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Rh (E) phenotype among pregnant women in Sokoto, North Western Nigeria.

Erhabor Osaro[1], Kabiru Salisu Adamu[1], Abdulrahaman Yakubu[1], Okwesili Augustine[1], Onuigwe F[1], Isaac ZI[1],

1. Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
2. Department of Obstetrics and Gynaecology Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

The Rhesus blood group system is second to the ABO blood group system among the clinically significant red cell antigens. The Rhesus blood group system has been incriminated in cases of haemolytic transfusion reaction and haemolytic disease of the foetus and newborn. In this present study, we investigated 155 pregnant women aged 18 to 45 years and mean age 27.19 ± 4.70 attending antenatal clinic in Usmanu Danfodiyo University Teaching Hospital Sokoto for their Rhesus E phenotype using Lorne Laboratories (United Kingdom) anti-E reagent. Out of the 155 pregnant women tested, 44(28.4%) were positive for Rh (E) whereas 111(71.6%) tested negative. Subjects were classified based on ethnicity. Pregnant women of Hausa ethnic group was found to have the highest frequency (60.6%), followed by Fulani (12.3%), Igbo (11.6%), others (9.7%) and Yoruba (5.8%). Subjects were stratified based on age groups. The age range of 26-35yrs was found to have the highest frequency 76 (49%), followed by 15-25 yrs 70 (45.2%) and 36-45yrs 9 (5.8%). Subjects were also categorized based on their educational status. Subjects that attended tertiary institutions had the highest frequency 42.6%, followed by secondary 31.6%, primary 21.9% and non formal 3.9%. We recommend that all pregnant women be routinely tested for clinically significant red cell antigen including Rhesus E during pregnancy. Pregnant women who are Rh E negative who require a transfusion should be transfused with Rh E negative red cells to prevent alloimmunization and HDFN in future pregnancies. Pregnant women should be tested routinely for the presence of clinically significant alloantibodies. Those positive for alloantibodies should be transfused with red cells that are negative for antigens to which the antibody is specific.

Keyword: Rhesus (E) phenotype, pregnant women, Sokoto, Nigeria, haemolytic disease of the new-born, haemolytic transfusion reaction.

*Corresponding Author Email: n_osaro@yahoo.com
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INTRODUCTION

The Rh (Rhesus) blood group system is one of the most clinically significant blood group system in the leagues of thirty-three existing human blood group systems. It is the most important blood group system after ABO blood group system. The Rhesus (Rh) blood group system was first described 60 years ago. A woman had a severe transfusion reaction when she was transfused with blood from her husband following the delivery of a stillborn child with erythroblastosis foetalis. Her serum agglutinated red blood cells (RBCs) of her husband and those of 80% of Caucasian ABO compatible donors [1]. Although more than 50 antigens have been described as part of the Rh blood group system, most cases of alloimmunisation following blood transfusions or pregnancy can be attributed to the five most common ones, (D, C, E, c and e) [2].

Women and girls that have child bearing potential should ideally receive Rhesus antigens (C, c, E and e) compatible red cell transfusions in addition to the K antigen of the Kell blood group to prevent Rhesus and Kell sensitization [3]. A previous report indicates that the majority of women with Rhesus antibodies were due to feto-maternal incompatibility [4]. Investigation of the phenotypic frequencies of Rh blood groups among 10,133 healthy voluntary Indian donors observed Rhesus E prevalence of 26.55% [5].

The determination of the proportion of Rh phenotypes in a population is vital in the generation of evidenced-based data required for the determination of predisposition to development of alloantibodies, determining the risk of Haemolytic Disease of the Foetus and Newborn (HDFN), determination of constitution of panel of blood donors and maintenance of optimum stocking of blood banks. Even after proper blood grouping and cross matching there is a possibility of alloimmunization and antibody production in the recipients against the Rh antigen particularly among recipients who receive units that are not Rhesus phenotyped and compatible units.

The determination of the prevalence of clinically significant red cell antigens is important to ensure the optimum stocking of Rh antigen negative red cells for transfusion to Rhesus antigen (C, D, E, c, e) negative women who may require a red cell transfusion. This can potentially help to prevent alloimmunization and production of Rh antibodies and by extension prevent the risk of HDFN and Haemolytic Transfusion Reaction(HTR). The aim of this present study is to determine the prevalence and ethnic distribution of Rhesus E antigen among pregnant women attending Antenatal Clinic (ANC) in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria.

MATERIALS AND METHOD

Description of the study area

The selected area for this study is the Haematology Department of Usmanu Danfodiyo University Teaching Hospital which is located in Wamakko Local Government within Sokoto Metropolis, Sokoto State. Sokoto is located in the Sudan Savannah of North-Western Nigeria and has a longitude of 5^0 14' East and latitude of 13^0 04' North. It covers a land area of about 60.33Km^2. It has a mean annual rainfall of 500-1300mm. Sokoto State shares borders with Kebbi State to the West and South-East, Zamfara State to the West and Niger Republic to the North. Report from the 2007 National Population Commission indicated that the state had a population of 3.6 million [6]. The residents are mainly Hausa/Fulani and other non-indigenous ethnic groups like Yoruba, Igbo, and Zabarma tribe from neighbouring Niger Republic. The main occupation of the people is trading, farming with few numbers of civil servants.

