

Research Article

## Rh (C) Phenotype Among Pregnant Women in Sokoto, North Western Nigeria

Erhabor O<sup>\*1</sup>, Kabiru Salisu Adamu<sup>1</sup>, Abdulrahaman Y<sup>1</sup>, Isaac Z<sup>1</sup>, Onuigwe F<sup>1</sup>, Kweifa I<sup>1</sup>, Buhari H<sup>1</sup>, Okwesili A<sup>1</sup>, Yakubu A<sup>2</sup>, Shehu CE<sup>2</sup>, Hassan M<sup>2</sup>, Singh S<sup>2</sup>, Yeldu MH<sup>3</sup>, Gwarzo S<sup>4</sup>

<sup>1</sup>Department of Haematology and Transfusion Science, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

<sup>2</sup>Department of Obstetrics and Gynaecology Usmanu Danfodiyo University, Sokoto, Nigeria.

<sup>3</sup>Department of Clinical Chemistry, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

<sup>4</sup>Department of Medical Laboratory Science, Bayero University, Kano, Nigeria.

*\*Corresponding author:* Prof. Erhabor Osaro, Department of Haematology and Transfusion Science, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria, E-mail: n\_osaro@yahoo.com

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### Abstract

Rhesus antigens play a significant role in blood transfusion and Haemolytic Disease of the Foetus and Newborn. This study investigated the prevalence of Rhesus C antigens among pregnant women in Sokoto, North Western, Nigeria. A total of 155 pregnant women aged 18 to 45 years and mean age  $27.19 \pm 4.70$  years attending ANC in UDUTH Sokoto were tested for Rh(C) phenotype using Lorne Laboratories of UK Anti-C reagent. Out of 155 subjects phenotyped, 40 (25.8%) were positive, while 115 (74.2%) were negative. The prevalence of Rhesus C phenotype was compared based on ethnicity. The prevalence of Rhesus C phenotype was highest among the Fulani ethnic group (47.4%) followed by the Igbo (27.8%), Hausa (23.4%), Yoruba (22.2%) and other minority ethnic groups (13.3%) ( $p=0.01$ ). The age distribution of subjects indicated that women in the 26-35yrs age group had the highest frequency 76 (49%), followed by 15-25 yrs 70 (45.2%) and 36-45yrs 9 (5.8%). Women educated to tertiary level (42.6%) and secondary level (31.6%) constituted a significant population of antenatal attendees compared to less educated women (21.9% and 3.9%) respectively for those with primary and non formal education ( $p=0.001$ ). We recommend that a large randomized nationwide survey be carried out to determine the distribution of Rhesus antigens and alloantibodies among pregnant women. Knowledge of the distribution of red cell antigens can help to prevent alloimmunisation and haemolytic transfusion reaction among pregnant women and multi-transfused patients as well as facilitate the provision of antigen negative blood for pregnant women and transfusion-dependent patients with alloantibodies. It can also facilitate the optimum stocking of blood banks in the area based of the relative prevalence of the Rhesus C and the various clinically significant red cell antigens in the population. We recommend that detailed phenotyping for all clinically significant red cell antigen including Rhesus C antigen be carried out routinely among all pregnant women in Nigeria. There is also the need to routinely screen all pregnant women for alloantibodies at antenatal booking to identify women at risk for Rhesus C HDFN as well as facilitate the selection of antigen negative units for those with clinically significant alloantibodies who may require a red cell transfusion during pregnancy or delivery.

**Keywords:** Rhesus (C) phenotype; Pregnant Women; Sokoto, Nigeria; Haemolytic Disease of the New-Born; Haemolytic Transfusion Reaction

## Introduction

The Rhesus (Rh) blood group system is one of thirty-three blood group systems and one of the mostly clinically significant human blood group system after the ABO blood group system [1,2].

The Rh blood group system is the most polymorphic of the human blood groups, consisting of at least 45 independent antigens and, next to the ABO blood group system, in terms of clinical significance in transfusion medicine and Haemolytic Disease of the Foetus and Newborn (HDFN) [3].

