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RESEARCH ARTICLE

STUDY OF RH-D, C,C,E & E ANTIGENS ON RED BLOOD CELLS TO FIND OUT THE PERCENTAGE OF PHENOTYPES AND ITS FREQUENCY IN AN INDIAN POPULATION

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ABSTRACT

The phenotype of a red cell is defined by the presence or absence of D,C,c,E, and e antigens. A detailed knowledge of Rh system gene frequencies in an Indian population is very important in blood transfusion service in area such as antenatal work, percentage testing and for selecting compatible blood in problem transfusion. From serological studies it is often impossible to determine the true genotype of person in the absence of family information, phenotypes are often symbolized as the most probable genotype deduced from known haplotype frequencies. Red blood cell transfusion in patients with clinically significant mixture of antibodies require the availability of matched blood units lacking the antigens to which antibodies are directed. This implies the need to phenotype RBC unit to one or several antigenic system since only ABO and RH-D are usually typed. The Rh phenotypes and its frequencies have been studied and established in many western countries, as well as in Asia, whereas very sporadic data is available on phenotyping and its frequency in Indian population.

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INTRODUCTION

Rh blood group phenotype is the most complex of the blood group system. It is comprised of 45 antigens numbered Rh- to Rh-51 with six antigens Obsolete. Rh antigen are encoded by two highly homologous, closely linked genes on theChromosome-1. RhD gene is responsible for producing D antigen whereas RhCE genes control the production of Cc and Ee antigen. D antigen is present on red cell membrane of about 85% of populations. The absence of D antigen generally result from a deletion of Rh-D and no antigen antithetical to D has been identified till date. C and c, E and e, represent two pairs of antithetical antigens, which are controller by the second gene of the Rh system. The phenotype of a red cell is defined by the presence or absence of D,C,c,E, and e antigens. A detailed knowledge of Rh system gene frequencies in an Indian population is very important in blood transfusion service in area such as antenatal work, percentage testing and for selecting compatible blood in problem transfusion. From serological studies it is often impossible to determine the true genotype of person in the absence of family information, phenotypes are often symbolized as the most probable genotype deduced from known haplotype frequencies. Red blood cell transfusion in patients with clinically significant mixture of antibodies require the availability of matched blood units lacking the antigens to which antibodies are directed. This implies the need to phenotype RBC unit to one or several antigenic system since only ABO and RH-D are usually typed. The Rh phenotypes and its frequencies have been studied and established in many western countries, as well as in Asia, whereas very sporadic data is available on phenotyping and its frequency in Indian population. Keeping these point in view, we have studied the Rh-phenotype and its frequency in Indian population.

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MATERIALS AND METHODS

Collection of Blood Samples:Blood samples were collected in CPDA-1 from Indian Red Cross Society (IRCS), New Delhi, where donors come from all parts of the Country. All blood samples were tested at the IRCS, New Delhi by ELISA method for HBsAg, HIV 1/2, VDRL etc. Non reactive samples were used for further studies. Collected samples were transported under prescribed guidelines and temperature.

Preparation of Red cells and plasma for grouping. sub-grouping & phenotyping

Whole blood was centrifuged for 1 minute at 1000 rpm. Red blood cell and plasma were separated using 5 ml transpipette. Red blood cells were washed three to four times (clear supernatant) by normal saline (0.9% Nacl analytical grade). Finally 2 and 40 % cell suspension were prepared in normal saline for grouping, sub- grouping and phenotyping as per standard procedure. Grouping, Sub grouping & Phenotyping: ABO and Rh-D group of blood cells are confirmed by test tube method using 2% cell suspension and anti-sera e.g. Anti-A, Anti-B, Anti-A B, Anti-D. Anti-Ai (Lectin) was used for sub-grouping of A and A B blood group cells. After grouping and sub-grouping, phenotyping was done by test tube method using rare anti-sera e.g. Anti-C, Anti-c, Anti-E, Anti-e as per standard procedure 15, in brief, 100 ul of RBC suspension to be grouped, sub grouped and phenotyped and 100 ul of Anti-A, Anti-B, Anti-A B, Anti-D, Anti-Al(Lectin), Anti-C, Anti-c, Anti-E, Anti-e in respective tubes were taken. The contents of the tubes were mixed and centrifuged at 1000 rmp for 1 minute. Agglutination was noted as positive reaction. All the negative reaction were confirmed by an inverted microscope.

