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Rhesus (Rh) blood group antigens in multiparous women attending antenatal clinic in tertiary hospital, port harcourt, Nigeria

Abstract

The routine blood screening test carried out for pregnant women attending antenatal clinic in Tertiary Hospital Port Harcourt, Nigeria especially with regards to blood group system is limited to ABO/Rh D antigen screening. This cross-sectional, hospital-based study was aimed at determining the prevalence of the Rh C, c, E and e blood group antigens in multiparous women attending antenatal clinic in Tertiary Hospital Port Harcourt, Nigeria. A total of one hundred and twenty (120) pregnant females within the age range of 18-40years were recruited randomly into the study between February-June, 2022. Blood samples were aseptically collected by venipuncture into EDTA sample bottle; Rh C, c, E and e blood group antigens were assay for using microwell agglutination technique. Data generated was statistically analyzed by simple percentage calculation and defining the percentage frequency of Rh antigens. Results obtained showed the percentage distribution of 17.5% for Rh C, 89.2% for Rh c, 39.2% for Rh E and 90.8% for Rh-e. Percentage distribution of Rh antigens amongst studied population based on parity showed that G3P2 group had the highest percentage positivity of 8(6.7%) for Rh-C antigen while those carrying their first pregnancy (prime) had the highest percentage positivity of 33(27.5%), 15(12.5%) and 36(30.0%) for Rh-c, Rh-E and Rh-e respectively. Distribution of Rh antigens with respect to previous transfusion history in studied participants showed 6 (5.0%), 15 (12.5%), 6 (5.0%) and 17 (14.2%) percentage positivity for Rh-C, Rh-c, Rh-E and Rh-e respectively. This study revealed a percentage positivity of 17.5%, 89.2%, 39.2 % and 90.8% for Rh-C, Rh-c, Rh-E and Rh-e respectively with high percentage expression for Rh-c, E and e antigens found on the red cells of Antennal Women carrying their first pregnancy. Furthermore, there is a high percentage expression of Rh antigens in previously transfused pregnant women in this study. Although routine phenotyping of these blood group antigens will be a financial burden in a resource limited country like Nigeria. It is expedient and needful to take into cognizance the fact that the presence of Rh C, c, E and e antigens may likely be the cause of some delayed transfusion reactions and haemolytic disease of the foetus and new born. Therefore, there is need for the inclusion of Rh C, c, E and e in routine antigen typing for pregnant women in other to help ameliorate red blood cell alloimmunization and delayed haemolytic transfusion reaction during pregnancy. Furthermore, there is need to promulgate policies that promote the optimum stocking of Rh-e, c antigen negative blood in blood banks in the area for emergency use.

Keywords: rhesus (Rh) antigens, multiparous women, parity, alloimmunization

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Introduction

The routine blood screening test carried out for pregnant women attending antenatal clinic in Tertiary Hospital Port Harcourt, Nigeria especially with regards to blood group system is limited to ABO/Rh D antigens. Research have shown that other Rh antigens such as C, c, E and e are highly implicated in transfusion reactions and haemolytic disease of the newborn,[1–4] but yet remain unaccessed amongst multiparous women attending Antenatal Clinic in Tertiary Hospital Port, Harcourt, Nigeria.

The Rhesus (Rh) blood group system remains the second most clinically significant blood group system after the ABO blood group system in the field of transfusion medicine,[5,6] with over 49 described antigens capable of causing haemolytic transfusion reaction (HTR) and haemolytic disease of the newborn (HDN) all attributable to its complex genetic basis.[1,3,5–7] Five of the Rh family protein falls into functional distinct group responsible for ammonia transport (RhAG, RhBG and RhCG) and the non transporting/non-glycosylated Rh proteins (RhD and RhCE) highly recognized for its key role in blood group incompatibility.[8]

Although the Rh antibodies do not occur naturally, they exist all as immune antibodies resulting from specific antigen stimulation either through transfusion, pregnancy or by injection of the antigen.[3] They are mostly of the IgG classs with subclasses IgG1 and IgG3, IgG2 and IgG4 also detected while some sera have an IgM component.[7] Rh antibodies rarely, if ever, bind complement, and therefore RBC destruction is mediated almost exclusively via macrophages in the spleen.[9]

The Rh-c antigen is considered the most clinically significant Rh antigen after D and is associated with severe haemolytic transfusion reaction.[2] Anti-c antibodies arise through previous exposure, such as fetomaternal haemorrhage or transfusion, and can produce acute and delayed haemolytic reactions.[10] As with the D antigen, pregnant women and girls are usually sensitized by the c-antigen during an initial pregnancy, and complications occur with repeat exposure during subsequent pregnancies. Similar to the other Rh antibodies, anti-c is also primarily of the IgG type. IgM anti-c, however, has been reported, as well as other Rh IgM antibodies.[2]