HDFN is caused by maternal IgG antibody crossing the placenta, binding to the foetal antigen- positive RBCs, and initiating their destruction, thereby causing anaemia [4]. Therefore the investigation of pregnant women for these Rh antigens is very important for better evidenced-based management of pregnant women to facilitate the prevention of alloimmunization, reduce the risk of HDFN and reduce the risk of transfusion reaction. The determination of the prevalence of clinically significant red cell antigens is important to justify the need for transfusion laboratory to stock optimum numbers of Rh antigen negative red cells for transfusion to Rh antigen negative women who may require red cell transfusion in a bid to prevent them from being sensitized to produce Rh antibodies and prevent the risk of HDN. The aim of this present study is to determine the prevalence and ethnic distribution of Rhesus C antigen among pregnant women attending antenatal clinic (ANC) in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria.

## Materials and Methods

### Description of the Study Area

The selected area for this study is 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° 14' East and latitude of 13° 04' North. It covers a land area of about 60.33Km<sup>2</sup>. 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 (NPC, 2007) [5]. The residents are mainly Hausa/Fulani and other non-indigenous ethnic groups like Yoruba, Igbo, and Zabarma tribe from neighbouring Niger Republic. Sokoto is a cosmopolitan city in North-western Nigeria. The Usmanu Danfodiyo University Teaching Hospital is a Federal Government of Nigeria- funded 500-bed tertiary health facility. It offers specialist medical care to people of Sokoto State and surrounding states of Kebbi, Zamfara and people from the neighbouring Benin Republic. A significant number of pregnant women receive antenatal care in the hospital while a small number of women seek antenatal care from the State Government-funded Specialist hospital also in Sokoto metropolis. Majority of people in Sokoto practice farming and animal husbandry. Others work in other private organizations and government ministries as

civil servants, workers and traders.

## 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 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

5kq= 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.

$$145 + 15 = 160$$

## Sampling Method

Consecutively recruited pregnant women who met the eligibility criteria for this study were recruited as subjects for this case study to avoid bias.

## 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. The participating hospital was involved in recruitment of the subjects, collection of blood samples as well as the laboratory testing.

## Eligibility Criteria

All consenting, consecutively recruited legal adults ( $\geq 18$

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 Socio-demographic information was collected using a questionnaire. 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 C phenotype determination. Samples that were not tested the same day were stored at 2-8°C till the following day.

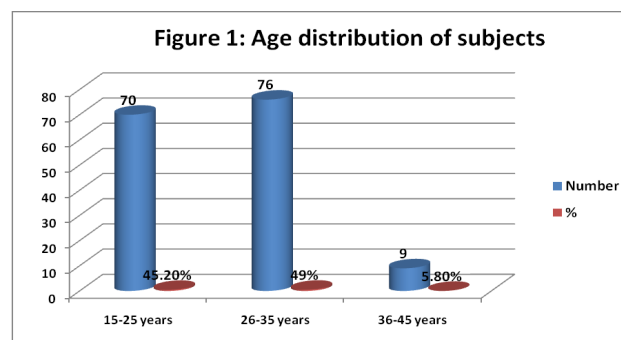
**Method**

The frequencies of Rhesus C antigen among pregnant women subjects attending the antenatal clinic in UDUTH, Sokoto was determined using standard serologic technique (tube method) using Lorne Diagnostics anti- C reagents (Lorne Diagnostics, UK). The principle is based on the ability of Lorne Diagnostic anti-C reagents to cause a direct agglutination of the test RBCs that carry the corresponding Rhesus C antigen. Agglutination indicated the presence of the group specific Rhesus C antigen to which the Rhesus antibody is specific. No agglutination generally indicates the absence of the corresponding Rhesus C antigen.

**Result**

A total of 155 blood samples was collected from pregnant women aged 18 to 45 years and mean age 27.19 ± 4.70 attending ANC in UDUTH Sokoto were studied. The age distribution of subjects indicated that women in the 26-35yrs age group had the highest frequency 76 (49%), followed by 15-25 yrs 70 (45.2%) and 36-45yrs 9 (5.8%). Figure 1 show the age distribution of subjects.

