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Original Article

Pathology Section

Prevalence of Principal Rh Blood Group Antigens in Blood Donors at the Blood Bank of a Tertiary Care Hospital in Southern India

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ABSTRACT

Introduction: Rhesus (Rh) antigen was discovered in 1940 by Karl Landsteiner and Wiener. Due to its immunogenicity along with A, B antigens, Rh D antigen testing was made mandatory in pre-transfusion testing. Presently there are more than 50 antigens in Rh blood group system but major ones are D, C, E, c, and e. Very few reports are available regarding their prevalence in India and no reports are available from Andhra Pradesh.

Aim: To study the prevalence of principal Rh blood group antigens like D, C, E, c & e in the voluntary blood donors attending our blood bank.

Materials and Methods: A prospective cross-sectional non interventional study was carried out on 1000 healthy blood donors from August 2013 to July 2014 at our blood bank. Donors were grouped and typed for ABO and Rh major antigens using monoclonal blood grouping reagents as per the manufacturer's instructions. Statistical analysis was carried out using SPSS version 16. Comparison of categorical data between antigen positive and negative individuals was done using Chi-square test. Descriptive statistics for the categorical variables were performed by computing the frequencies (percentages) in each category. Incidence was given in proportion with 95% confidence interval.

Results: A total of 1000 blood samples from donors were phenotyped. Among Rh antigens, e was the most common antigen (98.4%), followed by D-94.1%, C-88%, c-54.9% and E-18.8% with DCe/DCe (R1R1) (43.4%) being the most common phenotype and the least common phenotype is r'r' (0.1%).

Conclusion: Database for antigen frequency to at least Rh blood group system in local donors helps to provide antigen negative blood to patients with multiple alloantibodies, minimize alloimmunization rate, and thereby improve blood safety.

Keywords: Alloimmunization, Phenotype, Rh blood group antigens, Transfusion

INTRODUCTION

Prevalence of any blood group is the occurrence of permanent inherited characteristic at the phenotypic level in any given population [1]. Thus, the phenotype of a blood group of an individual is the observable expression of the genes inherited by the person and reflects the biologic activity of the genes. The presence or absence of antigens on red cells as determined by serological testing represents the phenotype [2].

Eighty percent of D negative patients exposed to D positive red cells may develop anti-D IgG antibodies that may persist for the rest of their lives, which can cause Haemolytic Transfusion Reaction (HTR) and Haemolytic Disease of Foetus and Newborn (HDFN) [3]. It is not practically feasible to match for all the minor antigens before transfusion so as to avoid alloimmunization. Though there are more than 50 Rh antigens, the five principal Rh antigens, i.e., D, C, c, E, and e are responsible for majority of clinically significant antibodies. After anti-D Rh antibodies, most commonly found antibody in the sera of alloimmunized individuals are anti-E >anti-c >anti-e >anti-C. Patients with alloantibodies must receive corresponding antigen negative blood. It has been observed that antibodies against Rh, Kell blood group systems are found to be more common and frequently encountered during compatibility testing [4,5]. The reported prevalence of the different Rh group antigens varies with race [6]. For example, the prevalence of D antigen in Indians is 93.6% where as in our neighboring country China, it was observed to be 99% [7]. India being vast country with diverse population groups, it is required that phenotypic frequencies be studied in different parts of India. Few such reports are available from North India [6,8-10]. Though one such study is available from one of the state of southern India [11]; no such reports are available from our state.

AIM

To study the prevalence of principal Rh blood group antigens like D, C, E, c & e in the voluntary blood donors attending our blood bank.

MATERIALS AND METHODS

This is a prospective, cross-sectional analytical study conducted at the Department of Transfusion Medicine attached to the tertiary care referral teaching hospital, Tirupati for a period of one year from August 2013 to July 2014 after obtaining approval from the Institutional ethical committee. Thousand blood donors who were eligible as per Drugs and Cosmetics Act, 1940 and Rules, 1945 [12] and willing to donate blood were selected for the study after obtaining informed consent. At the end of donation, blood samples were collected in 2mL ethylene diamine tetra acetic acid (EDTA) vials. Before proceeding to extended Rh phenotyping, the donor's ABO grouping was done and Rh D type was tested using antisera from two different companies (Tulip diagnostics Pvt. Ltd., Verna, Goa, India and Span diagnostics Pvt. Ltd., Surat, India); one containing monoclonal IgM antibody and the other one containing both monoclonal IgM and IgG respectively. All Rh 'D' negative samples were subjected to weak D testing by an Indirect Antiglobulin Test (IAT) according to our standard operating procedures. Detection of other major Rh antigens was done using monoclonal blood grouping reagents anti-C (anti-RH2), anti-E (anti-RH3), anti-c (anti-RH4), anti-e (anti-RH5) antisera (Ortho BioVue System, Raritan, New Jersey) in reverse diluent cassette of Ortho BioVue System (Ortho clinical diagnostics,1001 US Highway 202, Raritan, NJ 08869 USA) as per the manufacturer's instructions.

