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Distribution and frequency of principal Rh blood group antigens (D, C, c, E, and e) and their phenotypes in the blood donors attending blood bank in a tertiary care hospital in Barpeta district of Assam

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Abstract:

BACKGROUND: The ABO and Rhesus grouping system antigens have been found to have the highest immunogenicity and propensity to produce alloantibodies that cause most of the transfusion reactions. The Rhesus antigens that produce most of the immunogenic transfusion reactions are D, C, c, E, and e. Knowledge of the distribution of these Rh antigens in a population helps to render compatible blood in alloimmunized patients.

AIM: The aim was to study the distribution and frequency of principal Rh blood group antigens (D, C, c, E, and e) and their phenotypes in the blood donors attending blood bank in a tertiary care hospital in Barpeta district of Assam.

MATERIALS AND METHODS: The study was conducted in 315 voluntary blood donors in the blood bank of a tertiary care center. Rh-D typing was done by conventional tube method. Specific monoclonal antisera, i.e., anti-C, anti-c, anti-E, and anti-e, were used and tests were performed by conventional tube method for detection of the presence of rest of the major Rh antigens.

RESULTS: The samples were analyzed for the five major Rhesus antigens. "D" antigen was found to be the most common antigen (99.05%), followed by e (97.14%), C (92.38%), c (51.43%), and E (20.95%). In order of descending frequency, the most common phenotypes were DCCee – 45.71%, DCcee – 30.48%, DCcEe – 11.43%, DccEe – 4.76%, DCcEE – 1.90%, DCCEe – 1.90%, Dccee – 1.90%, DCCEE – 0.95%, and dccee – 0.95%.

CONCLUSION: D antigen is the most common antigen in our study population, whereas "e" antigen is the most common in most of the studies done from other parts of India. Data on frequencies of major Rh antigens in the local donor population will help in transfusing alloimmunized patients with corresponding antibody-negative blood ensuring blood safety.

Keywords:

Blood group, C antigen, c antigen, D antigen, E antigen, e antigen, Rhesus antigen

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Introduction

Rh blood group system is clinically relevant in blood transfusion services as anti-D IgG antibodies may develop in

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Rh-negative patients who receive Rh-positive blood transfusion which can later cause hemolytic disease of fetus and newborn and also hemolytic transfusion reaction.[1]

The Rh system is one of the most complex blood group systems, and Rh (D) antigens have greater immunogenicity than all other red cell antigens except A and B antigens. In 1939, Levine and Stetson described an antibody in the serum of a group O mother who delivered a stillborn fetus and subsequently developed symptoms of hemolytic transfusion reactions when transfused with her husband's group O blood.[2] In 1940, Landsteiner and Wiener immunized rabbits and guinea pigs with red cells of Rhesus monkey. The serum of the immunized rabbits contained an antibody, anti-Rh, which agglutinated 85% of human red cells.[3] At that time, it was thought that both antibodies have the same pattern of reactivity which led to the discovery of the Rh system. However, in 1963, Levine *et al*. established that the animal anti-Rhesus of Landsteiner and Wiener was not identical to the human antibody, anti-Rh antibody.[4] Anti-Rhesus formed by animals was renamed as anti-LW in honor of Landsteiner and Wiener.[5] However, the name Rh was retained for the human-produced antibody. 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 RBCs.[6] As no "d" antigen has been found on RBC, so "d" gene is considered as an amorphous gene. Although there are more than 50 antigens in the Rh system, D, C, c, E, and e are the most commonly identified and most significant antigen in blood transfusion services as these five principle antigens are responsible for majority of clinical significant antibodies.