Out of the 155 pregnant women phenotyped for Rhesus C antigen, 40 (25.8%) out of 155 were positive, while 115(74.2%) of the samples were negative. Table 1 show the distribution of Rhesus C phenotype among pregnant subjects.



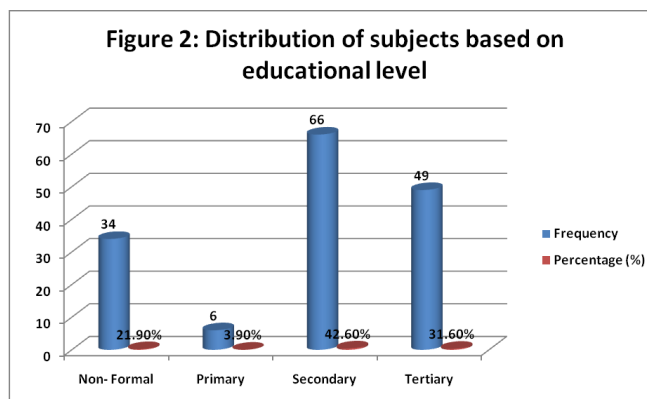
**Table 1.** Prevalence of Rh (C) among Subjects.

| Rh (C) Antigen Status | Number | %    |
|-----------------------|--------|------|
| Positive              | 40     | 25.8 |
| Negative              | 115    | 74.2 |
| Total                 | 155    | 100% |

The prevalence of Rhesus C phenotype was compared based on ethnicity. The prevalence of Rhesus C phenotype was highest among the Fulani ethnic group (47.4%) followed by the Igbo (27.8%), Hausa (23.4%), Yoruba (22.2%) and other minority ethnic groups (13.3%). Table 2 shows the distribution of Rhesus C phenotype among subjects based on ethnicity.

**Table 2.** Distribution of Rhesus C positive phenotype based on ethnicity.

| Ethnic groups | Number (%) Tested | Number Rh C Positive | C % Positive | p-value |
|---------------|-------------------|----------------------|--------------|---------|
| Hausa         | (60.6) 94         | 22                   | 23.4         | 0.01    |
| Fulani        | (12.3) 19         | 9                    | 47.4         |         |
| Yoruba        | (5.8) 9           | 2                    | 22.2         |         |
| Igbo          | (11.6) 18         | 5                    | 27.8         |         |
| Others        | (9.7) 15          | 2                    | 13.3         |         |



Subjects were stratified based of their level of educational attainment. It was found out that those that attended tertiary institutions have the highest frequency 42.6%, followed by secondary 31.6%, primary 21.9% and non formal 3.9% ( $p=0.001$ ). Figure 2 show the distribution of subjects based on their level of educational attainment.

## Discussion

The Rhesus blood group system is the second most clinically significant red cell antigen system after the ABO blood group system. The Rh system is involved in haemolytic disease of the foetus and new-born, haemolytic transfusion reaction and in autoimmune haemolytic anaemia and in forensic work [6]. The determination of Rhesus C status is of critical importance in the field of transfusion to prevent haemolytic transfusion reaction and obstetric medicine to prevent HDFN [7,8].

Currently in Sokoto State, pregnant women are only routinely tested for their ABO and Rh (D) status. Rhesus (C) phenotype testing is not routinely done. Also the Rhesus phenotyping of blood donated for transfusion purposes in Sokoto State are not routinely phenotyped for antigen C and other clinically significant red cell antigens. The Rhesus C phenotypes of pregnant women in the area are not known [9]. Pregnant women who are Rhesus C negative and are married to C positive men run the risk of carrying a C positive baby which can potentially put their mothers at risk of production of C antibody following any sensitizing events during pregnancy or delivery. This antibody-C can put future C positive pregnancies at risk of Haemolytic Disease of the Foetus and Newborn (HDFN). Similarly anaemic pregnant women who are antigen C negative run the risk of being transfused with ABO compatible C positive donor units. This can potentially put them at risk of developing antibody C which can cause HDFN in subsequent C positive pregnant as well as Haemolytic Transfusion Reactions (HTR) in subsequent C positive red cell transfusion. In this present study, we observed a prevalence of Rh (C) phenotype of 25.8%. Our finding is consistent with previous report by Gwaram and Abdullahi<sup>9</sup> who reported Rh (C) prevalence of 28% among their cohort of 113 blood donors in Kano, North- Central Nigeria. Our study is also consistent with a previous report by Nwauche and Ejele [10] who studied 65 subject made up of 35 pregnant women and 30 blood donors in the Niger Delta of Nigeria and obtained Rh (C) prevalence of 21.35%. Our observed prevalence is however lower than the prevalence observed in a previous report to determine the presence of clinically significant blood group antigens in the Lao population which indicated an Rh (C) antigen prevalence of 60.43% [11].