The Rh-E is found in 30% among the white and 21% in the blacks and is not as immunogenic as the Rh D antigen. Antibodies of the Rh E antigen are particularly IgM in nature although those of the IgG class exist. An antibody of Rh E is also capable of causing haemolytic transfusion reaction and haemolytic disease of the newborn.[11] The Rh-e is not as frequent as the Rh E antigen and its antibodies have been seen to cause haemolytic transfusion reaction and haemolytic disease of the newborn.[10] To prevent allo-immunization, women of reproductive age are given red cell transfusions compatible for Rh antigens such as C, c, D, E and e. This requirement may also be indicated for transfusion-dependent patients who receive regular red cell transfusions to prevent allo-immunization against the Rhesus antigens. Since alloantibody production can compromise future transfusion in these patients and make it more difficult and time consuming to provide compatible units for these patients, it is expedient and necessary that complete Rh antigen screening be carried out on pregnant women attending antenatal clinics in tertiary hospitals in Port Harcourt Nigeria and thus the need for this research.

Materials and methods

Study design and population

This cross-sectional, hospital-based study involved a total of one hundred (120) pregnant females within the age range of 18-40years recruited between February-July, 2022 at tertiary Hospital within Port Harcourt Metropolis. Informed consent was obtained from all studied participants via a well-structured questionnaire.

Collection of blood samples, storage and transportation

4 mls of Venepuncture blood sample was obtained aseptically from the antecubital fossa of each participant with the use of vacutainer containing 0.5 mL of 1.2 mg/mL of dipotassium Ethylene Diamine Tetra-Acetic Acid (EDTA), it was well mixed and used for the serological determination of Rh-C, Rh-c Rh-E and Rh-e blood group antigens respectively.

Determination of Rh-C, Rh-c, Rh-E and Rh-e blood group antigen using anti Rh-C, anti Rh-c, anti-Rh-E and anti Rh-e monoclonal, lorne laboratories microtitre agglutination techniques

Phenotyping of red cells was done using Micro-titre Agglutination technique as describe by Lorne laboratory Ltd. A 5% suspension of red blood cell was prepared using normal saline. 20 µl of Rh-C, Rh-c, Rh-E and Rh-e antibodies were added unto separate micro-titre plate, and 20 µl washed red cell was added into the micro-titre plate containing the Rh-C, Rh-c, Rh-E and Rh-e antibodies. The sample was incubated for 15 minutes with intermittent rocking and observation for agglutination every 30 seconds. If no agglutination found after 30 minutes, 20 µl of LISS antibody was added and observed for 15-30 minutes. Confirmation of agglutination and no agglutination was done by placing the sample on a slide and viewed microscopically. Presence of agglutination indicates a positive result and absence of agglutination indicates negative result

Data obtained were statistically analyzed by simple percentage calculation Statistical Analysis and defining the percentage frequency of Rh antigens with results presented in tables.

Results

Demographic data of studied population

A total of one hundred and twenty (120) pregnant female subjects within the age range of 18-45 years were recruited for this study. 31 of the total participants representing 25.8% were between the ages of 18-27 years, 77 (64.2%) were between the ages of 28-37years while 12 (10.0%) had ages >37 respectively. 20 participant have been previously transfused while 100 participant have no blood transfusion history. The number of studied participant with first pregnancy (prime) were 37 (30.8%), G2P1 were 30 (25.0%), G2P2 was 1(0.83%), G3P0 were 4 (3.3%), G3P1 were 2 (1.7%), G3P2 were 16 (13.3%) and G4P1 and above were 30 (25.0%) respectively as shown in Table1.

Table 1 Demographic data of studied population

Parameter	frequency	percentage
Age(years)		
18-27	31	25.8
28-37	77	64.2
>37	12	10
Transfusion history		
Yes	20	16.7
No	100	83.3
Parity		
Prime	37	30.8
G2P1	30	25
G2P2	1	0.83
G3P0	4	3.3
G3P1	2	1.7
G3P2	16	13.3
G4P1 and above	30	25

Distribution of Rh antigens in studied participants

Distribution of Rh Antigens in Studied Participants as presented on Table 2 showed that 21 (17.5%) expressed Rh C antigen, 107 (89.2%) expressed Rh c antigen, 47 (39.2%) expressed Rh E antigen and 109 (90.8%) expressed the Rh e antigen while 99 (82.5%), 13 (10.8%), 73 (60.8%) and 11 (9.2%) did not express Rh-C, Rh-c, Rh-E and Rh-e respectively.

