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RESEARCH ARTICLE

FREQUENCY OF WEAK RHESUS D PHENOTYPE IN RHESUS D-NEGATIVE BLOOD DONORS IN CONGO BRAZZAVILLE

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ABSTRACT

Background*:* The Rhesus (RH) system is of major clinical importance due to the high degree of immunogenicity of its antigens, particularly the D antigen. There are different variants of the D gene (weak D, partial D and Del). Screening for the weak D antigen can prevent the risk of alloimmunisation in Rh D-negative recipients. The aim of this study was to estimate the frequency of the weak D phenotype in D-negative blood donors in the Congolese population. ***Material and methods****:* This study was carried out on rhesus-negative blood donors received at the various collection stations of the national blood transfusion centre (Brazzaville and Pointe-Noire), from 1er February to 31 November 2018. Sociodemographic data were collected from the blood donor's clinical form. ABO and RH blood groupings were performed using the opaline plate haemagglutination technique. The weak phenotype D was determined using the indirect Coombs assay with anti-human polyglobulin antibody. ***Results****:* A total of 73 Rh D negative blood donors were selected for this study (52 in Brazzaville and 21 in Pointe-Noire). The O-negative phenotype was the most frequent (n = 54; 73.97%). In the RH system, the *ddcce*phenotype complex was the most common (89.04%). The frequency of the weak D phenotype was 19.18%, with men predominating (78.57%). This phenotype was predominantly found in blood group A donors (45.45%) and relatively found in groups B and O (12.5 and 14.81%, respectively). ***Conclusion***: This study reveals a relatively high frequency of the weak D phenotype in Rh D negative blood donors in the Republic of Congo. Systemic screening for this phenotype in Rh-negative blood donors at blood transfusion centres should therefore be considered in order to prevent alloimmunisation.

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INTRODUCTION

The Rhesus (RH) system was first described in 1940 by Landsteiner and Wiener[1] . Clinically, it is the second most important blood system after the ABO system. It is also the most polymorphic of the blood groups: in addition to the major antigens RH1 (D), RH2 (C), RH3 (E), RH4 (c) and RH5 (e), more than 55 antigens have been serologically defined to date[2,3] . The D antigen is clinically important in transfusion medicine and obstetrics, as anti-D antibodies are responsible for severe acute and delayed haemolytic transfusion reactions and haemolytic disease of the newborn[4,5] .Classically, an RH1 haematocyte has between 10,000 and 30,000 D antigenic sites. A distinction is therefore made between the Rh-positive phenotype in people who possess the major D antigen, and the Rh-negative phenotype in people who do not express it[6] . However, genetic alterations and mutations in the Rh D gene can cause quantitative or qualitative changes in the expression of the D antigen, resulting in a variety of D alleles which are classified as weak D, partial D and Del[7] The weak D phenotype is characterized by weakened expression of the D antigen on the surface of red blood cells.

Individuals with a weak RhD phenotype should be considered Rh positive. Therefore, detection of this phenotype is a pre-transfusion requirement to ensure adequate blood transfusion[8,9,10]. To date, there are serological tests using human antiglobulins that are very sensitive and can reveal the weak D phenotype[8]. It is reported that the frequency of weak D antigen varies from 0.2 to 1% in Caucasians and 4.6 to 7% in blacks[11,12]. To the best of our knowledge, our study is primary in estimating the frequency of weak phenotype D in the Republic of Congo. Our data could allow health authorities to put in place measures to reduce the risk of alloimmunization in RhD-negative recipients.

MATERIALS AND METHODS

Design and study population: This was a descriptive cross-sectional study carried out on 73 Rh D-negative blood donors, recruited from the database of the Brazzaville National Blood Transfusion Centre (CNTS) (52 donors) and the Pointe-Noire Interdepartmental Blood Transfusion Centre (CIDTS) (21 donors), during the period from 1 February 2018 to 30 November 2018.

Rh D-negative blood donors who had made at least 3 donations in the year and had given written consent to take part in the study were included. Participants' sociodemographic data were collected using individual information sheets.

Determination of ABO /RHCE phenotypes: Approximately 5 ml of blood was taken from each participant by phlebotomy of the vein in the crease of the elbow into an EDTA (Ethylene diamine tetra-acetate) tube, in compliance with good practice and asepsis. The ABO blood group was determined in duplicate (Beth-Vincent red blood cell technique and Simonin serum technique), using commercially available antisera (anti-A, anti-B, anti-AB and anti-D monoclonal antibodies from CYPRESS Diagnostics®, Belgium) and freshly prepared A and B test red blood cells respectively. The techniqueconsisted of placinga drop (50µl) of each reagent, anti-A, anti-B, anti-AB and anti-D, on a white opaline plate. A drop of whole blood was then added close to each drop of reagent (Beth-Vincent method). For the Simonin technique, a drop of test haematopoietic A and a drop of test haematopoietic B were placed separately on a white opaline plate. A drop of donor serum was placed on each drop of test haematoma. Finally, using an applicator stick, the blood and reagent were mixed and the opaline plate was gently rotated for 30 seconds. The appearance of clumping indicated the presence of the corresponding blood group antigen or antibody. The RhD, RhC, RhE, Rhc and Rhe phenotypes were tested using IgM, anti-D, anti-C, anti-E, anti-c and anti-e monoclonal antibodies, respectively, as recommended by the manufacturer (*CYPRESS Diagnostics®*, Belgium).

