Uncommon Rh Phenotypes in a Cross Section of Nigerian Antenatal Women: Implications for Molecular Genotyping of Blood Groups

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Abstract

Background: This study aimed to determine the frequency of rare blood group antigens among pregnant women in Port Harcourt Metropolis.

Methods: The frequencies of Rh antigens and phenotypes of the pregnant women in Port Harcourt Metropolis, Nigeria, were determined, using standard serologic techniques.

Results: Of the 374 pregnant women studied, the frequencies of the Rh antigens within the population were D (89.0%), c (82.0%), e (54.0%), C (24.3%), E (20.1%). The frequencies of the Rh antithetical antigens were Dd/Dd (91.2 %), Cc (19.5%), cc (84.5%), Ee (13.9%), ee (54.3%), CC (25.1%), EE (19.8%) and dd (10.4%). Seven (1.9%) were found to be Rhnull, sixteen (4.3%) were found to be D– or exalted D. Phenotypes without RhD reactivity were –c– (2.9%), -C- (0.3%), -C- (0.3%), -Ee (0.5%) and –E- (0.3%). The most frequently occurring Rh phenotype was Dccee with a frequency of (25.8%). RhD-ve was found to be significantly associated with HDN outcome (2=6.605 and P=0.01).

Conclusion: We concluded that: 1) There is the presence of uncommon Rh phenotypes in our population. 2) Rhnull exists in this study population of which similar studies have not been reported in Nigeria. 3) There is need to conduct studies in other parts of Nigeria in order to compare the frequencies of these rare blood groups in Nigerian population. 4) Molecular studies to establish reasons for deletion and Rhnull phenotypes among Nigerian and Africans in general are hereby advocated.

Keywords: Rh phenotype; Blood groups; RhD negative; RhD positive

Introduction

The Rh blood group has been reported to be the most complex and highly polymorphic of all the blood group systems [1]. Apart from the importance of the Rh antigens in blood transfusion and Haemolytic Disease of the New born (HDN)[1], Rh proteins are involved in transporting ammonium across the RBC membrane [2]. It follows that RBCs which lack Rh antigens will have abnormal shape, increased osmotic fragility and shortened lifespan, resulting in haemolytic anaemia that is usually mild in nature [1]. These patients are also at risk of adverse transfusion reactions because they may produce antibodies against several of the Rh antigens. The D antigen contains over 30 epitopes. Variations of the D phenotype arise when these epitopes are weakly expressed or missing resulting in uncommon phenotypes which are usually very rare. The frequencies of these uncommon Rh phenotypes are yet to be reported in this part of the globe. In this paper, we report some uncommon Rh phenotypes which we encountered during our study of Rh phenotyping of antenatal women.

Materials and Methods

Subjects

A total of 374 pregnant women of various ages and ethnic background who were attending antenatal clinics in Braithwaite Memorial Specialist Hospital (BMSH) and Churchhill Primary Health Care Centre, Port Harcourt City were recruited into the study. The study population consisted of eight ethnic nationalities: Ijaw, Ibo, Ibibio, Ikwerre, Yoruba, Ogoni, Hausa, Ekpeye and others who could not define their nationalities. The 374 pregnant women were in their various trimesters, attending antenatal clinics in both hospitals within a period of 6 months.

Study design

A prospective cross-sectional design was used in this study. Samples were collected randomly after obtaining informed consents from the study participants. The study received institutional ethical approval from the Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Nigeria.

Blood collection

Two milliliters (2 ml) of venous blood was collected from each of the 374 pregnant women through veni puncture using the antecubital vein into an Ethylenediamine Tetracetic Acid (EDTA) of 1.5 ml concentration. Samples that were not phenotyped the same day were archived at 2-8°C till the following day.

Procedures

The test procedure was based on the principle of haemagglutination...
where the red cells possessing the specific antigen will agglutinate when tested with the corresponding reagent (antibody).

**Rhesus (Rh) Phenotyping**

RBCs were phenotyped for D, C, E, c, e, antigens according to standard serologic protocols with IgG/IgM anti-D (11167/2), IgM MoAbs specific for C. (AC2329), c (Ac2368), E (ME2031) and e (Ac3469) (BIOTEC; IPSWICH, SUFFOLD, UK). Eight tubes were arranged in a row and labeled anti-D, C, c, E, e, and autocontrols for each of 374 patients. Two drops of antiserum and one drop of a 5% washed RBC suspension were added to each tube. This was followed by incubation at 37°C for 30 minutes. At the end of 30 minutes, the tubes were centrifuged and the agglutination was read macroscopically. All of the negative results of Rhesus (D) were confirmed microscopically. Albumin and Anti-Human Globulin (AHG) tests were used as control and for weak D determination. To determine the presence of weak D antigen, one drop of anti-human globulin reagent and one drop of RBC suspension were added to a labeled test tube and incubated at 37°C for 30 minutes.

