.5%, dCcee- 1.3%, dccEe- 1.3%, dccEE - 0.3%, dCCee - 0.3%, dCCEe - 0.2%, dCcEE - 0.1% while in 11 (1.1%) samples we reported deletion of antithetical antigen E/e i.e. 6 cases of DCC- - and five cases of DCc- -. Most common phenotype in study was DCCee 41% while in Rh D negative samples it was dccee 5.6% (Table 5).

In our study the most common probable genotype was DCe/DCe (R_1R_1) - 41% followed by DCe/Dce (R_1R_0)-25.5% (*p=.000005)*. Third most common genotype was dce/dce (rr) - 5.6 % belong to RhD negative group. Next probable genotypes in order of descending frequency were R_2R_0-5.5%, R_2R_2-4.7%, R_2R_Z-3.3%, R_1R_2-3.1%, R_0R_0-3.0%, R_1Rz-2.2%, RzRz-1.5%, r′r-1.3%, r″r-1.3%, DC-/DC- 0.6%, DC-/Dc- 0.5%, r″r″-0.3%, r′r′-0.3%, $r'r^y$-0.2% and $r''r^y$-0.1% (Table 6).

We had also screened serum samples for the irregular antibodies and it was found in 21 samples belonging to multi transfused patients and multi-Para females. The identification of antibodies was not done in the present study because of non availability of facilities.

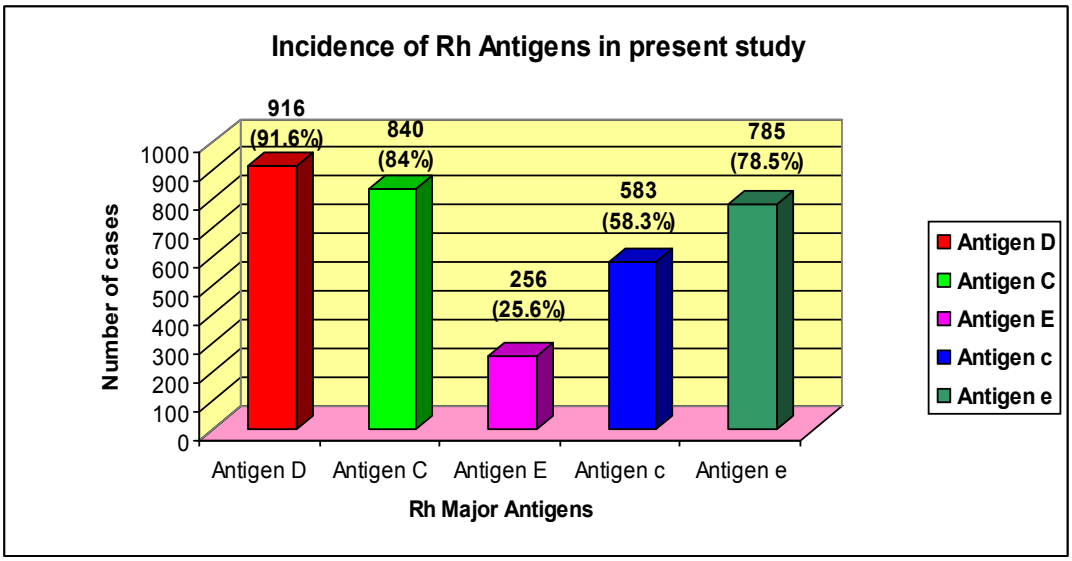

Fig. 3 Incidence of five major Rh antigens in the present study

Table 5. Incidence of Rh Phenotype in the present study

Rh Positive			Rh-Negative		
Phenotype	**Total Case**	**Percentage**	**Phenotype**	**Total Case**	**Percentage**
DCCee	410	41%	dccee	56	5.6%
DCcee	255	25.5%	dCcee	13	1.3%
DccEe	55	5.5%	dccEe	13	1.3%
DccEE	47	4.7%	dccEE	3	0.3%
DCcEE	33	3.3%	dCCee	3	0.3%
DCcEe	31	3.1%	dCCEe	2	0.2%
Dccee	30	3.0%	dCcEE	1	0.1%
DCCEe	22	2.2%	dCcEe	Cases was not detected	
DCCEE	15	1.5%	dCCEE	Cases was not detected	
*DCC - -	6	0.6%			
*DCc - -	5	0.5%			

** Deletion of antithetical antigens E/e.*

Table 6. Reaction pattern with antisera, phenotype, possible genotypes and probable genotypes in the present study