Determination of the weak D phenotype: All Rh-negative samples with anti-D reagent (*CYPRESS Diagnostics®*, Belgium) were tested for weak D antigen using the Combs indirect polyvalent human antiglobulin tube and cassette technique (*Ortho Biovue® France).* For the tube technique, 50 µl of a 5% suspension of washed red blood cells was placed in a haemolysis tube with 50 µl of Anti-D Blend serum (IgM + IgG). This mixture was incubated at 37ºC for 15 minutes, then washed three times with 0.9% saline. After aspirating the last supernatant, the red blood cells were resuspended with 100 µl of human antiglobulin (AHG), and centrifuged at 1000 rpm for one minute. The appearance of agglutination, indicating the presence of weak D antigen, was checked macroscopically by gently shaking the tube. In contrast, for the cassette technique (*Ortho Biovue*Systhem® France), 50µl of 5% red cell suspension to be tested was placed in each appropriate reaction chamber (containing the antiglobulin), together with 50 µl of Anti-D Blend serum (IgM + IgG, CYPRESS Diagnostics®, Belgium). The cassette was then incubated at 37°C for 30 minutes. Finally, after incubation, the cassette was centrifuged at 1000 rpm for five minutes using an *Ortho Biovue System* centrifuge. Both sides of the columns were read for an agglutination reaction. *Statistical analysis.* Categorical variables were expressed as headcounts and percentages. Statistical significance between the two sexes was determined with GraphPad Prism version 7 software using the Chi-squared test. A value of $p \leq 0.05$ *was* considered statistically significant with a confidence interval (CI) of 95%.

RESULTS

The study population included 73 blood donors, 58 of them were male (79.45%) and 15 female (20.55%). The sex ratio was 3.86 in favour of men. The mean age of the study population was 42.05 ± 8.60 years, with extremes ranging from 21 to 58 years (Table I).

Table I. Socio-demographic characteristics of the study population

Variables	Data	*p-value*
Age (years)		
Mean ± SD	42,05 ± 8,60	
Median (Min - max)	43 (21 - 58)	
Gender (n (%))		
Male	58 (79,45)	
Female	15 (20,55)	
Sex ratio (M/F)	3,86	*<0,0001*

The majorityof participants was blood group O (73.97%) while the groups A and B accounted for only 15.07% and 10.96%) in the study population, respectively (Table II).

Table II. Distribution of ABO antigens in the study population

Blood group	Number of RhD negative donors (N = 73)	Frequency (%)
A	11	15,07
B	8	10,96
AB	0	0
O	54	73,97

RhD, Rhesus D

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AB	0	0
O	54	73,97

Regarding the RhDCE phenotypes, *ddcce* was the most represented with a high frequency (89.04%), followed by the dCce (9.59%) and dCcEe (1.37%) phenotypes (Table III).

Table III. Distribution of the Rhesus DCE phenotypes in the study population

Rhesus phenotype	Number of RhD negative donors (N = 73)	Frequency (%)
ddcce	65	89,04
dCce	7	*9,59*
dCcEe	1	*1,37*

Analysis of the results of the weak D antigen test showed that 14 of the 73 D-negative blood donors were positive for the weak D test, representing 19.18% of the study population (Figure 1), with a greater proportion of men (78.57%) than women (21.43%) (Figure 2). The frequency distribution of weak D antigen in the different blood groups A, B and O was 45.45, 14.81 and 12.5% respectively (Figure 3).