**Statistical method**

SPSS version 16 for window (Chicago Illinois) was used for frequency distribution for blood groups, Pearson Chi-square test to test the association of jaundice with blood groups.

**Results**

Table 1 shows demographic characteristics of the study participants. The Ijaw ethnic group was found to have the highest frequency (30.5%), followed by Ibo 99 (26.5 %), Ikwerre 48 (12.8%), others 37 (9.9%), Ibibio 30 (8.0%), Yoruba 16 (4.3 %), Ogoni 14 (3.7 %), Ekpeye 13 (3.5 %), and Hausa 3 (0.8 %). The frequencies of age range are also shown in the table. The age range of 25-29 yrs was found to have the highest frequency 148 (39.6 %), followed by 30-34 yrs 135 (36.1%), 20-24 yrs 42 (11.27%), and 35-39 yrs 38 (10.2%), and 40 yrs and above 5 (1.3%), another is the frequency of trimester. The second trimester was found to have the highest frequency, 221 (59.1%), followed by third trimester 110 (29.4%), and first trimester 43 (11.5%). The frequency distribution of Rh antigens is shown in table 2. The highest frequency among Rh antigens was found to be D (89.0%) followed by c (82.4%), e (54.0%), C (24.3%), E (20.1%) and D' (0.0%). Rh phenotype frequencies in the population are shown in table 3. Twenty five phenotypes were found to occur in the population, the most common being Dccee (25.8%), and dccEe (0.5%), and DCCEe (0.3%) were the rarest phenotypes in the population. Out of the twenty five phenotypes in the population, there were D- (6.5%), --D, D--, --E (0.8%) and Rhnull (1.9%). The frequency of distribution of neonatal jaundice among blood groups, are shown in table 4. Neonatal jaundice occurred more frequently among the RhD- group (22.9%) while it least occurred among C (7.7%).

Table 5 shows the antithetical antigen frequencies within the study population. The DD/Dd was found to occur more frequently (91.2%), followed by cc (84.5%), ee (54.3%), CC (25.1%), EE (19.8%), Cc (19.5%), Ee (13.9%) and dd (10.4%) respectively.

**Discussion**

Within the South-South region of Nigeria, Rhesus phenotypes in the general population has been studied and reported in Port Harcourt where the red cells possessing the specific antigen will agglutinate when tested with the corresponding reagent (antibody).

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**Discussion**

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was found to have the highest frequency (2.9%), followed by –e/--e haemolytic anaemia might be high in such population.

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anaemia. This was similar to the finding of Wagner and Flegel [8] the incidence of HDN, blood transfusion reaction and haemolytic activity in the Niger Delta can lead to such changes which may pose serious health threats to pregnant women. This study was carried out using haemagglutination procedures. Recently, DNA based assays have been used for Rh blood group typing, especially the genotyping for weak and Rh null [8,10-13]. However, in this study, molecular typing of the Rhesus Rh gene combination Number Percentage

Table 4: Distribution of Neonatal Jaundice among blood groups.

Rh gene combination Number Percentage

cc 316 84.5
Cc 73 19.5
CC 94 25.1
ee 203 54.3
EE 52 13.9
EE 74 19.8
DD 341 91.2
dd 39 10.4

Table 5: Rh antithetical antigens within the study Population.

Blood Group Antigens Rh No. of cases of Neonatal Jaundice No. of Blood Group Patients Percentage (%)

RhD+ve 36 298 10.8
RhD-ve 8 35 22.9
C 7 91 7.7
c 39 308 12.7
E 9 75 12.0
e 22 202 10.1

This finding was similar to other studies carried out [4-7]. They reported the following frequencies: Caucasians: D (85%), C (68%), E (29%), c (80%), and e (80%); Blacks: D (92%), C (27%), E (22%), c (96%) and e (98%); Asians: D (99%), C (93%), E (39%), c (47%), and e (96%).