Blood group prevalence in any given population is the presence of permanent inherited characteristics at the phenotypic level in that population.[7] The phenotype of a blood group of an individual denotes the observable expression of the genes inherited by the person. Common Rh antigens on red blood cells can be detected by antisera which represent their phenotype.[8]

Although antibodies of different blood group systems play a role in blood transfusion and pregnancy, all are not clinically significant. Clinically significant antibodies can cause hemolytic transfusion reactions following transfusion of blood. However, the current practice of providing compatible blood when emergency transfusion is required to alloimmunized patients in India is reliant upon random cross-matching of available units in the inventory as it is not practically feasible and also expensive to match for all minor antigens before transfusion so as to avoid alloimmunization. These alloimmunized patients who developed alloantibodies in their blood must receive corresponding antigen-negative blood to prevent transfusion-related reaction. It has been noticed that during compatibility testing, antibodies against Rh and Kell blood group systems are common.[9,10]

The reported prevalence of different Rh group antigens varies with race as the prevalence of D antigen in Indians is 93.6%, whereas in China, it was 99%.[11,12] There is wide variation in the distribution and frequency of Rh antigens throughout the world[12-15] and India.[11] Lack of study from North Eastern part of India, especially in the population of Assam, impelled us to do this study to identify the frequency of five major Rh antigens and its phenotype.

This research was conducted with the aim to study the distribution and frequency of principal Rh blood group antigens (D, C, c, E, and e) and their phenotypes in the blood donors attending blood bank in a tertiary care hospital in Barpeta district of Assam.

Materials and Methods

This is a prospective hospital-based observational study conducted in the Blood Bank, Department of Pathology, Fakhruddin Ali Ahmed Medical College and Hospital, Barpeta, Assam, for a period of 1 year from January 22, 2016, to January 21, 2017. Ethical approval from the Institutional Ethical Committee was obtained. Three hundred and fifteen blood donors who were eligible as per the Drugs and Cosmetics Act, 1940 and Rules, 1945 and willing to donate blood were selected for the study after obtaining informed consent. At the end of donation, blood samples were collected in 2 mL ethylenediaminetetraacetic acid vials. Before proceeding to extended Rh phenotyping, forward and reverse ABO grouping was performed by conventional tube method. For forward grouping, commercially available monoclonal blood group antisera, i.e., antiA, antiB, antiAB, antiH, and antiA_1 (Tulip Diagnostics Pvt. Ltd., Verna, Goa, India), were used, while for reverse grouping, 5% pooled cell suspension of A, B, and O cells prepared in our blood bank was used. Rh-D typing was done by tube methods using antisera from two different companies (Tulip Diagnostics Pvt. Ltd., Verna, Goa, India, containing monoclonal IgM antibody and Span Diagnostics Pvt. Ltd., Surat, India, containing both monoclonal IgM and IgG). All Rh "D-" negative samples were subjected to weak D testing by an indirect antiglobulin test according to standard operating procedures using a blended IgG and IgM anti-D antisera. Apart from antiD, for detection of status of rest of the major antigens of Rh system, specific monoclonal antisera, i.e., anti-C, anti-c, anti-E, and anti-e, were used and tests were performed by conventional tube methods as per the manufacturer's instruction (DiaMed,

Switzerland). False-positive and false-negative results were reduced as much as possible by taking quality control measures at each step.

Calculation of Rh red cell antigen and phenotype frequencies of the various blood group systems was calculated by summation of the number of donors positive for a particular antigen phenotype divided by the total number of donors screened. Results were expressed as a percentage. By using antisera D, C, E, c, and e, five major antigens for Rh system were tested in donors RBC, the phenotype of which is reflected in the results by using Wiener's nomenclature. Determination of exact genotype is not possible without testing parents and other family members or by DNA testing. For this reason, the most probable genotype is determined from gene frequency estimates.

Statistical analysis

Descriptive statistical methods were used to describe the findings of the study.

Results

The sample size of this study is 315, as collected from voluntary blood donors of our Blood Bank. The age of the donors varied from 18 years to 50 years. The mean age of the donors was 35 years. Out of 315 samples, males were 288 (91.43%) while females were 27 (8.57%). The percentage of Rh-D antigen positivity is shown in Table 1. No sample was reported as DU variant.

Of the five major Rh antigens, "D" antigen was found to be the most common antigen (99.05%), followed by e (97.14%), C (92.38%), c (51.43%), and E (20.95%) [Table 2]. Gender-wise distribution of principal Rh-antigens is shown in Table 3.

