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ORIGINAL RESEARCH

Rhesus blood group haplotype frequencies among blood donors in southwestern Uganda

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Aim/objective: The study was undertaken to determine the Rhesus blood group system and Rhesus haplotype frequencies among blood donors at Mbarara Regional Blood Bank.

Materials and methods: We included ethylene-diaminetetra-acetic acid-containing plasma samples and serum samples from recruited consented blood donors. The Rh blood group system and the Rh haplotypes was established by the incubation of appropriate antisera (anti-D, anti-E, anti-C, anti-e, and anti-c) and cells at a temperature of 24°C in microplates for 1 hour and the reaction was read by gentle shaking and examining for agglutinations. Donors were asked to fill in questionnaires, after we obtained the informed consent, to assess their demographics.

Results: Among the 386 participants, 233 were males (60%) and 153 (40%) females. The Rh negative blood group percentage was 3.8%, while the Rh haplotype frequencies were as follows: D*ce dce* 68.1%, *dce dce* 2.8%, CD*e dce* 13%, *cD*E *dce* 12.4%, DC*e Dc*E 1.6%, *Dc*E *Dc*E 1%, dC*e dce* 0.8%, and *Dc*E DC*e* 0.3%.

Conclusion: Given this frequency, a high prevalence of anti-D alloantibody formation among those transfused is possible and could cause diverse effects, especially in the Rh D positive women. We recommend additional research studies on the role of autoimmunity to the transfused on the occurrence of Rh D variants plus their implications on hemolytic disease of the fetus and newborn in Uganda. This study recommends that the blood bank includes Rhesus haplotyping in its protocols and that the finding be disseminated to donors and blood users.

Keywords: Rhesus blood group, Rhesus genotype frequencies, blood donors, Uganda

Introduction

Rhesus (Rh) blood group system is the second most important system in blood transfusion after the ABO system. This system was discovered by Landsteiner and Wiener after experiencing problems in transfusion, as even when the ABO groups were matching they still had problems, including hemolytic disease of the fetus and newborn.[1,2]

The Rh blood group system, unlike the ABO system, has been shown to have 6 common antigens listed as C, *c*, D, *d,* E, and *e* as originally suggested by Fisher after Landsteiner.[1,3] There are racial differences in the distribution of these antigens. Rhesus D negative is more common in Caucasians (15%), whereas R (*DCe*) is found in ~48% Afro-Americans but is uncommon in Caucasians with a prevalence of approximately <2%.[4]

From the 6 common Rh antigens, 8 different haplotypes can occur; dC*e*, *Dce*, *dce*, *dc*E, dC*e*, DCE, and dCE. Haplotype dC*e* combination is relatively common in ~46% of the people.[5]

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It must be noted that the determination of the presence of the D antigen alone in a potential blood donor is not sufficient.[6] Testing the D positive patients of childbearing age with anti *c* reagent is not common though necessary where those who are negative should be transfused with blood of the same haplotype, as this would protect the patient from Rh alloimmunization of the *c* antigen.[7] It also ensures that antibody against anti-c should not be produced, which could cause complications should the patient later become pregnant and carry a c-positive fetus.[6]

This study aimed at establishing the Rhesus haplotype frequency among blood donors in Mbarara Regional Blood Bank by carrying out Rhesus blood group grouping, haplotyping on the donor blood from recruited blood donors and establishing the Rhesus haplotype frequencies among these blood donors.

Materials and methods

The study was mainly a cross-sectional one which covered 6 districts of southwestern Uganda with a population of 6,836,862 covering a 32,476 km^2 area, of which 85% is covered by Mbarara Regional Blood Bank. A systematic sampling procedure was done on donors after informed consent for the study was obtained and recruitment for blood donation at Mbarara blood bank. All blood from the blood donors recruited was included in the study excluding all the hemolyzed samples.

The study was approved by Faculty Research and Ethics Committee of Mbarara University of Science & Technology. All the blood donors recruited for the study signed a written informed consent prior to the taking of blood.

Sample analysis

Test procedure involved the incubation of appropriate amount of antisera and cells at a temperature of 24°C in a "U" microplate for 1 hour, and the reaction was read by gentle shaking and examining for the agglutinations. The reaction proceeded in 2 stages, that is sensitization or combination stage in which antibody attached itself on the antigenic sites of the red cells, and second the visible or agglutination stage, which was much influenced by the type of fluid medium in which the cells were suspended.

At the end of the reaction, the microplates were gently agitated to loosen the cell sediments at the bottom of the wells. Strength of reaction shown by agglutination was recorded as +, ++, weak, no reaction (–). The phenotype was obtained from the reaction of each antiserum. Data are presented as frequency of appearance of the haplotypes obtained on analyzing the results from the grouping.

Results

Out of 386 participants recruited in the study, 233 were males (60%).The study participant recruitment per district was as follows: Mbarara 43.7%, Bushenyi 9.3%, Kabale 51%, Ntungamo 13.7%, Rungiri 9.6, and Kanungu 10.4%, as shown in Table 1.