Calculation of red cell antigen and phenotype frequencies of the various blood group systems was calculated by totaling the number of donors positive for a particular antigen phenotype divided by the total number of donors screened. Results were expressed as a percentage. The allele frequencies were calculated under the standard assumption of Hardy-Weinberg equilibrium, using the counting method of Cepellini et al., [13]. 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 & e, phenotype of the patient is reflected in the results using Wiener nomenclature. Determination of exact genotype is not possible without testing parents and other family members or by DNA testing. For this reason, most probable genotype is determined from gene frequency estimates.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS version 16. Comparison of categorical data between antigen positive and negative individuals was done using Chi-square test. Descriptive statistics for the categorical variables were performed by computing the frequencies (percentages) in each category. Incidence was given in proportion with 95% confidence interval.

RESULTS

During the study period, antigen typing was done on 1000 voluntary blood donors. Of the five major antigens, "e" antigen was found to be the most common antigen (98.4%), followed by D (94.1%), C (88%), c (54.9%) and E (18.8%). Blood group wise and gender wise distribution of Rh principal antigens is shown in [Table/Fig-1,2]. Prevalence of antigens is similar in both the genders. There was no difference in distribution of antigens irrespective of their blood group. The most common phenotype observed was R1R1 (DCCee) followed by R1r (DCcee)> R1R2 (DCcEe)> R2r (DccEe)> rr(dce) and the least common being r'r' among the total study population. Most common phenotype in Rh positives is R1R1 and among Rh negatives is rr. Allele frequency is shown in [Table/Fig-3].

Blood Group	Rh antigens				
	D %	C %	E %	c %	e %
A (n=186)	94.6% (176)	87.09% (162)	23.11% (43)	52.7% (98)	96.2% (179)
B (n=339)	93.5% (317)	87.31% (296)	15.6% (53)	53.09% (180)	98.5% (334)
AB (n=60)	95% (57)	86.6% (52)	18.33% (11)	46.6% (28)	100% (60)
O (n=415)	94.2% (391)	89.1% (370)	19.56% (81)	58.45% (243)	99.03% (411)

[Table/Fig-1]: Blood group wise distribution of Rh principal antigens in the study population (n=1000).

Gender	D	C	E	c	e
Males (n=976)	920 (94.2%)	860 (88.11%)	182 (18.6%)	532 (54.5%)	960 (98.3%)
Females (n=24)	21 (87.5%)	20 (83.33%)	6 (25%)	17 (70.83%)	24 (100%)
Total	941	880	188	549	984

[Table/Fig-2]: Gender wise distribution of Rh principal antigens in the study population (n=1000).

Allele	Frequency
D	0.756
d	0.244
C	0.33
c	0.67
E	0.1
e	0.9

[Table/Fig-3]: Presumptive allele frequency of Rh antigens in the study population (n=1000).

	D(%)	C(%)	E(%)	c(%)	e(%)	Reference
Present study	94.1	88	18.8	54.9	98.4	
Caucasians	85	68	28	80	98	[14]
Blacks	92	27	22	96	98	[14]
Chinese	99	93	39	47	96	[7]
Sharma et al.,	91.6	84	25.6	58.3	78.5	[15]
Makroo et al.,	93.6	87	20	58	98	[6]
Kahar et al.,	84.34	81.74	21.74	56.52	100	[10]

[Table/Fig-4]: Comparison of prevalence of Rh antigens worldwide.