In order of descending frequency, the most common phenotypes were DCCee, followed by DCcee, DCcEe, DccEe, DCcEE, DCCEe, Dccee, DCCEE, and dccee. The most common phenotype in Rh positive was DCCee – 45.71%, while in Rh-D-negative sample, it was dccee – 0.95% [Table 4].

In our study, the most common probable genotype was DCe/DCe (R_1R_1) – 45.71% followed by DCe/Dce (R_1R_0) – 30.48%, DCe/DcE (R_1R_2) – 11.43%, DcE/Dce (R_2R_0) – 4.76%, DcE/DCE (R_2R_Z) – 1.90%, DCe/DCE (R_1R_Z) – 1.90%, DCE/DCE (R_ZR_Z) – 0.95%, and dce/dce (rr) - 0.95% [Table 5]. The allele frequency of Rh antigens in the study population is shown in Table 6.

Table 1: Distribution of Rh-D positive and negative in the present study (315 samples)

Rh-D positive (%)	Rh-D negative (%)
312 (99.05)	3 (0.95)

Table 2: Distribution of five major Rh antigens in the present study (315 samples)

D (%)	C (%)	E (%)	c (%)	e (%)
312 (99.05)	291 (92.38)	66 (20.95)	162 (51.43)	306 (97.14)

Discussion

In our study, the age group of donors varied from 18 years to 50 years, whereas the male-to-female ratio was 10.67:1. Khattak *et al*. reported a male-to-female ratio of 3.02:1 from Pakistan.[13] The variation is explainable from the fact that sample was drawn from voluntary blood donors at the blood bank.

As observed from the present study, the most prevalent antigen is D. The overall positivity of Rh-D antigen is 99.05%, while Rh-D-negative blood group accounted for 0.95% of total blood donors. Rh-D antigen frequency varies in different parts of the world with the highest rate in the Japanese and Burmese population (99%–100%) and lower rate in European population (85%).[14] Karim *et al*. and Anwar *et al*. from Pakistan reported 97% and 95%, respectively, for Rh-D antigen frequency.[15,16] Similarly, studies done in Bangladesh by Shil *et al*. and Nepal by Pramanik *et al*. reported 94.6% and 96.7%, respectively.[17,18] Studies done in Iran, Saudi Arabia, Palestine, and the UAE reported prevalence of Rh-D antigen as 90.2%, 93%, 92%, and 91.1%, respectively.[19-22] Most of the studies in India showed the Rh positivity within the range of 91%–97%. Thakral *et al*. (North India), Sharma *et al*. (Central India), Gundrajukuppam *et al*. (South India), and Basu *et al*. (Eastern India) reported Rh-D positivity as 91.6%, 93.3%, 94.1%, and 96.60%, respectively.[23-26] Kahar *et al*. and Gajjar *et al*. have obtained a Rh-D-negative prevalence of 15.65% and 16%, respectively.[27,28] Similarly, the prevalence of Rh-D-negative phenotype is higher in Caucasian 15% but lower in a neighboring country, China (1%).[12,14] Studies done in different parts of India have shown a higher Rh-D-negative prevalence ranging from 4.29%–5.80% than the present study. Garg *et al*. from Delhi have reported 93.8% for Rh positive and 6.20% for Rh negative.[29] A study conducted by Gupta *et al*. has also documented similar

Table 3: Gender-wise distribution of principal Rh antigens

	Rh antigen	D	C	E	C	e
Male	Positive	285 (98.96)	264 (91.67)	63 (21.87)	150 (52.08)	279 (96.87)
Female	Positive	27 (100)	27 (100)	03 (11.11)	12 (44.44)	27 (100)
Total		312	291	66	162	306

findings with 94.2% for Rh-D positive and 5.80% for Rh-D negative.[30] Rh antigen frequency in our study group was compared with other studies in the world [Table 7] and different populations of India [Table 8].