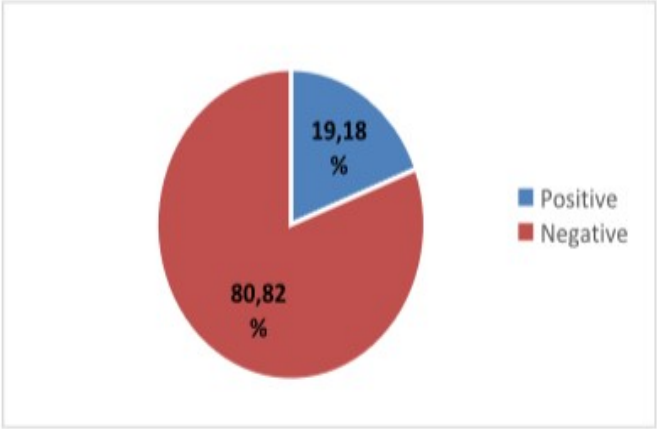

Figure 1. Frequency of weak Rhesus D phenotype in the study population

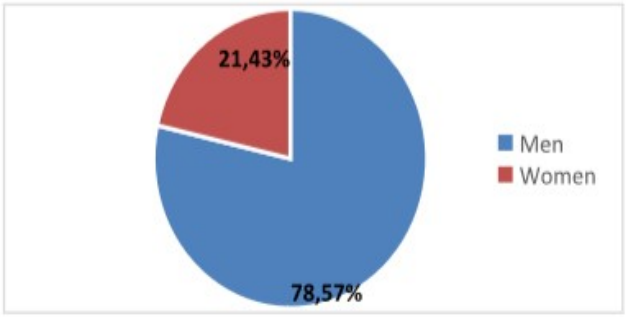

Figure 2. Frequency of weak Rhesus D phenotype in men and women

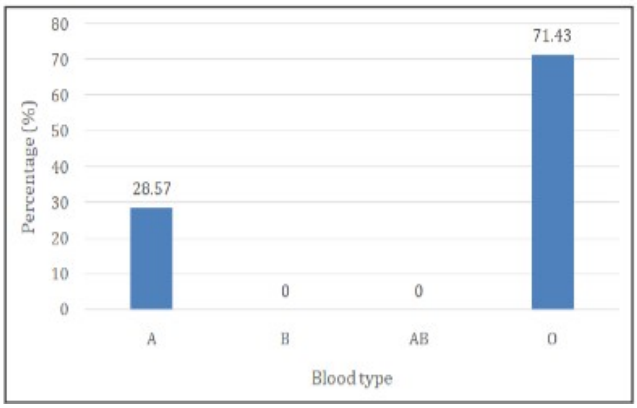

Figure 3. Distribution of weak D antigen by blood group in the study population

DISCUSSION

The aim of this study was to determine the frequency of weak D antigen in a population of D-negative blood donors at the Brazzaville and Pointe Noire blood transfusion stations in the Republic of Congo. We used conventional serological tests to identify ABO blood groups and the human antiglobulin technique to detect the weak D antigen. Seventy-three (73) RhD-negative blood donors were included in this study. The size of our sample may be a limiting factor in extrapolating the results compared with other studies. These differences could be explained by the sampling method and the low frequency of Rh-negative blood donors (known as rare blood groups). Despite this limitation, the results of this preliminary study deserve some comment and some comparison with other authors. In our study, the average age of the study population was 42.05 ± 8.60 years, with extremes ranging from 21 to 58 years. The majority of participants (79.45%) were men. Several authors working on larger samples also reported this male predominance[12,13] .Gynaeco-obstetrical constraints (pregnancy, breast-feeding, menstrual period, etc.) probably contribute to this predominance of men. In this study, blood group O predominated (73.97%) followed by blood group A (15.07%). Other studies on the ABO system with larger sample sizes have also reported a predominance of blood group O, followed by groups A, B and AB[13,14,15,16] . The most frequent phenotype in our study population was the (*dccee)* phenotype (RH:-1;-2;-3;4;5) with 89.04%, followed by the (*dCcee)* phenotype (RH:-1;2;-3;4;5) with a frequency of 9.59%. These results are similar to those reported in other studies[14,17,18,19] . The study by Eiman Hussein *et al* (2013) suggested that D variants are much more common in phenotyped blood donors (*ccee)*, which is also the case in our study. The frequency of the weak D phenotype in our study population was 19.18%. These results are similar to those reported by Wafi *et al*. in Morocco[17] , i.e. 15.87% in a population of 60 donors typed Rh D weak. Other studies have reported frequencies varying between 0.2 and 1%[8,11] , 2.26% in Algeria[20] and 6.45% (Opoku-Okrah *et al*., 2008). The weak D antigen was found predominantly in men (78.57%). This variability in the frequency of the weak D phenotype could be explained by the sampling method, the size of the population and the techniques used to detect this antigen. Analysis of the distribution of weak D antigen according to ABO blood groups shows a predominance of group O (71.43%). This trend differs from that observed by Khachaa *et al*, on a sample of 252 RhD weak blood donors[14] .

CONCLUSION

Our study reports the first data on the frequency of the weak D phenotype in a population of RhD-negative blood donors. This relatively high frequency (19.18%), dominated by blood group O donors, needs to be confirmed by other studies carried out on a larger sample. The present results could, however, help to establish a systematic screening strategy for the weak Rh D phenotype, to minimise the risk of anti-D alloimmunisation and ensure the transfusion and obstetrical safety of Rh D negative recipients.

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Declaration of interests: The authors declare that they have no conflicts of interest in relation to this study.

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