The Dcee phenotype was found to be the most common in the population with a frequency of (25.8%) and the least DCCee (0.3%). This observation agreed with a similar study carried in Calabar [4]. They reported the following frequencies: Dcee (73.6%), DccEe (13.9%), ccce (5.27%), DccEE (4.4%), DccCe (1.94%), DcCe (0.56%) and CcCe (0.28%). We also observed some rare phenotypes among the participants with RhD deletion frequencies of -c- (2.9%), followed by -Ee (0.5%), -Cc (0.3%), -C- (0.3%) and -E- (0.3%) respectively. It was observed that –c- had the highest frequency of (2.9%), -Cc-, -C- and -E- (0.3%) each as least RhD deletions. In such population there may be a high incidence of HDN, blood transfusion reaction and haemolytic anaemia. This was similar to the finding of Wagner and Flegel [8] the reason is not clear.

D-- or exalted D phenotypes were also observed which demonstrate no Cc or Ee reactivity. This is similar to the finding of Harmingen [9]; this was also similar to the findings of Huang et al. [10] Clierif-Zahar et al. [11]. The incidence of blood transfusion reaction, HDN and haemolytic anaemia might be high in such population.

In this study a variety of rare genotype frequencies were also observed as in the case of the phenotypes with RhD deletions. --/--c- was found to have the highest frequency (2.9%), followed by --/--e-- (0.5%), --/--Cc (0.3%), -Cc/--- (0.3%), and -E/E-- (0.3%) respectively. The highest Rh probable genotype frequency in the study population was found to be Dcc/dce (25.8%), followed by CD/-/C (18.6%), cdc/ cde (0.3%) and DcE/dCe (0.3%) respectively. We observed also that some individual within the study population failed to express any of the Rh antigens on the RBC surface, otherwise known as Rhnull with a frequency of (1.9%). This agreed with the findings of Avent and Reid [12], on the review of the molecular basis of the Rh blood group phenotype [11]. They all reported that such phenotypes exist in some populations but they did not show any documented evidence.

Exalted (D--) genotype was also observed in the population with a frequency of (4.3%), this is similar to other studies, [8-14]. There was no statistical data given, but they all reported that such genotypes exist in some populations, especially in the blacks. Such population may have a high incidence of HDN and transfusion reactions. The Rh gene combination was studied also in the population. The following findings were observed: the DD/Dd was found to have the frequency of (91.2%), followed by cc (84.5%), ee (54.3%), CC (25.1%), EE (19.8%), Cc (19.5%), Ee (13.9%) and dd (10.4%) respectively. These values obtained were similar in some Rh genes combination and others differ in another study carried out in Port Harcourt and [3,4].

It is pertinent to note that the knowledge of the individual differences in genetic variability as well as their frequency and distribution in a given population is very vital to the understanding of the genetic basis of diseases. As is the case with many D variants, modified RhD protein can have important implications for transfusion related antigenicity. The essential clinical difference between Rhesus positive and Rhesus negative hinges on the presence or absence of the RhD protein in the erythrocyte membrane. Apart from this lack of RhD protein, the D negative phenotype could be caused by a series of changes (mutations) in the RhD protein which in turn change the phenotype of the D antigen. These changes are classified by partial D, weak D, exalted D or DEL. It is possible that environmental factors occasioned by oil exploration activities in the Niger Delta can lead to such changes which may pose serious health threats to pregnant women. This study was carried out using haemagglutination procedures. Recently, DNA based assays have been used for Rh blood group typing, especially the genotyping for weak and Rh null [8,10-13]. However, in this study, molecular typing of the Rhesus RhD gene could not be done to confirm the possible deletions encountered in this serological procedures. It is hoped that our feature research will take care of this. This study was designed to phenotype the red cells for five antigens (C, D, c, e, C) of the Rhesus blood group system. Their corresponding antibodies were therefore obtained were similar in some Rh genes combination and others differ in another study carried out in Port Harcourt and [3,4].

Conclusion

In conclusion, the findings presented in this study further shows that the Rh blood groups are highly polymorphic in nature. This study concluded as follows: 1) RhD-- or exalted RhD, Rh deletions and other forms of deletion exist in our typical black population. 2) R_hnull exists in this study population of which similar studies have not been reported in Nigeria. 3) There is need to conduct studies in other parts of Nigeria in order to compare the frequencies of these rare blood groups in Nigerian population. 4) Molecular studies to establish reasons for deletion and Rhnull phenotypes among Nigerian and Africans in general are hereby advocated.
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