Frequency Occurrence and Percentage Distribution of Rh C, Rh c, Rh E and Rh e Blood Group Amongst Pregnant Women Attending Antenatal Clinic in Port Harcourt, Nigeria

Serekara Gideon Christian, Evelyn Mgbeoma Eze, Barizoge Monsi Badom, Ibiere Allwell Pepple, and Christopher Aloy Simeon

ABSTRACT

Background: The Rhesus (Rh) blood group is one of the most complex blood groups in humans comprising mainly of Rh D, C, c, E and e. However, only Rh D is routinely screened for in Nigeria despite the fact that other Rh antigens are clinically significant and can cause haemolytic disease of the newborn and delayed haemolytic transfusion reactions.

Aim: The aim was to determine the frequency distribution of Rh C, c, E and e blood groups among pregnant women attending antenatal clinic in Port Harcourt, Nigeria.

Study Design: The study consisted of 147 apparently healthy pregnant women within the age range of 18-45 years, attending a selected Primary Healthcare Centre (Obio Cottage Hospital) in Port Harcourt. The study was carried out from January 20, 2020 to March 27, 2020. The presence of Rh C, c, E and e blood groups were investigated using Anti-C, c, E and e monoclonal antibody in the same order.

Results: Rh C, c, E and e were observed in 38.09%, 100%, 17.68% and 100% in the same order.

Conclusion: The study indicated dominance of Rh c and Rh e over Rh C and Rh E among the studied pregnant women. It is necessary to take into cognizance the fact that the presence of Rh C, c, E and e antigens may be the cause of some delayed transfusion reactions and haemolytic disease of the foetus and newborn. Therefore, routine antigen typing for Rh antigens may help in decreasing red blood cell allo-immunization and delayed haemolytic transfusion reaction during pregnancy.

Keywords: Rh C, Rh c, Rh E, Rh e, Rhesus Blood Group, Nigeria.

Submitted : April 08, 2021

Published : June 03, 2021

ISSN: 2593-8339

DOI: 10.24018/ejmed.2021.3.3.808

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I. INTRODUCTION

The Rhesus blood group is one of the most complex blood groups in humans. From its discovery over 60 years ago, it has become second in clinical importance only to the ABO blood group in the field of transfusion medicine [1]. The Rh blood group system was discovered in 1940 by Karl Landstainer and Weiner who at that time believed that antibodies of these system cause haemolytic transfusion reaction (HTR) and haemolytic disease of the newborn (HDN) [2]. As early as 1941, it was obvious that Rh was not a simple single antigen system. With 49 antigens so far described, it is the largest of all blood group systems. The unusually large number of Rh antigens is attributable to its

complex genetic basis [3]. The Rh system differs from the ABO system in several ways and is second only to the ABO system in importance in transfusion medicine [1].

The Rh antigens are expressed as part of a protein complex in the red blood cell (RBC) membrane. This complex is only expressed in cells of the erythroid line, and therefore Rh antigens are only expressed in the membrane of red blood cells [2]. The complexity of the Rh blood group antigens begins with the highly polymorphic genes that encode them. The first Rhesus gene, the RHCE gene, was discovered in 1990. The RHD gene was found two years later, and the total deletion of this gene ascertained as the cause of the Rh D negative phenotype [4]. More than 170 alleles have been found on the RHD gene since then [4]. The two genes,

designated RHD and RHCE, encode the Rh proteins. Individuals who are Rh-positive have both genes, whereas most Rh-negative white people have only the RHCE gene [2]. The genes are 97% identical. Each gene has 10 exons and is the result of a gene duplication on chromosome 1p34–p36.26 [2].

The RhD protein carries the D antigen, and the RHCE protein carries various combinations of the CE antigens (ce, cE, Ce, or CE) [5]. RhD differs from RhCE by 32 to 35 amino acids (depending on which form of RhCE is present), and both are predicted to span the membrane 12 times. They are covalently linked to fatty acids in the lipid bilayer of erythrocytes [6].