Sharma *et al*. from Gwalior and Chambal region have detected 1.6% cases of D^u variant.[24] In our study, no cases of D^u variant was detected while Makroo *et al*. from Delhi have reported incidence of weak D as 0.12% among Rh-negative individuals.[11]

The frequency of other major antigens in Rh-D positives and negatives is shown in Table 9. It is seen that "e" antigen is always associated with both Rh-D positive and Rh-D negative (nearly 100%), but "E" antigen is less common in both the cases.

The frequency of Rh-e antigen in the study population was 97.14%. In most of the population of the world, high frequency of Rh-e antigen is seen accounting for approximately 98% [Table 8]. Makroo *et al*., Thakral *et al*., Gundrajukuppam *et al*., Kahar *et al*., Garg *et al*., Gupta *et al*., and Sarkar *et al*. reported similar frequency of Rh-e antigen from different regions of India [Table 8]. However, Sharma *et al*. from Central India reported the number of cases of Rh-e as 78.5%.[24] It would be difficult to find "e" antigen-negative donor for a patient with alloimmunization against this antigen since 98% of the population has the "e" antigen. Anti-e is often seen as autoantibody which will also make it difficult to find compatible blood.

Table 4: Distribution of Rh phenotype in the present study

Phenotype	Number of cases (%)
DCCee	144 (45.71)
DCcee	96 (30.48)
DccEe	15 (4.76)
DCcEE	6 (1.90)
DCcEe	36 (11.43)
DCCEe	6 (1.90)
Dccee	6 (1.90)
DCCEE	3 (0.95)
dccee	3 (0.95)

The frequency of Rh-C antigen in this study was 92.38%, which is similar to the findings reported by different authors from India as well as from other countries with a range from 87% to 93%.[11,12,16,25,29,30]

The frequency of Rh-c antigen (51.43%) in this study is similar to the other studies reported from India [Table 8]. However, it varies among different populations of the world. In Asia, it occurs in a frequency of 62.8%, 73.9%, 81%, and 71% in Pakistan, Iran, Palestine, and the UAE, respectively.[16,19,21,22] Other studies in Europe and Africa have documented 80% and 99.8%, respectively.[14,31]

Least prevalent antigen in our study was "E" (20.95%) which is similar to the studies by Makroo *et al*. (20%), Gundrajukuppam *et al*. (18.8%), Kahar *et al*. (21.74%), Garg *et al*. (21.1%), and Gupta *et al*. (18.6%).[11,25,27,29,30] Rh-E is also the least common Rh antigen worldwide as a study conducted by Karim *et al*. in Pakistan, Anwar *et al*. in Pakistan, Janan Y Taha in the UAE, and Jeremiah ZA *et al*. in Nigeria has reported 19%, 22.6%, 21%, and 20.5%, respectively.[15,16,22,31] "E" is a strong immunogenic antigen, but due to its low frequency in population, transfusion reaction due to E antigen is least common among Rh antigens.

Sharma *et al*. reported that the most common antigen was Rh-D (91.6%) followed by Rh-C (84%), Rh-e (78.5%), Rh-c (58.3%), and Rh-E (25.6%).[24] In our study also, Rh-D was the most common Rh antigen (99.05%). Makroo *et al*., Thakral *et al*., Gundrajukuppam *et al*., Kahar *et al*., Garg *et al*., and Gupta *et al*. have documented the frequency of Rh antigens in the following order, Rh-e, RhD, Rh-C, Rh-c, Rh-E.[11,23,25,27,29,30] A study conducted on the population in China has reported that the most common Rh antigen was Rh-D (99%) followed by Rh-e (96%), Rh-C (93%), Rh-c (47%), and Rh-E (39%).[12]

The most common phenotype in Rh-positive samples in our study was DCCee (45.71%) while the least common was DCCEE (0.95%). In Rh-negative sample, dccee (0.95%) was the only sample in this study population. Similar to the present study, other studies

Table 5: Reaction pattern with antisera, phenotype, possible genotype, and probable genotypes in the present study