Anti-C is a rare antibody and commonly produced in combination with anti-D by (cde/cde pregnant women). It can also be produced in combination with anti-e by  $R_2R_2$  (cDE/cDE) individuals. It is usually a combination of IgG and IgM antibodies. Anti-C seldom causes HDFN and when it does, the disease is usually mild. The finding from this study reinforces the advocacy to provide pregnant women and women with child bearing potential red cell transfusion of their ABO,

Rhesus and Kell phenotypes. This has the potential to reduce the risk of alloimmunization to Rhesus and Kell antigens in donor units that are lacking in the recipient. A previous report [12] suggest matching the red cell phenotype other than ABO and D (C, E, c and K) among the transfusion- dependent patients in an attempt to prevent alloimmunization. Similarly Singer and colleagues [13] have reported that patients who received blood matched for Rhesus (C, D, E, c and e) and Kell system from their first transfusion, have relatively lower rate of alloimmunization among them. Previous report recommends that all multiply- transfused patients should be phenotyped for the Rh system and that Rh phenotype specific blood is provided in order to limit alloimmunization [14].

In this present study, we observed that 74.2% of our cohort of pregnant women in Sokoto, Nigeria was negative for Rhesus C phenotype. This has obstetric (high risk of anti-C related HDFN) and blood transfusion (risk of anti-C related haemolytic transfusion reactions) implications. Alloantibody C is prevalent among pregnant Nigerian women. In a previous report, Jeremiah and colleagues identified antibodies in the serum of 17 (3.4%) of their cohort of 500 pregnant women. The specificity of the antibodies was as follows: anti-C 6 (1.2%), anti-E 3 (0.6%), anti-Jsb 3 (0.6%), and anti-K 5 (1.0%) [15]. Anti-C have been reported in pregnancies of Rh D negative (ccddee) women and can lead to severe haemolytic disease of the foetus and new-born (HDFN) [16-21].

Alloimmunization occurs when incompatible (foreign) red cell antigens are introduced (pregnancy, transfusion and transplant) to an immune-competent host. This foreign antigen can potentially sensitize the host immune system leading to an immune response which culminates in the production of the alloantibody. Alloimmunization against RBCs can result in haemolytic transfusion reactions and haemolytic disease of the foetus and newborns. Development of alloantibodies thus complicates and limits transfusion therapy, contributing to a delay in getting compatible units, technical complications and oftentimes morbidity and mortality [22-25]. Routine pre-transfusion testing of pregnant women during pregnancy is one of the important safety measures to detect the presence of clinically significant red cell antigens and unexpected red cell antibody in the patient's serum to prevent the risk of immediate and delayed haemolytic transfusion reaction as well as HDFN [26].

Blood group genotyping is gaining widespread adoption in blood centres and transfusion services [27]. It is hoped that blood group genotyping information may be used as a donor recruitment tool in future. This will allow recipients to receive blood of their own Rhesus genotype and thus limit the chances of transfusion-related Rh alloimmunization. This is particularly useful in multi-transfused and transfusion-dependent patients [28].