The single gene, RHAG, located at chromosome 6p11–p21.1 encodes Rh-associated glycoproteins (RhAG) or Rh50 glycoprotein- to reflect its apparent molecular weight [7]. The Rh-associated glycoproteins is a 409-amino acid glycosylated protein that co-precipitates with RhD and RhCE. Rh-associated glycoproteins is 47% identical to the RH genes and also has 10 exons [8]. Rh50 shares 37% amino acid identity with the RhD and RhCE proteins and has the same predicted membrane topology. Rh-associated glycoprotein is not polymorphic and does not carry Rh antigens. It is important for targeting the RhD and RhCE to the membrane, because mutations in, or lack of expression of RhAG results in a lack of Rh antigen expression (Rh-null) or a marked reduction of Rh antigen expression (Rh-mod) [9].

Studies to estimate the number of D, C/c, and E/e antigen sites on RBCs found differences between Rh phenotypes. The number of D antigens ranges from 10,000 on Dce/ce RBCs to 33,000 on DcE/DcE. The number of C, c, and e antigens per RBC varies from 8500 to 85,000 [10]. Because C or c and E or e are carried on the same protein, their numbers should be equivalent. Results of tests with monoclonal antibodies to high-incidence Rh antigens suggest that the total number of Rh proteins (RhD and RhCE) per RBC is 100,000 to 200,000. The number of RhAG is also estimated to be 100,000 to 200,000 [11], consistent with predictions that Rh and RhAG may be present in the membrane as a tetramer of two molecules of each [12].

Amongst the 49 defined blood group antigens of the Rh blood group system, five are most important and they are C, c, E, e and D [1]. The antigens C, D, E, c and e (except d) are antigenic. They are capable of stimulating production of antibodies if introduced into the body of an individual whose red cells lack them. However, the Rh antigens vary in their degree of antigenicity. The D antigen is the most immunogenic of them [13].

Unlike ABO antibodies, Rh antibodies do not occur naturally. All Rh-antibodies are immune antibodies resulting from specific antigen stimulation either through transfusion, pregnancy or by injection of the antigen. Most Rh antibodies are IgG, subclasses IgG1 and IgG3, IgG2 and IgG4 have also been detected, and some sera have an IgM component [14]. Rh antibodies do not activate complement, although two rare exceptions have been reported. The inability to activate complement by Rh antibodies is thought to be due to the distance between antigens, but is probably due to a lack of mobility [10].

There are no natural Rh antibodies, hence antibody typing is not possible in the Rh system [15]. Therefore, all Rh-typing

methods depend upon antigen typing using known antiserum. Reactivity of Rh antibodies is enhanced by enzyme treatment of the test RBCs, and most react optimally at 37 °C. Some Rh-antibodies cannot be detected in saline suspension of red blood cells. If protein rich medium such as serum albumin is used, the antibodies can agglutinate the respective red cells, hence they are called incomplete or albumin active antibodies [16].

Rh antigens are highly immunogenic and are capable of stimulating an immune response and this is due to their large differences in amino acids. Whereas most blood types are determined by red cell antigens that differ by one or two amino acids, the Rh blood group contains the D antigen which differs from the C/c and E/e antigens by 35 amino acids [17]. Most of the Rh antibodies should be considered as potential causes of haemolytic transfusion reactions (HTR) and haemolytic disease of the new born (HDN) [17]. The majority of antibodies formed against the Rh antigens are of the IgG type. They are capable of causing significant HTR and HDN. Rh antibodies rarely, if ever, bind complement, and therefore RBC destruction is mediated almost exclusively via macrophages in the spleen [17].

Most times, pregnant women that register for antenatal in hospitals go through normal routine tests which do not include these other Rhesus blood group types. The normal routine test carried out for pregnant women in Nigeria include only the Rh D and there are other transfusion reactions and haemolytic disease of the newborn that can be caused by other Rh antigens of C, c, E and e, hence the essence of this research.

II. MATERIALS AND METHODS

A. Study Design

This was a cross sectional study performed to determine the prevalence of the Rh C, c, E and e blood groups in pregnant women and it cuts across different Nigerian ethnic groups. The study was carried out from January 20, 2020 to March 27, 2020. Only apparently healthy pregnant women within the age bracket of 18 to 45 years, who gave verbal consent were recruited based on convenient sampling method.