DISCUSSION

The knowledge of various blood group antigen and phenotype frequencies in a population is important in day to day work in a transfusion service in areas such as antenatal serology, paternity testing, and selecting compatible blood in problematic transfusions. While such data in Caucasian and Black races is readily available, information in Indian population is limited. Rh antigen prevalence among the study population was compared with that of other populations in [Table/Fig-4]. As observed from the present study, the most prevalent antigen is 'e' with 98.4% which is similar to studies from the other parts of India except that from Central India where it is lower (78.5%) [14]. Similar prevalence of 'e' antigen is observed in all the studies in India as well as outside India as seen in [Table/Fig-4]. Thus, it would be difficult to find 'e' antigen negative donor or for a patient with alloimmunization against this antigen. Least prevalent antigen being 'E' with 18.8%, but it is reported to be higher (25.6%) in a study from Central India [14]. The prevalence of Rh D antigen varies in different parts of world, with highest rate in the Japanese and Burmese population (100-99%) and least rate in the South France and Northern Spain (60-80%) [9]. Prevalence of D negative phenotype in our study was observed to be 5.9% and observed to be higher in Caucasians (15%) [15], lower in neighboring people, the Chinese (1%)[7]. Our observation is in correlation with other studies in blood donors in South India who have reported a similar prevalence rate [16,17]. The prevalence has been reported to be higher in a study by Gajjar et al., from Ahmedabad (West India) have phenotyped about 200 donors by tube technique and obtained a Rh D negative prevalence of 16% [18]. Other studies from North India show a slightly higher prevalence ranging from 6.4% to 16% [6,8-10,18,19]. A recent multicentre study involving different zones of India has shown a Rh D negative status in the range of 4.76% to 7.02% [19].

Next to D antigen, "c" is the most immunogenic antigen [20]. In our study we observed a frequency of 54.9%. Almost similar prevalence of "c" antigen was observed in various studies from India [8-10]. In contrast, phenotypic profile of Rh antigens, c and e of the Western world ranges from 80-99% [14].

Prevalence of other major antigens in Rh D positives and negatives is shown in [Table/Fig-5]. It is seen that c and e antigens are always associated with Rh D negatives in all the studies except in studies from Divjot singh [21] and Kahar et al., [10]. E antigen is found to be less common [21] and not seen in a study by Thakral et al., [8]. Rh phenotypes involving major antigens comparing with other studies in India and in different races are shown in [Table/Fig-6]. The commonest phenotype in our study was R1R1 constituting 43.4% of the whole study population and the least common phenotype being r'r' with 0.1%. In contrast, the predominant Rh phenotype reported in Whites is R1r - 34.9% and the least common being RZRZ (0.01%). In Blacks, the most common is R0r and the least common is R2R2 with 0.2% [15]. Most common phenotype

	In Rh D Positives (%)				In RhD negatives (%)				Reference
	C	E	c	e	C	E	c	e	
Present study	92.5	19.4	52.1	98.3	15.25	8.47	100	100	
Divjot singh	90.8	22.8	59.6	98.9	10	4.3	98.6	100	[21]
Thakral	90.15	18.9	49.48	98.1	8.54	-	100	100	[8]
Kahar et al.,	93.81	22.68	50.52	100	16.67	16.67	88.89	100	[10]

[Table/Fig-5]: Prevalence of other principal antigens in Rh D Positives.

	R1R1	R1r	R2r	R2R2	R2RZ	R1R2	R0r	R1RZ	RZRZ	rr	r'r	r"r	r'r'	r'r"	Reference
Present study n=1000	43.4	31.2	0.5	0.7	0.2	10.7	1.2	1.3	0.4	4.7	0.6	0.3	0.1	0.2	
Thakral et al., n=1240	43.8	30	8.95	1.45	-	8.22	0.97	-	-	5.81	0.56	3	-	-	[8]
Makroo et al., n=3073	42.6	32.2	0.1	0.8	1.1	14.5	1.3	0.5	-	4.6	0.3	0.2	-	-	[6]
Sharma et al., n=1000	41	25.5	5.5	4.7	3.3	3.1	3.0	2.2	1.5	5.6	1.3	1.3	0.3	-	[14]
Whites	18.5	34.9	11.8	2.3	0.1	13.3	2.1	0.2	0.01	15.1	0.8	0.9	-	5	[14]
Blacks	2.0	21	18.6	0.2	-	4.0	45.8	-	-	6.8	-	-	-	-	[14]

[Table/Fig-6]: Comparison of prevalence of Rh phenotypes worldwide in percentage.

among Rh positives is R1R1 with (46.12%) and the least common phenotype is R2RZ (0.21%). Among Rh negatives, most common phenotype is rr with a frequency of 79.6% and the least common is r'r' with 1.69% in our study. Variability in Rh phenotype distribution of different populations is probably responsible for the reported differences in the frequencies of alloimmunization. Knowledge of the phenotype in a particular population may be helpful in formulating population specific transfusion guidelines. The factors responsible for alloimmunization are complex; RBC antigenic difference between the blood donor and the recipient being one among them. In thalassemia, alloimmunisation rate ranges from 4-50% and it is still lower in more homogenous populations [22-26]. Dhawan et al., reported an alloimmunization rate of 5.64%. They reported that 52.17% belong to the antibodies of the Rh system (anti-E 17%, anti-D 13% and anti-C 13%) [5]. These antibodies may result in clinically significant haemolytic transfusion reactions particularly delayed haemolytic transfusion reactions (DHTR), difficulty in cross matching of blood, decreased red cell survival and increase in transfusion requirements. Agnihotri A in a study from Delhi had observed a frequency of 0.8% alloantibodies using an Asian cell panel during routine pre transfusion testing. The most common antibodies detected were those of the Rh system (41.6%) [27].