Reaction with test sera					Phenotype	Number of cases (%)	Possible genotype	Most probable genotype
D	C	c	E	e				
+	+	−	−	+	DCCee	144 (45.71)	R1R1, R1r'	DCe/DCe (R1R1)
+	+	+	−	+	DCcee	96 (30.48)	R1R0, R1r, R0r'	DCe/Dce (R1R0)
+	−	+	+	+	DccEe	15 (4.76)	R2R0, R2r, R0r''	DcE/Dce (R2R0)
+	+	+	+	−	DCcEE	6 (1.90)	R2RZ, RZr'', R2rY	DcE/DCE (R2Rz)
+	+	+	+	+	DCcEe	36 (11.43)	R1R2, RZr, R2r', R1r'', RZR0, R0rY	DCe/DcE (R1R2)
+	+	−	+	+	DCCEe	6 (1.90)	R1RZ, RZr'', R2rY	DCe/DCE (R1Rz)
+	−	+	−	+	Dccee	6 (1.90)	R0R0, R0r	Dce/Dce (R0R0)
+	+	−	+	−	DCCEE	3 (0.95)	RZRZ, RZRY	DCE/DCE (RzRz)
−	−	+	−	+	dccee	3 (0.95)	Rr	dce/dce (rr)

done in India by Makroo *et al*., Thakral *et al*., Sharma *et al*., Gundrajukuppam *et al*., Basu *et al*., and Kahar *et al*. have also reported that DCCee was the most common phenotype with 42.6%, 43.8%, 41%, 43.4%, 49.02%, and 40.87%, respectively[11,23-27] [Table 10]. In contrast, the predominant Rh phenotype reported in White is DCcee (34.9%) and the least common was DCCEE (0.01%).[14] In Black, the most common Rh phenotype reported is Dccee (45.8%) and the least common is DccEE (0.2%).[14] Studies done by Karim *et al*. (41%) from Pakistan have also observed similar findings.[15]

Table 6: Presumptive allele frequency of Rh antigens in the study population

Allele	Frequency
D	0.834
D	0.166
C	0.61
C	0.39
E	0.2
E	0.8

Table 7: Rh antigen frequencies in our study are compared with other studies in different populations of the world

Particulars	D (%)	C (%)	E (%)	c (%)	e (%)
Present study	99.05	92.38	20.95	51.43	97.14
Chinese[12]	99	93	39	47	96
Caucasian[14]	85	68	29	80	98
Blacks[14]	92	27	22	96	98
Karim *et al*.[15]	97	87	19	57	99
Anwar *et al*.[16]	95	89.6	22.6	62.8	97
Keramati *et al*.[19]	90.2	75.9	29.5	73.9	97.79
El-Wahhab Skaik[21]	92	69	38	81	97
Taha[22]	91.1	73.2	21	71	97.3
Jeremiah and Buseri[31]	95	17.7	20.5	99.8	98.7

Table 8: Comparison of frequency of Rh antigen in the present study with different populations in India

Particulars	D (%)	C (%)	E (%)	c (%)	e (%)
Present study	99.05	92.38	20.95	51.43	97.14
Makroo *et al*.[11]	93.6	87	20	58	98
Thakral *et al*.[23]	93.3	84.76	17.9	52.82	98.3
Sharma *et al*.[24]	91.6	84	25.6	58.3	78.5
Gundrajukuppam *et al*.[25]	94.1	88	18.8	54.9	98.4
Kahar and Patel[27]	84.34	81.74	21.74	56.52	100
Garg *et al*.[29]	93.8	91.8	21.1	55.2	98.7
Gupta *et al*.[30]	94.2	88.6	18.6	54.8	98.2
Sarkar *et al*.[32]	92.25	87.55	26.55	51.06	98.42

There are different factors responsible for alloimmunization in multiple transfused cases. Difference in RBC surface antigenic profile between blood donor and recipient is one among them. Antibody specificity is also dependent upon age, sex, number, and time interval between transfusions.[33] However, the frequency of alloimmunization may also vary with underlying pathophysiology of the transfused patients. The overall frequency of alloimmunization in case of multiple transfused cases is approximately 2%–6%.[34] In thalassemia, the alloimmunization rate ranges from 4% to 7% in India.[35]