RESULTS

Results of Rh-D positivity cells have been summarized in table 1. It showed that 90.75% of the Indian population are RhD positive and 9.25% population are RhD negative. Incidence of percentage of Rh-C, Rh-c, Rh-E and Rh-e have been given in table 2. A percentage of 84.71% population have Rh-C antigen where as 61.34% population are Rh-c positive. 98.53% individuals are Rh-e antigen positive and only 22.49% of people are Rh-E antigen positive. Gene frequency refers to the presence of a specific gene in a population. The frequency of C gene in an Indian population is0.8471. The gene frequency of c in an Indian population is 0.6134 %. The frequency of E gene in an Indian population is0.2249%. The gene frequency of e in an Indian population is 0.9853%. When antigen are paired such as C and c, the gene frequency always add 1. "For quality control evaluation of rare Rh blood grouping reagents the respective antigen positive and negative cells are required for potency specificity and avidity testing. The phenotyping of Rh antigens are also necessary for the transfusion of antigen negative cells where the antibody is present in recipient. In an Indian population the commonest RH phenotype is DCe/DCe (R1R1) about 37.68%.

Table 1. Positivity of Rh-D antigen in Delhi Population

Rh-D antigen	Number of Samples	Percentage (%)	Frequency
POSITIVE	932	90.75	0.9075
NEGATIVE	95	9.25	0.0925
TOTAL	1027		

Samples were collected from Delhi, but Delhi being the capital of India where the peoples from all part of the India are residing.

Table 2. Detection of individual Rh-D, Rh-C, Rh-E, & Rh-E antigen on RBC in North Indians

S. No.	Rh Antigen	Presence of Individual antigen on RBC	% of individual antigen	Frequency
1	RH-D	932	90.75	0.9075
2	RH-C	870	84.71	0.8471
3	RH-C	630	61.34	0.6134
4	RH-E	231	22.49	0.2249
5	RH-E	1012	98.53	0.9853

Table 3. Homozygous / Heterozygous C and E antigens (n = 9999)

S.No.	Phenotype	Homozygous / Heterozygous C and E antigens		Percentage
1.	Homozygous of CC antigen	397	38.66	38.64%
2.	Homozygous of Cc antigen	473	46.06	46.45%
	Homozygous of cc antigen	157	15.28	14.91%
3.	Homozygous of EE antigen	15	1.46	1.50%
	Homozygous of Ee antigen	226	22.00	22.02%
4.	Homozygous of ee antigen	786	76.53	76.48%

Table 4. Phenotyping of Blood sample (n = 9999)

Phenotype of Blood sample		No of samples positive for Individual Phenotype	Percentage (%) of Individual Phenotype
Dce/dce	(R0r)	11	1.07
DCe/dce	(R1r)	299	29.11
DCe/DCe	(R1R1)	387	37.68
DCe/DcE	(R1R2)	162	15.77
DCe/DCE	(R1RZ)	08	0.78
DcE/dce	(R2r)	50	4.87
DcE/DcE	(R2R2)	13	1.27
DcE/DCe	(R2RZ)	02	0.19
dce/dce	(rr)	78	7.59
dce/dCe	(r'r)	09	0.88
dcE/dce	(r''r)	05	0.49
dCe/dcE	(r'r'')	01	0.09
dCe/dC	(r'r')	02	0.19

The approximate frequencies of the next commonest phenotypes in order DCe/dce(Rlr) 29.11%, DCe/DCE(RlR2) 15.77%, DcE/dce(R2r) 4.87%, DCe/DCE (R1RZ) 0.78%, DcE/DcE (R2R2) 1.27%, Dce/Dce (ROr) 1.07% & DcE/DcE(R2RZ) 0.19%. So far as D negative individuals are concerned the commonest phenotype dce/dce (rr) is approximately 7.59% and the approximate frequency of the next commonest Rh-D negative individual are dCe/dce(r'r). 88%, dcE/dce (r"r) 0.49% and dCe/dCe (r'r') 0.19% as shown in Fig 1.