Priya S from Pondicherry had observed a prevalence of red cell antibodies among antibody screening in antenatal women as 0.8%. Anti-D was the most common (50%) antibody identified, anti D along with anti C was the next most common antibody [28]. In patients with Warm Autoimmune Haemolytic Anaemia (WAIHA), most of the antibodies appear to be "non specific". Many have specificity to an Rh antigen notably to "e" [29]. Auto antibodies sometimes, have been observed to have the specificity for an antigen such as "E"; they are termed as mimicking antibodies as they seem to be allo antibodies with anti-E specificity [30]. So, phenotyping for at least Rh blood group antigens can minimize alloimmunisation to some extent.

Results of typing do not define genotype. They can only be arrived. It is a powerful adjunct to serological testing for resolution of D status but routine implementation of it cannot be considered until Rh D genotyping methods become automated and readily available. Other molecular methods are reported to be more powerful today. The allelic diversity in this Rh system is a potential problem for a reliable genotyping by PCR based assays. Haemagglutination is still a powerful, practical and economical test with a specificity and sensitivity that is appropriate for clinical applications. However, the use of haemagglutination in conjunction with molecular techniques undoubtedly can enhance approaches for the treatment of Rh incompatibility [29].

Though blood group antibodies play a role in blood transfusion and pregnancy, all are not clinically significant. Clinically significant antibodies can cause mild to severe HDFN, haemolytic transfusion reactions following transfusion of incompatible blood. It is often difficult and expensive to provide antigen negative blood for transfusion to patients with clinically significant blood group antibodies in case transfusion support is required immediately [31]. Thus, phenotyping of donor RBCs assumes importance when clinically significant antibody to a particular antigen is present in the patient. In such a case corresponding antigen negative blood can be provided without much delay.

CONCLUSION

Reports available in literature regarding the incidence of alloimmunization, DHTRs, auto antibodies in WAIHA stress the need for phenotyping. So phenotyping of red cells if routinely done, improves the blood safety, prevents alloimmunization not only in multi transfused patients (thalassemia, oncology patients) but also in other groups of patients like women with post partum haemorrhage, in life threatening AIHA, in natural calamities, to tackle emergency situations for antigen negative blood, and for predicting genotype. Like blood group typing, if the phenotyping of the person is known, even if he/she travels to other parts of the world, suitable blood can be made available in appropriate time.

In a resource limited country like ours, phenotyping of all the minor blood group antigens could be a financial constraint. In all the reports regarding alloimmunization, predominant antibodies observed belong to Rh blood group system. Hence, routine antigen typing at least for Rh antigens may help in decreasing the RBC alloimmunization and DHTR.

REFERENCES

[1] Francis CL. Blood group genetics. In: Roback JD, Grossman BJ, Hillyer CD editors. AABB Technical Manual 17th ed. Bethesda MD: aabb press; 2011:27-62.

[2] Kulkarni SS. Genetics of Rh Blood group system In: Snehalatha Gupte, Desai PK editors. Recent trends in Transfusion Medicine. published by Surat Raktadan Kendra and research centre 2002;98.

[3] Westhoff CM and Siegel DL. Rh and LW Blood Group Antigens In: Simon TM, Synder EL editors. Rossi's Principles of Transfusion Medicine 4th ed, Oxford. Wiley Blackwell aabb press 2009;109-20.

[4] Saied DA, Kaddah AM, Badr Eldin RM, Mohaseb SS. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent Egyptian thalassemic patients. *J Pediatr Haematol Oncol*. 2011;33:409-14.

[5] Dhawan HK, Kumawat V, Marwaha N, Sharma RR, Sachdev S, Bansal D, et al. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. *Asian J Transfus Sci*. 2014;8:84-88.

[6] Makroo RN, Bhatia A, Gupta R , Phillip J. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. *Indian J Med Res*. 2013;137:521-26.

[7] Lin-Chu M, Broadberry RE, Chan FJ. The blood group antigens and alloantibodies among Chinese in Taiwan. *Transfusion*. 1988;28:350-52.