In this present, we observed that a significant variation in the distribution of Rhesus C phenotypes among the various ethnic group in Sokoto, North Western Nigeria. Blood group antigens can be distributed differently within different nationalities. In this present study we observed Rhesus C phe-



notypes in 25.8% of our cohort of 155 pregnant women in Sokoto North Western Nigeria. The prevalence obtained in this present study is at variance with a 28%, 21.35% and 60% prevalence obtained respectively in Kano North Western Nigeria, Port Harcourt in the Niger Delta of Nigeria and among Lao population [9-11]. Determination of the distribution of red cell antigen in a population is vital for several reasons; it facilitates the optimum stocking of blood banks with red cells that are negative for clinically significant red cell antigens, it enable the determination of the risk of HDFN and HTR occurring, it allow policy makers to plan for the obstetric and neonatal needs of their population and it allows obstetricians and neonatologist to effectively manage the risk of HDFN. This study re-emphasizes the importance of determining the frequency of red cell blood group phenotypes among individuals of different ethnic backgrounds. Over the last 20-30 years, there has been a change in the demography of most countries due to increased mobility and immigration. A previous report indicates that blood transfusion services in most countries particularly those whose population was previously primarily of European Caucasian ancestry face a significant challenge in meeting the blood transfusion needs of increasing number of patients of Blacks and Oriental extraction [29]. Knowledge of a patient's ethnic background can sometimes be used to accelerate the identification of an antibody. The knowledge of the differences in antigen frequency among different populations is also vital in meeting the long-term transfusion need of some transfusion-dependent patients.

In this present, we observed 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 pregnant woman's educational status seems to influence the degree to which she seeks antenatal care in hospitals. Education seems to play a very important role in antenatal attendance. Better educated women are more likely 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 [30].

Rhesus D antigen is the most clinical significant red cell antigen in the Rhesus blood group system. Rhesus C antigen is also a clinically significant red cell antigen. Antibody to antigen C has been incriminated in cases of HDFN and HTR. Although Rh(C),(D) and (E) are closely linked, they are inherited independently of each other. We have not included the prevalence of Rh(D) phenotype of pregnant women this present study having published a paper on the prevalence of Rhesus D phenotype among pregnant women in Sokoto in a previous report [31].

### Conclusion and Recommendation

In this study, we observed a prevalence of Rh (C) phenotype of 25.8% among our cohort of pregnant women in Sokoto, North Western Nigeria. We recommend that a large ran-

domized nationwide survey be carried out to determine the distribution of Rhesus antigens and alloantibodies among pregnant women. Knowledge of the distribution of red cell antigens can help to prevent alloimmunisation and haemolytic transfusion reaction among pregnant women and multi-transfused patients. It can also facilitate the provision of antigen negative blood for pregnant women and transfusion-dependent patients with alloantibodies as well as facilitate the optimum stocking of blood banks in the area based of the relative prevalence of the Rhesus C and the various clinically significant red cell antigens in the population. We recommend that detailed phenotyping for all clinically significant red cell antigen including Rhesus C antigen be carried out routinely for all pregnant women in Nigeria. There is also the need to routinely screen all pregnant women for alloantibodies at antenatal booking to identify women at risk for Rhesus C HDFN as well as facilitate the selection of antigen negative units for those with clinically significant alloantibodies who may require a red cell transfusion during pregnancy or delivery. This will facilitate the optimum obstetric management of HDFN in pregnant women who have a clinically significant alloantibody.

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### Limitations

In this present study, we excluded pregnant women who has had a red cell transfusion within the last 4 months. The reason for excluding women who have had transfusion in the last 4 months (120 days) was to avoid the possible detection of the C phenotype of the transfused donor blood. The half life of red cells in circulation is 120 days. C phenotyping of a transfused pregnant woman can produce erroneous result.

In this present study, we only included demographic date of age, ethnicity and educational status of the pregnant women studied. We did not include any demographic data of the occupation. This information may have added value to the result presented in this study.

### Conflict of Interest

Authors declare that there are no conflicting interest with this article.

**Authors Contribution:** Osaro Erhabor, Kabiru Salisu Adamu, Abdulrahman Y, Isaac Z designed the study. Yakubu A,

Shehu CE, Hassan M and Singh S facilitated the recruitment and counselling of the pregnant subjects while Onuigue F, Kweifa I, Buhari H and Okwesili A were responsible for obtaining informed consent, sampling and laboratory testing of samples. Yeldu MH and Gwarzo S did the statistical analysis. All authors read and approved the final report.

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