Study design

One hundred and fifty five consecutively- recruited pregnant women visiting the antenatal clinic of Usmanu Danfodiyo University Teaching Hospital in Sokoto, North Western, Nigeria constituted the subjects for this case study. The aim of the study was to investigate the prevalence of Rhesus E antigens among the pregnant subjects.

Sampling method

One hundred and fifty five consecutively -recruited pregnant women who met the eligibility criteria for this study (age ≥ 18 years, confirmed pregnant by a consultant obstetrician, willingness to give written informed consent and no history of recent transfusion in the last 4 months) were recruited as subjects for this case study to avoid bias. Their Rhesus blood group antigen status was determined. The sample size was estimated using the formula, $N= Z^2 pq \div d^2$.

Where,

N= sample size, Z= standard deviation of normal, p= prevalence of event in the population

q= 1-p

d= confidence interval

By using the formula,

$N= (1.96)^2 \times 0.95 \times (1-0.95)/ (0.05)^2 = 145$

Due to attrition, 10% of 145 is calculated and added to the sample size. 145+ 15= 160

Statistical analysis

The data collected was recorded on an Excel spreadsheet and later subjected to statistical analysis using a statistical software SPSS version 18.0. Statistical analysis included descriptive statistics of

mean and bivariate analysis of t- test and chi- square. Correlation was compared using linear regression analysis. Differences were considered significant when $p \leq 0.05$.

Study site and participating hospital

The study was carried out in the Faculty of Medical Laboratory Sciences (FMLS) of Usmanu Danfodiyo University Sokoto (UDUS) in collaboration with the Department of Obstetrics and Gynaecology as well as Haematology Department of UDUTH. The laboratory in UDUTH is a service laboratory equipped with facilities for the analysis of Rhesus antigens status of pregnant subjects.

Eligibility criteria

All consenting, consecutively recruited legal adults (≥ 18 years) and confirmed pregnant women (by a consultant obstetrician) attending the antenatal clinic (ANC) in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto constituted the subjects of this study.

Exclusion criteria

The following women who did not meet the inclusion criteria were excluded from ; women who were not pregnant, pregnant but not consenting, pregnant women < 18 years of age and pregnant women who have had a history of a recent blood transfusion in the last 4 months.

Informed consent

Written informed consent was obtained from all pregnant women participating in this study, together with socio-demographic information. Ethical clearance was obtained from the ethical committee (UDUTH/HREC/2014/No 198) of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto North Western, Nigeria.

Sample collection

Five milliliters of whole blood was collected using a syringe and needle into EDTA anticoagulated tube and used for the determination of Rhesus phenotype (E) using Lorne Laboratories (United Kingdom) anti-E reagents. Samples that were not tested the same day were stored at 2-8^{0}C till the following day.

Method

The frequencies of Rhesus E antigens among pregnant women attending the antenatal clinic in UDUTH, Sokoto was determined using standard serologic technique (tube method) using Lorne Diagnostics anti- E reagents (Lorne Diagnostics, UK). The principle is based on the ability of Lorne Diagnostic anti-E reagents to cause a direct agglutination of the test RBCs that carry the corresponding Rhesus antigen. Agglutination indicated the presence of the group specific Rhesus

antigen to which the Rhesus antibody is specific. No agglutination generally indicates the absence of the corresponding Rhesus antigen.

RESULTS AND DISCUSSION

A total of 155 blood samples was collected from pregnant women aged 18 to 45 years and mean age 27.19 ± 4.70 attending Antenatal clinic in Usmanu Danfodiyo University Teaching Hospital Sokoto. Samples were investigated for their Rhesus E phenotype status. Out of the 155 pregnant women tested, 44(28.4%) of the samples phenotyped were positive for Rh (E) whereas 111(71.6%) were negative. Table 1 show the prevalence of E antigen among subjects. Subjects were classified based on ethnicity. Women of Hausa ethnic group was found to have the highest frequency (60.6%), followed by Fulani (12.3%), Igbo (11.6%), others (9.7%) and Yoruba (5.8%). Table 2: shows the distribution of subjects based on ethnicity. Subjects were stratified based on age groups. Majority of the subjects 76 (49%) were in the 26-35years age group followed by the 15-25 years 70 (45.2%) and 36-45years 9 (5.8%). Table 3 show the distribution of subjects based on their age group. Subjects were also categorized based on their educational status. Subjects that attended tertiary institutions have the highest frequency 42.6%, followed by secondary 31.6%, primary 21.9% and non formal 3.9%. Table 4 show the distribution of subjects based of educational status.