B. Study Area

This study was conducted in Port Harcourt, Rivers State, Nigeria. Port Harcourt the capital of Rivers State is located at latitude 4.75°N and longitude 7.00°E and lies along Bonny River on the Niger Delta [18]. As at 2016, the Population of the area was 1,865,000 [19]. All participants were recruited in Port Harcourt and samples were collected at the Primary Health Care Centre - Obio Cottage Hospital in Port Harcourt, Rivers State. Obio Cottage Hospital (OCH) was established in 1978 by the Rivers State government. It started as a primary health centre (PHC) providing preventive and curative health care services to mostly indigenes of Obio/Akpor local government area. More than two decades later Shell Petroleum Development Company (SPDC) started supporting the hospital as part of its social infrastructure program. Shell Petroleum Development Company upgraded and rehabilitated the facility converting the four-bed health care center operating on a small twin bungalow building with

13 staff to a 40-bed facility with over 100 staff.

C. Study Population

One hundred and forty-seven subjects, consisting of 147 pregnant females within the age range of 18-45 years participated in the study. They were apparently healthy and free from transmissible infections after they tested negative to HIV, hepatitis and syphilis.

D. Collection of Blood Samples, Storage and Transportation

After pre-test counseling and explanations, venous blood was drawn from the antecubital fossa of the subjects with the use of vacutainer as described by Cheesebrough (2018) [20]. Three millilitres of venous blood was drawn from each participant into a sample container that contains 0.5ml of 1.2 mg/ml dipotassium ethylene diamine tetracetic acid. It was well mixed for the serological identification of Rh C, Rhc, RhE and Rh e blood groups. Blood samples were analysed within 24 hours of collection.

E. Methodology

1. Determination of RhC Blood Group using Anti-C, Anti-c, Anti-E, and Anti-e Monoclonal, Lorne Laboratories Ltd, UK

Method: Standard Tube Method.

Standard tube technique was used for phenotyping of red cells as described by Lorne Laboratories [21]-[24]. Three percent (3%) red cell suspensions was prepared using isotonic saline. One volume of the Lorne anti-C, anti-c, anti-E, and anti-e reagent were added to one volume of the prepared 3% red cell suspension and properly mixed and centrifuged for 20 seconds at 1000g for the respective Rh blood groups. The red cell button was gently re-suspended and read macroscopically for the presence of agglutination. Tubes that indicated a negative result were incubated for 15 minutes at room temperature, centrifuged again and then observed macroscopically for agglutination. Presence of agglutination indicated a positive result, while absence of agglutination indicated a negative result.

F. Statistical Analysis

Data collected were statistically analyzed by simple frequency and percentage calculation. Results obtained are presented in tables.

III. RESULTS

A. Demographic Details of Study Population

A total number of 147 subjects from different tribes within the age of 18-45 years were recruited for the study and the total number of subjects from each tribe was calculated. Details are shown in Table I.

B. Frequency Occurrence and Percentage Distribution of the Study Population Based on Ethnic Group

The demographic characteristics of the subjects revealed that the Ijaws recorded the highest number of participants (35), followed by the Ogonis (28), the Orashi people (23), the Etche people (16), the Ikwerre people (15), the Igbos and Kalabari people (14 each), while the Ibibios recorded the least. Details are shown in Table II.

TABLE I: DEMOGRAPHIC DETAILS OF STUDY POPULATION

Parameters	Frequency (n)
Total Number of Subjects in the Study	147
Total Number of Subjects from Ogoni	28
Total Number of Subjects from Ijaw	35
Total Number of Subjects from Orashi (Ahoada)	23
Total Number of Subjects from Etche	16
Total Number of Subjects from Ikwerre	15
Total Number of Subjects from Igbo	14
Total Number of Subjects from Kalabari	14
Total Number of Subjects from Ibibio	2
Age Range (Yrs)	18-45

TABLE II: FREQUENCY OCCURRENCE AND PERCENTAGE DISTRIBUTION OF THE STUDY POPULATION BASED ON ETHNIC GROUP

Tribe	Frequency (n)	Percentage
Ogoni	28	19.04
Ijaw	35	23.80
Orashi (Ahoada)	23	15.65
Etche	16	10.88
Ikwerre	15	10.20
Igbo	14	9.52
Kalabari	14	9.52
Ibibio	2	1.36

C. Frequency Occurrence and Percentage Distribution of Rh C, Rh c, Rh E and Rh e Blood Groups in the Study Population

The study revealed that Rh c and Rh e recorded the highest frequency and a percentage distribution of 100%. Percentage distribution for Rh C and Rh E were 38.09% and 17.68% in the same order. Details of other Rh blood groups are shown in Table III.