Reaction with Test sera					Phenotype	Incidence numbers (%)	Possible genotypes	Most probable genotype
D	C	E	c	e				
+	+	-	-	+	DCCee	410 (41%)	R_1R_1, R_1r'	DCe/DCe(R_1R_1)
+	+	-	+	+	DCcee	255 (25.5%)	R_1R_0, R_1r, R_0r'	DCe/Dce (R_1R_0)
-	-	-	+	+	dccee	56 (5.6%)	rr	dce/dce (rr)
+	-	+	+	+	DccEe	55 (5.5%)	R_2R_0, R_2r, R_0r''	DcE/Dce(R_2R_0)
+	-	+	+	-	DccEE	47 (4.7%)	R_2R_0, R_2r''	DcE/DcE (R_2R_2)
+	+	+	+	-	DCcEE	33 (3.3%)	R_2R_z, R_zr'', R_2r^y	DcE/DCE (R_2R_z)
+	+	+	+	+	DCcEe	31 (3.17%)	R_1R_2, R_zr, R_2r',R_1 r'', R_ZR_O, R_Or^y	DCe/DcE (R_1R_2)
+	-	-	+	+	Dccee	30 (3.0%)	R_0R_0, R_0r	Dce/Dce (R_0R_0)
+	+	+	-	+	DCCEe	22 (2.2%)	R_1R_z, R_zr'', R_2r^y	DCe/DCE (R_1R_z)
+	+	+	-	-	DCCEE	15 (1.5%)	R_zR_z, , R_zr^y	DCE/DCE (R_zR_z)
-	+	-	+	+	dCcee	13 (1.3%)	$r'r$	dCe/dce (r'r)
-	-	+	+	+	dccEe	13(1.3%)	rr''	dce/dcE (rr")
-	-	+	+	-	dccEE	3 (0.3%)	$r''r''$	dcE/dcE (r"r")
-	+	-	-	+	dCCee	3 (0.3%)	$r'r'$	dCe/dCe ($r'r'$)
-	+	+	-	+	dCCEe	2 (0.2%)	$r'r^y$	dCe/dCE ($r'r^y$)
-	+	+	+	-	dCcEE	1 (0.1%)	$r''r^y$	dcE/dCE ($r''r^y$)
+	+	-	-	-	*DCC - -	6 (0.6%)	DC-/DC-, DC-/dC-	DC-/DC -
+	+	-	+	-	*DCc - -	5 (0.5%)	DC-/Dc-, DC-/dc-, Dc-/dC-	DC-/Dc-

** Deletion of antithetical antigens E/e.*

4. DISCUSSION

In our study, age of patients varied from 01 day to 72 years, Male and female ratio was 94.9:5.1 and incidence of RhD positive was 91.6% and 8.4% belong to RhD negative. Khattak ID et al. [39] reported male to female ratio 74.86: 25.14 from Pakistan. Enosolease ME et al. [40] observed 6.01% RhD negative phenotype in Nigeria. de Zoysa NS [41] had also reported similar distribution of rhesus phenotype from Shrilanka as reported by Indians. Geographically higher incidence of RhD positive was reported in Japanese population while it was lower in European population [42].

Table 7. Incidence of RhD positive and negative from different country [42]

Country	Rh-D Positive in percentage	Rh-D Negative in percentage
Europe	83	17
West Africa	97	3
India	90	10
Japan	99.7	0.3
China	93	7

In our study 16 (1.6%) cases of D^u variant were detected while Makroo RN et al. [43] reported incidence of weak D as 0.12% among Rh-negative individuals from Delhi, India. Frequency of major Rh-antigen in the present study was D - 91.6%, C -84%, E -25.6%, c-58.3%, and e - 78.5% , while it was D-85%, C-68%, E-29 %, c-80% and e-98% in European countries [44]. Study conducted by Jeremiah ZA et al. [45] observed that most frequently occurring antigen was c (99.8%) followed by e (98.7%), D-(95%), E (20.5%) and finally C (17.7%), While Jenan Y Taha [46] from UAE reported that most frequently occurring antigen was found to be e (97.3%), followed by D (91.1%), C (73.2%), c (71%) and E (21%). Thakral et al. [47] from north India, amongst Rh antigens, e was the most common (98.3%) followed by D, C (84.76%), c (52.82%) and E (17.9%). Younis Abed EL [48] reported the percentage of Rh antigens; D+, D−, C, c, E and e in the total sample was 92%, 8%, 69%, 81%, 38% and 97%, respectively [Table 8].

Table 8. Frequency of five major Rh antigens in different populations

	Rh Antigens	Frequency in present study (%)	In Europe an Study (%)	Jeremiah ZA et al. Nigeria (%)	Jenan Y Taha from UAE (%)	Thakral et al. north India (%)	Younis Abed EL et al. Palestine (%)
1	D (Rho)	91.6	85	95	91.1	84.7	92
2	C (rh')	84	68	17.7	73.2	84.7	69
3	E(rh")	25.6	29	20.5	21	52.8	38
4	c(hr')	58.3	80	99.8	71	17.9	81
5	e (hr")	78.5	98	98.7	97.3	98.3	97

The D antigen is the product of the D gene and the proposed allelic gene *d is* considered as amorphous because **no 'd'** antigen or anti-d antibody is ever discovered. In our study RhD negative cases was 8.4%. The antigens C and c are the products of the co-dominant alleles *C* and *c.* Antigens E and e are the products of the co-dominant alleles *E* and *e*. E is a strong

immunogen (almost as strong as D) but this is least effective immunogen due to its low frequency **(antigenicity D>c>E>C>e)**.