Clinical importance of Rh alloimmunization

Antenatal antibody screening mainly focuses on detection of anti-D in Rh-D-negative mothers as anti-D in Rh-D-negative women as Rh alloimmunization is a major cause of severe hemolytic disease of the fetus and newborn (HDFN). It has been found that women are more likely to have alloantibodies than men as they get sensitized during pregnancy.[36] The incidence in patients who received multiple transfusions due to various causes has been reported to vary from 8% to 76% among different countries.[37] In India, the reported prevalence of alloimmunization in multitransfused patients is comparatively low varying from approximately 3% to 10%.[38-40] A study done by Pahuja *et al*., Dhawan *et al*., and Datta *et al*. has reported a low rate of alloimmunization (3.79%, 5.64%, and 5.6%, respectively) which may be explained by presumed high phenotypic compatibility between blood donors and the patients.[10,41,42] Sahoo *et al*. show the rate of alloimmunization as 3.6% in pregnant women which is higher in comparison to other parts of India studied by Varghese *et al*. (1.48%), Pahuja *et al*. (1.25%), Suresh *et al*. (1.1%), and Das *et al*. (2.27%).[43-47] As reported by Handa *et al*., the overall rate of alloimmunization in the study was 7% which is similar with the study done by Pimpaldara *et al*.[48,49] However, Handa *et al*. reported that the rate of alloimmunization in thalassemia was 7.4% as similar results were observed by Pradhan *et al*. (8%) and Gupta *et al*. (9.48%) on thalassemia.[35,48,50] The alloimmunization rate reported by Varghese *et al*., Das *et al*., and Sahoo *et al*. in Rh-D-negative blood group was 9.43%, 6.9%, and 4.42%, respectively, and in Rh-D-positive blood group was 0.08%, 1.1%, and

Table 9: Frequency of other principal antigens in Rh-D positive/Rh-D negative

	In Rh-D positive (%)				In Rh-D negative (%)			
	C (%)	E (%)	c (%)	e (%)	C (%)	E (%)	c (%)	e (%)
Present study	93.27	21.15	50.96	97.11	0	0	100	100
Thakral *et al*.[23]	90.15	18.9	49.48	98.1	8.54	-	100	100
GundrajuKuppam *et al*.[25]	92.5	19.4	52.1	98.3	15.25	8.47	100	100
Kahar and Patel[27]	93.81	22.68	50.52	100	16.67	16.67	88.89	100

Table 10: Comparison of prevalence of Rh phenotypes worldwide in percentage

Weiner	R_1R_1 (%)	R_1R_2 (%)	R_1R_0 (%)	R_zR_z (%)	rr (%)	R_0R_0 (%)	R_1R_z (%)	R_2R_0 (%)	R_2R_z (%)	R_2R_2 (%)
Fisher race	DCCee	DCcEe	DCcee	DCCEE	dccee	Dccee	DCCEe	DccEe	DCcEE	DccEE
Present study	45.71	11.43	30.48	0.95	0.95	1.90	1.90	4.76	1.90	-
Thakral *et al*.[23]	43.8	8.22	30	-	5.81	0.97	-	8.95	-	1.45
Makroo *et al*.[11]	42.6	14.5	32.2	-	4.6	1.3	0.5	0.1	1.1	0.8
Sharma *et al*.[24]	41	3.1	25.5	1.5	5.6	3.0	2.2	5.5	3.3	4.7
GundrajuKuppam *et al*.[25]	43.4	10.7	31.2	0.4	4.7	1.2	1.3	0.5	0.2	0.7
Whites[14]	18.5	13.3	34.9	0.01	15.1	2.1	0.2	11.8	0.1	2.3
Blacks[14]	2.0	4.0	21	-	6.8	45.8	-	18.6	-	0.2
Basu[26]	49.02	13.74	27.75	0.26	2.75	0.98	0.72	3.40	0	0.72
Kahar and Patel[27]	40.87	13.91	23.48		11.30	0.87	0.87	4.35	-	-