A total of 15 (3.86%) participants were Rh D negative. The highest Rhesus haplotype incidence was *Dce dce* with 68.1%, and others being *dce dce* 2.8%, DC*e Dc*E 1.6%, d*Ce dc*e 0.8%, DC*e dce* 13%, *Dc*E *Dc*E 1%, *Dc*E *dce* 12.4%, and DC*e* DC*e* 0.3%. All the haplotypes appeared in all the districts as shown in Table 2.

Discussion

In the study, the participants were the usual blood donors who turned up freely for the donor sessions at the donor recruitment sites of Mbarara regional Blood Bank, which covers southwestern Uganda. The majority (60.4%) were men, because men easily qualify through the selection exercise as given in the donor recruitment form.

The haplotype incidences were found to be quite different from a study done in the UK[8] and among the Cau-

Table 1 General distribution of Rh genotype frequency in southwestern Uganda districts

Phenotype	Genotype	Bus	Mba	Ntu	Kab	Ruk	Kan
Dce	*Dce dce*	23	115	35	40	21	30
Dce	*dce dce*	2	3	2	2		2
DCcEe	DC*e Dc*E	1	3	1	1		
d*Cce*	d*Ce dce*		2	1			
DC*ce*	DC*e dce*	4	19	9	2	9	6
*Dc*E	*Dc*E *Dc*E		2		1	1	
*Dc*Ee	*Dc*E *dce*	5	24	5	5	6	2
DCe	*Dc*E DCe		1				
	Total	36	169	53	51	37	40

Abbreviations: Bus, Bushenyi; Mba, Mbarara; Ntu, Ntungamu; Kab, Kabale; Ruk, Rukungiri; Kan, Kanungu.

Table 2 Comparison of Rhesus genotypes frequencies percentage in this study, UK, and in Caucasians

Phenotype	Genotype	In this study (%)	In UK (%)	Caucasians (%)
Dce	*Dce dce*	68.1	1	3
Dce	*dce dce*	2.8	15	15
DCcEe	DC*e Dc*E	1.6	13	10
dCce	d*Ce dce*	0.8	1	1
DC*ce*	DC*e dce*	13	31	33
*Dc*E	*Dc*E *Dc*E	1	3	1.5
*Dc*Ee	*Dc*E *dce*	12.4	13	10
DCe	*Dc*E DCe	0.3	16	19

Note: Data from Regan.[16]

casians. The most frequent haplotype is DC*e dce* (31%) in the UK and 33% in Caucasians, but there is a correlation in incidence of some haplotypes, especially dC*e dce*, *Dc*E *Dc*E, and *Dc*E *dce*. These results were still not similar to a study done in Bangladeshis[5,8] that had the most frequent haplotype as DCe DcE with 39.75% and rarely *dce dce*, which was at 1.8%.[9]

These above variations in Rh haplotype frequencies are due to the ethnic origin, which influences the haplotype frequency of a given population. This arises from the encoding of the *RHD* & *RHCE* genes, which results in the expression of the respective antigens like C, *c*, E, *e,* and D as the phenotype.[4] This variation between races is based on the highly homologous gene arrangement on the responsible chromosome (chromosome 1) in the different races.[10] On encoding, they determine the expression pattern indicated. While in most D-negative Caucasians, there is deletion of the *RHD* gene in 15% of this population. In other populations, notably African blacks and Japanese, Chinese, Maeris, Burmese, Malaysians, Eskimos, and American Indians, the D-negative phenotype is associated with a grossly normal *RHD* gene.[11,12]

The frequencies were consistent and similar from high to lowest in all the districts. Being genetically determined, intermarriages within the population have not diluted much of the trends in the expression and they are similar to the Rhesus genotype incidence in other blacks.[11,13,14]

Most of the participants (96%) expressed D antigen, but a small percentage of about 4% (*dce dce* 2.8% and dC*e dce* 0.8%) did not express the D antigen, and this can lead to alloimmunized. In some areas like in UK and USA, the percentage of those not expressing D antigen is 15%, in other places like Bangladesh, People's Republic of China, and Japan it is at 1.8%, while some Mongoliods do not express it all.[7,9,15] These expressions are due to the position of the genes in the chromosome, which influence their being passed over to the offspring and their expression.[9,12] C, c was expressed in all of the haplotypes, and it is the second most immunogenic Rhesus antigen following D; this is closely related to other populations.[9]

With the presence of *dce dce* and dC*e dce* which easily leads to alloimmunization, there is need for proper management of these cases during transfusion and pregnancy. Properly cross-matching Rh blood group to the haplotype level, particularly in females of childbearing age, is of much importance. Studies relating these haplotypes to antibody production leading to Rh alloimmunization are highly recommended.

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Author contributions

YM participated in study conception and design, data acquisition, analysis and interpretation, manuscript drafting, and revising. EM participated in data interpretation, manuscript drafting, and revising. GM participated in study design, data analysis and interpretation, manuscript drafting, and revising.

Disclosure

The authors report no conflicts of interest in this work.

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