Anti-D is most common antibody seen in Rh-D negative people. Anti-E is least common antibody seen in Rh positive people as only 30% of the population has the antigen. The anti-C or anti-c is less common. Anti-e is often seen as autoantibody and this will make it difficult to find compatible blood since 98% of the population has the small e antigen. Anti-C, e or anti-c, E are often seen in combination and if a patient lacks both C & e and has made an anti-C antibody then enhanced technique should be used to make sure that an anti-e is also not present. Study done by Hassab AH et al. [49] detected high incidence of anti-E in 23.8% in alloimmunized patients. Chu HP et al. [50] reported an unusual severe case of intrauterine hemolysis resulting from rare anti-c, and it may be life threatening.

In our study most common phenotype was DCCee (41%) while least common was DCCEE (1.5%) in Rh positive samples. In Rh negative samples dccee (5.6%) was most common and dCcEE (0.1%) was least common. Most common frequency reported in white was DCCee - 42 % while it was Dccee in black - 44% [44]. No sample of Rh_{null} was reported in present study while in 11 samples (1.1%) deletion of antithetical antigen E/e was found.

In our study most common probable genotype was 41% -DCe/DCe (R_1R_1) fallowed by in decreasing order was 25.5% - DCe/Dce (R_1R_0), 5.6% - dce/dce (rr), 5.5% - DcE/Dce (R_2R_0), 4.7% - DcE/DcE (R_2R_2), 3.3% - DcE/DCE (R_2R_z), 3.17% - DCe/DcE (R_1R_2), 3.0% - Dce/Dce (R_0R_0), 2.2% - DCe/DCE (R_1R_z), 1.5% - DCE/DCE (R_zR_z), 1.3% - dCe/dce (r'r), 1.3% - dce/dcE (rr''), 0.3% - dcE/dcE (r''r''), 0.3% - dCe/dCe (r′r′), 0.2% - dCe/dCE (r′r^y) while least common was 0.1% - dcE/dCE(r″r^y). Hassan FM et al. [51] reported decreasing order of frequency of Rh genotype from Saudi Arabia were R1R2, R1r'', R2r', R0R0, R0r, RzR1, R1r'', R2r'', R1r, R1R0, R0r', R1R1, R1r', R0, R0r, r'r'. Rahman M et al. [52] from Bangladesh reported that most prominent Rhesus genotype was CDe/cDE (R1R2), i.e., 39.75%, while Rhesus genotype cde/cde (rr) was found to be only 1.75% in his study. The most common genotype reported in whites was DCe/dce (R_1r) i.e. 39.4%, in blacks it was Dce/dce (R_0r) i.e. 45.8% and in Asians it was DCe/ DCe (R_1R_1) i.e. 51.8% [53].

Rh genotype is used in paternity testing, in hemolytic disease of new born (HDN) and predictably HDN by testing the father's Rh genotype. This helps to predict likelihood of HDN due to RhD antigen when mother has anti D. The most common Rh-genotype of the father will indicate whether the baby has 0%, 50% or 100% probability of being RhD positive. When undertaking RhD typing of patients and selecting blood donors, consideration must be given to the qualitative and quantitative variations in the expression of RhD antigen. During RhD antigen typing of patients it is important to detect all the weaker form of D, so that the patient is not unnecessarily transfused with Rh-D negative blood which would be wasting of scarce resource. When RhD typing of RhD negative women was done, it is important that women receive anti-D prophylaxis if the baby has a weak form of D. Patients with a partial D type will produce anti-D antibody when exposed to normal Rh-D positive donor blood. The partial D expression on a baby cell is poorly immunogenic and it is therefore not necessary to offer anti D prophylaxis to Rh-D negative mother of such infants. There is a different approach when D typing of blood donor is done, where it is necessary to detect partiality D type in order to avoid mistakenly transfusing Rh-D partial blood to RhD negative patients, as this may result in induction of anti-D antibody.

5. CONCLUSION

It was concluded through our study that: 1). Most frequent antigen amongst five major antigens of Rh system was RhD while the least common was antigen E. 2). Most common phenotype was DCCee. 3). The most frequent probable genotype was DCe/DCe (R_1R_1) while in Rh negative samples it was dce/dce (rr). 4). Phenotype and probable genotype showed wide range of variations in different races and religion. 5). Reliable population based frequency data of Rh antigens study has vital role in population genetic study, in resolving medico legal issues and most importantly in transfusion practice.

Here we recommend that Rhesus antigenic phenotyping and genotyping along with antibody screening and their identification prior to transfusion to patients with the history of multi-transfusion or multi-parity in females is most vital in transfusion practice in this modern era.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images on the institutional Patient Consent Form.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee of Gajra Raja Medical College, Gwalior, India, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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