TABLE III: FREQUENCY OCCURRENCE AND PERCENTAGE DISTRIBUTION OF RH C, RH c, RH E AND RH e BLOOD GROUPS IN THE STUDY POPULATION

Blood Groups	Frequencies (n)	Percentages (%)
Rh C Positive	56	38.09
Rh C Negative	91	61.90
Rh c Positive	147	100
Rh c Negative	0	0
Rh E Positive	26	17.68
Rh E Negative	121	82.31
Rh e Positive	147	100
Rh e Negative	0	0

D. Percentage Distribution of the Blood Group in the Study Population Based on Ethnicity

Table IV shows the frequency occurrence and percentage distribution across the various ethnic groups captured in the study. For Rh C, the Kalabari tribe recorded the highest percentage (57.14%), followed by the Orashi people (43.75%), the Ogonis and the Ijaws (42.85% each), the Etche people (37.50%), the Igbos (28.57%), and the Ikwerre people (26.70%). For Rh E, the Kalabari and Igbo people recorded 21.43% each, the Ijaws (20%), the Ogonis (17.86%), the Ikwerre people (13.33%), the Etche people (12.50%), and the Orashi people (8.70%). Only two participants were from Ibibio and they were Rh E positive, but because their number is too small compared to their population, the percentage is not significant to be a representation of the entire Ibibios.

TABLE IV: PERCENTAGE DISTRIBUTION OF THE BLOOD GROUP IN THE STUDY POPULATION BASED ON ETHNICITY

Ethnic Groups	Total Number	Rhc (n) %	RhC (n) %	RhE (n) %	Rhe (n) %
Ogoni	28	(28) Pos 100	(12) Pos 42.85 (16) Neg 57.15	(5) Pos 17.86 (23) Neg 82.14	(28) Pos 100
Ijaw	35	(35) Pos 100	(15) Pos 42.85 (20) Neg 57.14	(7) Pos 20 (28) Neg 80	(35) Pos 100
Orashi	23	(23) Pos 100	(7) Pos 30.43 (16) Neg 69.56	(2) Pos 8.70 (21) Neg 91.30	(23) Pos 100
Etche	16	(16) Pos 100	(6) Pos 37.5 (10) Neg 62.5	(2) Pos 12.5 (14) Neg 87.5	(16) Pos 100
Ikwerre	15	(15) Pos 100	(4) Pos 26.7 (11) Neg 73.3	(2) Pos 13.33 (13) Neg 86.67	(15) Pos 100
Igbo	14	(14) Pos 100	(4) Pos 28.57 (10) Neg 71.43	(3) Pos 21.43 (11) Neg 78.57	(14) Pos 100
Kalabari	14	(14) Pos 100	(8) Pos 57.14 (6) Neg 42.86	(3) Pos 21.43 (11) Neg 78.57	(14) Pos 100
Ibibio	2	(2) Pos 100	(2) Neg 100	(2) Pos 100	(2) Pos 100

IV. DISCUSSION

This study investigated the frequency distribution of Rh C, Rh c, Rh E and Rh e blood group amongst pregnant women attending antenatal in Port Harcourt. A total number of 147 subjects comprising of pregnant women from 8 different ethnic groups in Nigeria were recruited. The Ijaws formed the largest group with percentage distribution of 23.80% while the Ibibos formed the least group with 1.36%.

The most frequently occurring Rh phenotypes among the different groups was the Rh c and Rh e phenotypes which showed an overall frequency percentage distribution of 100% amongst all the groups, followed by Rh C (38.09%) and the least being the Rh E phenotype (17.68%). There was an ethnic variation in the distribution of Rh phenotypes among the pregnant women studied.