Table 2 Distribution of Rh antigens in studied participants

Rh antigens	Number of positives (%)	Number of negatives (%)
Rh-C	21 (17.5)	99 (82.5)
Rh-c	107 (89.2)	13 (10.8)
Rh-E	47 (39.2)	73 (60.8)
Rh-e	109 (90.8)	11 (9.2)

Distribution of Rh antigens with respect to parity in studied population

Rh C percentage distribution amongst studied population based on parity showed that individuals who are carrying their first pregnancy (Prime), G2P1, G2P2, G3P0, G3P1, G3P2, G4P1 and above respectively expressed Rh-C in a percentage of 4(3.3%), 5(4.2%), 0(0.0%), 1(0.8%), 0(0.0%), 8(6.7%) and 3(2.5%) respectively while 33(27.5%), 25(20.8%), 1(0.8%), 3(2.5%), 2(1.7%), 8(6.7%) and 27(22.5%) respectively did not expressed the Rh-C antigens on their red cells in Table 3

Rh c percentage distribution amongst studied population based on parity showed that individuals who are carrying their first pregnancy (Prime), G2P1, G2P2, G3P0, G3P1, G3P2, G4P1 and above respectively expressed Rh-c in a percentage of 33(27.5%), 24(20.0%), 1(0.8%), 2(2.5%), 2(1.6%), 15(12.5%) and 29(24.2%) respectively while 4 (3.3%), 6(5.0%), 0(0.0%), 1(0.8%), 0(0.0%), 1(0.8%) and

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1(0.8%) respectively did not expressed the Rh-c antigens on their red cells.

Table 3 Distribution of Rh antigens with respect to parity in studied participants

Rh antigen/parity	Number of positives (%)	Number of negatives (%)
Rh C		
Prime	4 (3.3)	33 (27.5)
G2P1	5 (4.2)	25 (20.8)
G2P2	0 (0.0)	1 (0.8)
G3P0	1 (0.8)	3 (2.5)
G3P1	0 (0.0)	2 (1.7)
G3P2	8 (6.7)	8 (6.7)
G4P1 and above	3 (2.5)	27 (22.5)
Rh c		
Prime	33 (27.5)	4 (3.3)
G2P1	24 (20.0)	6 (5.0)
G2P2	1 (0.8)	0 (0.0)
G3P0	3 (2.5)	1 (0.8)
G3P1	2 (1.7)	0 (0.0)
G3P2	15 (12.5)	1 (0.8)
G4P1 and above	29 (24.2)	1 (0.8)
Rh E		
Prime	15 (12.5)	18 (15.0)
G2P1	11 (9.2)	19 (15.8)
G2P2	1 (.08)	0 (0.0)
G3P0	2 (1.7)	2 (1.7)
G3P1	2 (1.7)	0 (0.0)
G3P2	5 (4.2)	10 (8.3)
G4P1 and above	11 (9.2)	19 (15.8)
Rh e		
Prime	36 (30)	1 (0.8)
G2P1	23 (19.2)	7 (5.8)
G2P2	1 (0.8)	0 (0.0)
G3P0	4 (3.3)	0 (0.0)
G3P1	2 (1.7)	0 (0.0)
G3P2	15 (12.5)	1 (0.8)
G4P1 and above	28 (23.3)	2 (1.7)

Key: G= Gravida, P = Para; G1P1 = Gravida 1, Para 1; G2P2 = Gravida 2, Para 2; G3P0 = Gravida 3, Para 0; G3P1 = Gravida 3, Para 1, G3P2 = Gravida 3, Para 2, Rh= Rhesus.

Rh E percentage distribution amongst studied population based on parity showed that individuals who are carrying their first pregnancy (Prime), G2P1, G2P2, G3P0, G3P1, G3P2, G4P1 and above respectively expressed Rh-E in a percentage of 15(12.5%), 11(9.2%), 1(0.8%), 2(1.7%), 2(1.7%), 5(4.2%) and 11(9.2%) respectively while 18(15.0%), 19(15.8%), 0(0.0%), 2(1.7%), 0(0.0%), 10(8.3%) and 19 (15.8%) respectively did not express the Rh-E antigens on their red cells.