The Rhesus blood group system is the second after the ABO blood group system in the league of clinically significant red cell antigen system [7]. The Rh system is involved in haemolytic disease of the foetus and new-born, haemolytic transfusion reaction, autoimmune haemolytic anaemia and in forensic work[8]. In this study among pregnant women in Sokoto, we observed Rhesus E phenotype among 44 (28.4%) out of our cohort of 155 pregnant women tested. Our finding is consistent with previous report by Gwaram and Abdullahi [9] who reported Rh (E) prevalence of 34% among their cohort of one hundred and three consecutively -recruited blood donors in Aminu Kano Teaching Hospital in Kano, North- Central Nigeria. Similarly, a previous report among 115 O blood group donors from three different blood banks in India reported Rh (E) prevalence of 21.74% [10]. Our observed prevalence is however higher than that obtained in a previous report by Nwauche and Ejele (2004) [11] who studied 65 subjects made up of 35 pregnant women and 30 blood donors in the Niger Delta of Nigeria and obtained Rh (E) prevalence of 16.92%. Our finding is also consistent with reports by Sarkar and colleagues [5] who reported Rh E antigen prevalence of 26.55% among their cohort of 10,133 healthy voluntary blood donors in India .

Anti-E is a more common antibody compared to anti-C. Anti-E is commonly produced in R_1R_1 individuals exposed to R_2R_2 or R_2r red cells and exist mostly in combination with anti-c. Anti-E

seldom causes HDFN and when it does the disease is usually mild. In a previous study involving Norwegian women, a significant one third of all new cases of alloimmunization to the Rhesus blood group system (anti- C, anti-D and anti- E) occurred among Rh (D) positive women [12].

Table 1: Prevalence of E antigen among subjects

E antigen status	Frequency	Percentage
Positive	44	28.4
Negative	111	71.6
Total	155	100

Table 2: Distribution of subjects based on ethnicity

Tribe	Frequency	Percentage (%)
Hausa	94	60.6
Fulani	19	12.3
Yoruba	9	5.8
Igbo	18	11.6
Others	15	9.7
Total	155	100

Table 3: Distribution of subjects based on their age range

Age Group	Number	%
15-25	70	45.2
26-35	76	49
36-45	9	5.8
Total	155	100

Table 4: Distribution of subjects based of educational status

Educational Level	Number	%
No formal	34	21.9
Primary	6	3.9
Secondary	66	42.6
Tertiary	49	31.6
Total	155	100

This present study shows that a significant number of women tested were negative for Rhesus antigen E (71.6%). This is an indication that there is significant risk of pregnant women in the area being sensitized to produce alloantibody -E particularly if they are exposed to Rhesus E positive foetus and E -positive red cell transfusion. A previous study that involved pregnant women in Port Harcourt in the Niger Delta of Nigeria [13] observed clinically significant antibodies in the serum of 3.4% of pregnant women studied. The specificity of the antibodies identified included; anti-C 6 (1.2%), anti-E 3 (0.6%), anti-K 5 (1.0%) and anti-Jsb 3 (0.6%). Rhesus antigens are well developed at birth and have been identified in early foetal life (as early as 38 days) as well as immunogenic (ability to stimulate antibody production) and therefore can easily cause haemolytic disease of the foetus and new-born if the baby has Rh E antigen that the mother lacks. Clinically significant alloantibodies including Rhesus Anti-E is prevalent among pregnant women in

Nigerian. A previous report in Port-Harcourt [14] indicates that anti-E antibody is common among pregnant women in Nigeria. Similarly, in a retrospective review of 28,303 (21,327 Chinese) antenatal attendances from 1997 to 2001, 213 (0.79%) indicated the presence of anti-E [15]. There is combined genetic inheritance between Rhesus antigen E and c. It is advisable particularly in all cases when anti-E is detected that the potential presence of anti-c be strongly suspected. It is best practice to select c-negative and E-negative red cells for transfusion to recipients who are positive for anti-E. These studies re-emphasises the need for routine screening of pregnant women for clinically significant red cell antigens and alloantibodies. This will facilitate the management of these women to prevent HDFN. It will also enable those that have a clinically significant alloantibody and who requires a red cell transfusion to be transfused with donor blood that is negative for antigens to which their antibody is specific.

Findings in this study indicates that a significant number of highly educated women (secondary and tertiary) attend antenatal clinic compared to less educated women (no formal and primary). The level of a patient's educational status seems to influence the degree at which they seek antenatal care in hospitals. Education seems to play a very important role. It helps women to make informed decision as well as access information on the several complications associated with non-attendance to ANC. Our finding is in agreement with previous report which indicated that less educated women and women of low socio-economic class are less likely to attend antenatal clinic and present for delivery in labour unbooked [16].

CONCLUSION

The Rh system play a significant role in the incidence of haemolytic disease of the new-born and haemolytic transfusion reaction. The incidence of Rhesus (E) disease of the new-born is mathematically related to the frequency of E negative individuals in a population. We recommend that all pregnant women be routinely tested for clinically significant red cell antigen including Rhesus E during pregnancy. Pregnant women who are Rh E negative who require a transfusion should be transfused with Rh E negative red cell to prevent alloimmunization and development of anti-E alloantibodies which can potentially cause HDFN in future pregnancies particularly those associated with babies who are positive for Rh E antigens. Pregnant women should also be tested routinely for the presence of clinically significant alloantibodies. Those positive for alloantibodies who require a red cell transfusion, should be transfused with red cells negative for antigens to which the antibody is specific.

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