All the Rh-D negative cells were tested for weak D by Indirect antiglobulin test. Not even a single individual was observed having weak RhD during the course of our studies.

DISCUSSION

The Rh-D antigen is the most immuno-genic and clinically significant red cell alloantigen. The clinical importance of Rh system is due to the antigenicity of these antigens and the antibodies produced against these antigens in circulation can cause red cell destruction or hemolytic diseases of the individual. Transfusion of antigen positive red blood cell to an antigen negative individual may result IgG production which may lead to extravascular hemolysis and a delayed hemolytic transfusion reaction upon subsequent transfusion of antigen positive blood. If a large volume of Rh-D positive blood is given to a Rh-D negative individual, in about 80% of such cases the recipient produce anti-D antibodies or immunoglobulins in respond to Rh-D antigen as a secondary immune response and it is the most common cause of severe HDN. Between 82 and 88% of Europeans and North American white people are D positive, around 95% of the black African are D positive. D is a high frequency antigen in East, reaching 100% in some populations; 99.7% of Hong Kong Chinese and similar percentage in Japanese appear D-positive. ln our study by normal blood groupingtechnique; we found the 90.36% of North Indian population are Rh-D positive and 9.72% are Rh-D negative. The phenotype frequency refers to the rate at which an observable trait is seen in population. The C antigen is expressed on the red cells of both homozygous (C/C, c/c) and heterozygous (C/c). Blood group antigens, in general, are expressed in a co-dominate fashion. Using the Rh C blood group antigen system as an example, 85% of Indians express C and 62% express c. The sum of phenotype frequencies is usually is more than 100% because there are people who express both phenotypes. C and c antigens are the product of alleles Cc and these antigens have a frequency of 68.0% and 81.0% respectively in English blood donor. In black Africans frequency of c antigen is much higher than that of C antigen, where as, in Eastern Asia the opposite is case i.e. C approaching 100% and c of very low incidence. Our studies showed that the frequency of C antigen is 84.87% and c has frequency of 61.58%. The C and c antigens are follow the same pattern as given for Eastern Asia . The antibody produced in recipient against c antigen is highly significant and react with approximately 80% of random samples from white population. This study suggest that the Rh phenotyping is important for transfusion and on the other hand it is also essential for the quality control testing of anti-C and anti-c blood typing reagents. Presence of Anti-C without anti-D is rare. Even in Rh-D negative persons, C antigen is poorly immunogenic because of absence of Rh-D antigen. Literature showed that if both anti-D and anti -C are present in serum of recipient, only D and C negative (cEe- r"rphenotype) sample can be transfuse. Frequency of 0.49 percentage in Indian population. It is again encouraging the Rh-phenotyping of red blood cells for transfusion purpose. Phenotype r"r is also equally important for Quality Control testing of anti-C and D reagents. E & e are another pair of allelic antigens with in Rh system, encoded by the same gene as that encoding C and c. In all population e has a significantly higher frequency than E. Our studies in North Indian population showed that the frequency of e antigen is 98.70% and 24.50% population have E antigen on RBC as compared to English population 98% and 29% respectively. Our data suggest that the frequency of E and e antigens are follow the similar pattern of English Population. The Rh- e antigen negative red blood cells are found very less and is only 1.30% as compared to other Rh antigens. The people should be thankful to the nature because the antigen e is less immunogenic in comparison to other Rh antigens. Cryopreservation for such rare type of red blood cells is necessary for emergency cases.

Conclusion

The phenotype of red blood cells is highly important for transfusion of blood to the recipient having clinical significant antibody in plasma. The work carried out in our laboratory clearly shows the significance of various Rh-antigens and also states the importance and requirement of Rh-phenotyping of Red Blood Cells for transfusion purpose. Even though the occurrence of such cases is rare, but implementing of Rh-Phenotyping in routine blood grouping of blood banks helps in life saving. Simultaneously the Quality Control testing of these rare blood grouping reagents is also necessary and it indirectly helps in proper blood transfusion. Rh-phenotyping of red blood cells is also required for the necessary reagent red cells of a particular Rh antigen to evaluate the respective rare blood grouping reagents for quality products and safe blood transfusion.

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