Rh e percentage distribution amongst studied population based on parity showed that individuals who are carrying their first pregnancy (Prime), G2P1, G2P2, G3P0, G3P1, G3P2, G4P1 and above respectively expressed Rh-E in a percentage of 36(30%), 23(19.2%), 1(0.8%), 4(3.3%), 2(1.7%), 15(12.5%) and 28(23.3%) respectively while 1(0.8%), 7(5.8%), 0(0.0%), 0 (0.0%), 0(0.0%), 1(0.8%) and 2(1.7%) respectively did not express the Rh-e antigens on their red cell in study population

Distribution of Rh antigens with respect to previous transfusion history in studied participants

Distribution of Rh Antigens with respect to previous transfusion history in studied participants showed that 6 (5.0%) for Rh-C, 15 (12.5%) for Rh-c, 6 (5.0%) for Rh E and 17 (14.2%) for Rh-e respectively among studied participants who have had previous transfusion while those with no history of previous transfusion showed 16 (12.5%), 92 (76.6%), 41 (34.2%) and 93(77.5%) for Rh-C, Rh-c, Rh-E and Rh-e respectively in the studied population in Table 4

Table 4 Distribution of Rh antigens with respect to previous blood transfusion in studied population

Transfusion history /Rh antigens	observation	frequency	percentage (%)
Rh C			
Yes	Positive	6	5
No	Positive	16	12.5
Rh c			
Yes	Positive	15	12.5
No	Positive	92	76.6
Rh E			
Yes	Positive	6	5
No	Positive	41	34.2
Rh e			
Yes	Positive	17	14.2
No	Positive	93	77.5

Discussion

This study investigated the frequency distribution of Rh-C, Rh-c, Rh-E and Rh-e blood group antigens amongst pregnant women attending antenatal clinic in Tertiary Hospital, Port Harcourt, Nigeria. A total of one hundred and twenty (120) pregnant females carrying their first pregnancy (prime) or with second, third or fourth pregnancy all within the age range of 18-40 years were recruited for the study. 31 of the total participants representing 25.8% were between the ages of 18-27 years, 77 (64.2%) and 12 (10.0%) were between the ages of 28-37 and >37 respectively.

In this study, the percentage positivity for Rh C, Rh-c, Rh-E and Rh-e was 17.5%, 89.2% 39.2% and 90.8% respectively .The most frequently occurring Rh phenotypes among the different groups was the Rh-e followed by Rh-c, Rh- E and the least being the Rh-C phenotype. The results in this study is in tandem with results obtained by Reid and Lomas-Francis,[12]and Daniels,[13] who revealed that the Rh C antigen was the least prevalent antigen with 27% behind the Rh E antigen (29%) in Blacks. Also, studies carried out by Jeremiah and Odumody,[14] in Calabar municipal showed that the overall frequency of Rh-E antigen (18.89%) was higher than the overall frequency of Rh-C antigen (2.78%) in the population. The findings in the present study revealed that the Rh-e antigen is the most prevalent in the study population before the Rh-c, Rh-E and Rh-C. The high percentage positivity of Rh-e phenotype observed in this study is slightly in agreement with the findings in our earlier study amongst descent of Bonny Kingdom,[3] were we found a percentage prevalence of 94.2% for Rh-e antigens.

Within the South-South region of Nigeria, Rh phenotypes in the general population has been studied and reported in Port Harcourt and Calabar. Jeremiah,[15] carried out a study in Port Harcourt, Nigeria, and found out that out of 374 pregnant women recruited for their study, the Rh-c phenotype showed a prevalence of 82.0%, which is lower than the prevalence indicated in the present study. Also studies by

Jacob,[2] revealed a prevalence of 93.3% in the total population with a frequency occurrence of 112 out of 120 subjects for the Rh-c blood group antigen. A study by Adewoyin,[16] also shows a high prevalence of the Rh-c and Rh-e antigen with a percentage distribution of 97.7% and 97.4% respectively. Erhabor,[17] carried out a study in Northern Nigeria, which showed a prevalence of 92% for the Rh-c antigen and 98.5% for the Rh-e antigen.

The high frequency of Rh-e and Rh-c in this study population might be due to the non-inclusion of Rh-e and Rh-c antigens screening during pre-transfusion/donation screening in men and women attending tertiary hospitals in Port Harcourt Nigeria thus sponsoring increase exposure to the antibodies. Also the rate of exposure to these antibodies must have occurred during delivery, blood transfusion or post-partum haemorrhage in pregnant women thus accounting for the high prevalence rate of 90.8% and 89.3% in the study population. The Rh-c is considered the most clinically significant Rh antigen after D and is associated with severe haemolytic disease of the new born (HDN). Anti-c antibodies arise through previous exposure, such as feto-maternal haemorrhage or transfusion, and can produce acute and delayed haemolytic reactions.