Within the South-South region of Nigeria, Rh phenotypes in the general population have been studied and reported in Port Harcourt and Calabar [25], [26]. One of such studies by Jeremiah et al. [27] carried out in Port Harcourt, Nigeria, in which 374 pregnant women were recruited for the study and of the 374 pregnant women studied; the Rh c and Rh e phenotype showed a prevalence of 82.0% and 54.0% respectively, which is lower than the prevalence indicated in the present study.

In another study carried out in Calabar municipal among the Ibibio, Efik, and Igbo ethnic nationalities in Calabar, the Rh c phenotype showed a percentage distribution of 100% which is similar with findings from the present study, while the Rh e phenotype showed a percentage distribution of 96.38% [26] which also indicated dominance of Rh e blood

group. A multi-ethnic population of healthy Nigerian individuals studied by Adewoyin et al. [28] also shows a high prevalence of the Rh c and Rh e antigen with a percentage distribution of 97.7% and 97.4% respectively. Similar findings are also seen in a study by Erhabor et al. [29] carried out in Northern Nigeria, which showed a prevalence of 92% for the Rh c antigen and 98.5% for the Rh e antigen. In India, the prevalence of Rh e phenotype was 85% and Rh c 79% [30]. This finding shows that although the Rh c and Rh e phenotype distributions are high among the general population, there is an ethnic variation in the distribution in different population.

The findings in the present study revealed that the Rh C antigen is the next most prevalent in the study population after the Rh c and Rh e antigen with 38.09%. This is in agreement with the observation in a study by Jeremiah et al. [27], where the Rh C antigen was the next common frequently Rh antigen with a prevalence of 24.3%. With similar findings observed in a multi-ethnic cohort study carried out by Adewoyin et al. [28], and a study carried out in Abidjan for antigens of the Rh blood group system amongst 651 blood donors [31]. However, the findings in the present study are in contrast to a study by Reid and Lomas-Francis [32] and Daniels [10] which revealed that the Rh C antigen was the least prevalent antigen with 27% behind the Rh E antigen (29%) in Blacks.

Different studies have shown variations in the prevalence of the Rh E antigen distribution. In the present study, it was observed that the Rh E antigen is the least prevalent (17.68%) of the Rh antigens amongst the 147 pregnant women studied. This is in concord with a study carried out by Jeremiah et al. [27], it was observed that the Rh E antigen was the least predominant antigen in the study population with 20.1% when compared to Rh c (82.0%), Rh e (54.0%) and Rh C (24.3%). A multi-ethnic population study by Adewoyin et al. [28], also agrees with the findings in the present study. Bogui et al. [31], also showed in a study that was carried out in Abidjan among 651 blood donors that the frequency distribution of the Rh E antigen appeared to be the lowest amongst the other Rh blood group antigens studied. However, a number of studies do not follow a similar pattern, one of such studies carried out by Jeremiah and Odumody [26] in Calabar municipal among the Ibibio, Efik and Igbo ethnic nationalities in Calabar 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. In another of such study carried out in Blacks to ascertain Rh phenotype distribution; it was observed that the Rh E antigen was the third most prevalent antigen after the Rh e and Rh c antigen in the study population [10], [32].

There is paucity of data showing the frequency distribution of the Rh phenotype among the ethnic groups in Nigeria. Hence, comparative study to measure the variations in this study and other studies is not possible. A study by Christian et al. [33] amongst the Ogonis, revealed a percentage distribution of 25.74% for Rh E antigen. The finding in the study was higher by 7.88% when compared with the percentage distribution of the Rh E antigen in the present study for the Ogonis.

The limitation of the study is that pregnant women from other tribes in Nigeria (Hausa/Fulani tribe, Yorubas, Tiv, and

only two participants from Ibibio) were not recruited. We therefore recommend that a larger population of pregnant women that should include people from other tribes and more participants should be studied.

V. CONCLUSION

The study indicated dominance of Rh c and Rh e among the studied pregnant women, while Rh C and Rh E blood groups were less in the studied subjects although routine phenotyping of these blood group antigens will be a financial burden in a resource limited country like ours. It will be necessary 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, routine antigen typing for Rh antigens may help in decreasing red blood cell allo-immunization and delayed haemolytic transfusion reaction during pregnancy.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from apparently healthy pregnant subjects prior to enrolment upon approval by the Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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