[8] Thakral B, Saluja K, Sharma R, Marwaha N. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in north Indian blood donors. *Transfus Apher Sci*. 2010;43:17-22.
[9] Sarkar RS, Philip J, Mallhi RS and Yadav P. Proportion of Rh phenotypes in voluntary blood donors. *Med J Armed Forces India*. 2013;69:330-34.
[10] Kahar MA, Patel RD. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis and Lutheran) in blood donors of south Gujarat, India. *Asian J Transfus Sci*. 2014;8:51-55.
[11] Hamsavardhini S. Phenotyping of rare blood group antigens. Abstract of 37th Annual Conference of Indian Society of Blood Transfusion & Immunohaemotology (ISBTI). *Asian J Transfus Sci*. 2011;5:63-109.
[12] Vijay Malik. Criteria for blood donation: Law Relating to Drugs & Cosmetics, 22nd ed.Eastern Book Company publishers, Lucknow 2011; pp. 323-24.
[13] Cepellini R, Siniscalco M, Smith CA. The estimation of gene frequencies in a random mating population. *Ann Hum Genet*. 1955;22:97-115.
[14] Reid ME, Francis CL. Blood group antigen factsbook. 2nd ed. San Diego: *Academic Press*. 2004.
[15] Sharma DC, Singhal S, Rai S, Iyenger S, Sao S and Jain B. Incidence of Rh antigens, phenotype & probable genotype in the population of Gwalior and Chambal region, Central India. *Int Blood Res Rev*. 2013;1:29-43.
[16] Das PK, Nair SC, Harris VK, Rose D, Mammen JJ, Bose YN, et al. Distribution of ABO and Rh-D blood groups among blood donors in a tertiary care centre in South India. *Trop Doct*. 2001;31:47–48.
[17] Periyavan S, Sangeetha SK, Marimuthu P, Manjunath BK, Seema DM. Distribution of ABO and Rhesus-D blood groups in and around Bangalore. *Asian J Transfus Sci*. 2010;4:41.
[18] Gajjar M, Adulkar D, Bhatnagar N, Sonani R, Patel T, Gupta S. Frequency Of ABO And Rh Blood Groups In Blood Donors: A Study From A Tertiary Care Teaching Hospital In India. *NJIRM*. 2013;4:69-73.
[19] Agrawal A, Tiwari AK, Mehta N, Bhattacharya P, Wankhede R, Tulsiani S, et al. ABO and Rh (D) group distribution and gene frequency; the first multicentric study in India. *Asian J Transfus Sci*. 2014;8:121-25.
[20] Davies SC, McWilliam AC, Hewitt PE, Devenish A, Brozovic M. Red cell alloimmunization in sickle cell disease. *Br J Haematol*. 1986;63:241-45.
[21] Lamba DS, Kaur R, Basu S. Clinically Significant Minor Blood Group Antigens amongst North Indian Donor Population. *Adv Haematol*. 2013;2013:215454.
[22] Wang LY, Liang DC, Liu HC, Chang FC, Wang CL, Chan YS, et al. Alloimmunization among patients with transfusion-dependent thalassemia in Taiwan. *Transfus Med*. 2006;16:200-03.
[23] Sirchia G, Zanella A, Parravicini A, Morelati F, Rebulla P, Masera G. Red cell alloantibodies in thalassemia major. Results of an Italian cooperative study. *Transfusion*. 1985;25:110-12.
[24] Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. *Blood*. 2000;96:3369-73.
[25] Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multitransfused thalassemics of Indian Origin. *Haematology*. 2010;15:174-77.
[26] Moreira Junior G, Bordin JO, Kuroda A, Kerbauy J. Red blood cell alloimmunization in sickle cell disease: the influence of racial and antigenic pattern differences between donors and recipients in Brazil. A*m J Haematol*. 1996;52:197-200.
[27] Agnihotri A. Antibody screening with the Asian cell panel: Can we still do away with cross-match? Abstract of 2nd Indian Society of Transfusion Medicine (ISTM) Annual Conference. *Asian J Transfus Sci*. 2014;8(Suppl S1):26-87.
[28] Priya S. Frequency of Rh and irregular antibodies among pregnant women and its implications. Abstract of 2nd Indian Society of Transfusion Medicine (ISTM) Annual Conference. *Asian J Transfus Sci.* 2014;8(Suppl S1):26-87.
[29] Avent ND, Reid ME. The Rh blood group system: a review. *Blood.* 2000;95:375-87.
[30] Duffy TP. Autoimmune Haemolytic Anaemia and Paroxysmal Nocturnal Haemoglobinuria. In: Simon TL, Snyder EL, Solheim BG, Stowell CP, Strauss RG and Petrides M editors. Rossi's Principles of Transfusion Medicine, 4th ed. USA, Blackwell Publications 2009; pp 323.
[31] Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. *Transfus Med Rev*. 2007;21:58-71.

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