Percentage distribution of Rh-C, Rh-c, Rh-E and Rh-e amongst studied population based on parity showed that individuals who are carrying their first pregnancy (Prime), G2P1, G2P2, G3P0, G3P1, G3P2, G4P1 and above respectively expressed a percentage of 4(3.3%), 5(4.2%), 0(0.0%), 1(0.8%), 0(0.0%), 8(6.7%) and 3(2.5) for Rh-C, 33(27.5%), 24(20.0%), 1(0.8%), 2(2.5%), 2(1.6%), 15(12.5%) and 29(24.2) for Rh-c, 15(12.5), 11(9.2), 1(0.8), 2(1.7), 2(1.7), 5(4.2) and 11(9.2) for Rh-E and 36(30), 23(19.2), 2(1.7), 5(4.2), 2(1.7), 13(10.2) and 28(23.3). The results indicates that those carrying their first pregnancy (prime) expressed more of the Rh-e followed by the Rh-c, Rh-E and Rh-C in that other and thus can be concluded that there is variation in the distribution of these antigens as the number of pregnancy increases. Antigen expression is peak at the first pregnancy and decline within second or third pregnancy and then peak again from the fourth pregnancy. Research by Joy,[18] amongst a total of 283 pregnancies identified with anti-E established that Anti-E alloimmunization can cause haemolytic disease of the fetus or newborn and thus requires prenatal intervention. Hackney,[19] has also reported similar observation after a review of 102 pregnancies managed at the Ohio State University from 1967 to 2001 for anti-c isoimmunization and concluded that Anti-c isoimmunization might cause significant fetal and newborn haemolytic disease

Distribution of Rh Antigens with respect to previous transfusion history in studied participants showed that 6 (5.0%) for Rh-C, 15 (12.5%) for Rh-c, 6 (5.0%) for Rh E and 17 (14.2%) for Rh-e respectively among studied participants who have had previous transfusion while those with no history of previous transfusion showed 16 (12.5%), 92 (76.6%), 41 (34.2%) and 93(77.5%) for Rh-C, Rh-c, Rh-E and Rh-e respectively in the studied population. Research done by Christian,[11] indicated that allo-immunization due to Rh antibodies may occur as a result of blood transfusion. However, this was not observed in our study. The reason may be as a result of most of our study populations 83.3% have not had any previous transfusion thus there no significant effect on the distribution of Rh antigens particularly the Rh-e and Rh-c among the studied participants further supporting the fact that the study population may not have been previously exposed to Rh-e and Rh-c antibodies in course of transfusion or deliveries.

Conclusion

This study revealed a percentage positivity of 17.5%, 89.2%, 39.2 % and 90.8% for Rh-C, Rh-c, Rh-E and Rh-e respectively with high percentage expression for Rh-c, E and e antigens found on the red cells of Antennal Women carrying their first pregnancy. Furthermore, there is a high percentage expression of Rh antigens in previously transfused pregnant women in this study. Although routine phenotyping of these blood group antigens will be a financial burden in a resource limited country like Nigeria. It is expedient and needful to take into cognizance the fact that the presence of Rh C, c, E and e antigens may likely be the cause of some delayed transfusion reactions and haemolytic disease of the foetus and new born. Therefore, there is need for the inclusion of Rh C, c, E and e in routine antigen typing for pregnant women in other to help in ameliorate red blood cell alloimmunization and delayed haemolytic transfusion reaction during pregnancy.

Recommendation

Prenatal immunohematologic care of pregnant women requires the investigation of unexpected RBC antibodies in their sera during pregnancy. When RBC antibody screening is positive, it is necessary to determine specificity of the antibody, its clinical importance, and the ability to cross the placenta and cause HDFN.

The finding from this study re-emphasizes the need for a policy to promote the optimum stocking of Rh-e, c antigen negative blood in blood banks in the area for emergency use. There is also the need for routine screening of pregnant women for clinically significant red cell antigens (including antigen C, c E and e) and alloantibodies (antibody C, c E and e). This will facilitate the management of women positive for antibody C, c, E and e to prevent HDFN associated with alloantibody. It will also enable those that have a clinically significant alloantibody e and c, and who requires red cell transfusion to be transfused with donor blood that is negative for these antigens to which their antibody is specific.

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None.

Conflicts of interest

The authors declare no conflicts of interest.

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