2.20%, respectively.[43,44,47] The rate of RhD-negative alloimmunization was less (4.42% vs. 10.4%) in the study done by Sahoo *et al.* than studied by Pahuja *et al*.[43,45] Published literature in India gives a different rate of alloimmunization among Rh-D-negative pregnancies which varies from 6.9% to 12.8%.[44,46,51] Pahuja *et al.*, Suresh *et al.*, and Sidhu *et al.* have reported 0.12%, 0.3%, and 0.45% as the rate of alloimmunization among Rh-D-positive patients.[45,46,52] As per studies conducted by Pahuja *et al.* and Sahoo *et al.*, 92.2% and 84.72% of the antibodies detected belong to Rh blood group system and 78.4% and 53.85% of the antibodies detected were anti-D antibody, respectively.[43,45] Other non-Rh-D antibodies that were found to cause HDFN are anti-C, anti-E, and anti-c. Sahoo *et al.* have reported 1.65% as the frequency of non-anti-D antibodies, and among them, anti-c (15.38%) was the most common antibody identified.[43] As reported by Varghese *et al.*, 64% of alloimmunized women have anti-D, c, E, e, C, and K antibodies which are commonly associated with HDFN.[44] Anti-E was the second most frequent antibody (28.5%) detected followed by anti-c (14.28%) as reported by Handa *et al*.[48] The most frequent and clinically significant antibody reported in the study done by Sahoo *et al*. was anti-D (1.93%) followed by anti-c (0.55%), anti-E (0.28%), and anti-D and anti-C combined (0.28%).[43] Sahoo *et al.* have also reported that the most common antibody detected among allosensitized D-negative women was found to be anti-D (63.64%) followed by combination of anti-D and anti-C antibody (9.09%).[43] In another study conducted by Rath *et al.*, the postnatal outcome of neonates with severe HDFN due to anti-C was found to be similar to HDFN due to Rh-D.[53] Among allosensitized-D-positive women, anti-c (33.33%), anti-E (33.33%), and anti-Lea (3.34%) antibodies were encountered in the study done by Sahoo *et al*.[43] Sankaralingam *et al.* have also reported that anti-E (85.7%) was the most common antibody in Rh-D-positive women.[54] This suggests that non-Rh-D antibodies were detected not only in Rh-D-negative mothers during pregnancy but also in Rh-D-positive women. Even though routine antibody screening in Rh-D-positive women is debatable, as the occurrence of allosensitization among Rh-D-positive women is low, yet whenever feasible, option of screening for such women should be made available. Despite established guideline by regulatory bodies, the use of anti-D prophylaxis in all enlisted events is low. As clinically significant antibodies can cause mild to severe HDFN and hydrops fetalis, non-Rh-D red cell antibodies associated with HDFN will also be a challenge to clinicians. As there is no definite guidelines for universal antibody screening for pregnant women in India, inclusion of such screening should also be advised to reduce the incidence of non-RhD-associated HDFN. In addition, extended Rh typing in a day-to-day clinical transfusion practice is also advisable. Regular screening for development of alloantibodies in multiple transfused patients will help in minimizing blood transfusion reaction and ensure blood safety to a great extent as corresponding antigen-negative blood can be given to such patients.

Conclusion

The knowledge of data on frequencies of antigens of Rh blood group system in local donor population helps in blood transfusion services, particularly in alloimmunized cases where clinically significant antibodies can be identified in patients' serum and hence corresponding antigen-negative blood can be given from donors' database. In our study, we have found D antigen as the most common in our population. We also found that e antigen is less common in our study population as found in Central India than other studies done from India in order of antigen frequency. Determination of Rh blood group phenotypic characteristics of subjects is of utmost importance to reduce transfusion-related reaction in the recipient and thereby improve blood safety.

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Conflicts of interest

There are no conflicts of interest.

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