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Rh (D) phenotype among pregnant women in Sokoto, North Western Nigeria. Implications on haemolytic disease of the new-born and haemolytic transfusion reaction

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Abstract

This study investigated the prevalence of Rhesus D antigens among pregnant women in Sokoto, North Western, Nigeria. A total of 155 blood samples from pregnant women aged 18 to 45 years and mean age 27.19 ± 4.70 years attending ANC in UDUTH Sokoto were tested for Rh(D) phenotype using Lorne Laboratories of UK Anti-D reagent. Out of 155 subjects tested, 144 (92.9%) were Rh (D) positive while 11(7.1%) were Rhesus (D) negative. The prevalence of Rh D positive phenotype was highest among the Yoruba ethnic group (100%) and lowest among the Ibo ethnic group (83.3%). The prevalence of Rh D negative phenotype was highest among the Hausa ethnic group (42.5%) and lowest among the Yoruba ethnic group (0%). Antibodies to Rhesus D antigens can cause haemolytic disease of the foetus new-born and haemolytic transfusion reaction. Pregnant women should be tested routinely for their Rhesus D phenotypes as well as for the presence of clinically significant alloantibodies during pregnancy. Facilities for the demonstration of FMH should be made available in laboratories in Nigeria. Healthcare staff looking after Rh (D) negative women should be trained on anti-D prophylaxis. Universal access to prophylactic anti-D should be provided for all Rh (D) negative women. Rh (D) negative women who are to have a termination of pregnancy or who suffer from miscarriages or any other potentially sensitizing events during pregnancy should be offered immunoglobulin D as a prophylactic measure to prevent alloimmunization.

1. Introduction

The Rh (Rhesus) blood group system is one of thirty-three current human blood group systems. It is the most important blood group system after the ABO blood group system. At present, the Rh blood group system consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e are the most

important. 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]. The following year Landsteiner and Wiener (1940) found that sera from rabbits (and later guinea pigs) immunized with RBCs from *Macaca mulatta* (*Macacus Rhesus* in the original paper) agglutinated 85% of the human RBC samples. Initially, it was thought that the animal and human antibodies identified a common factor (Rh) on the surface of Rhesus and human RBCs. It was soon realized that this was not the case [2]. Therefore the original terms (Rh factor and anti-Rh) coined by Landsteiner and Wiener, although being misnomers, have continued in common usage.

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 [3]. Apart from the importance of the Rh antigens in blood transfusion and Haemolytic Disease of the Foetus and Newborn (HDFN), Rh proteins are important in transporting ammonia across the RBC membrane [4]. It follows that RBCs which lack Rh antigens will have abnormal shape, increased osmotic fragility and shortened life span. This often results in haemolytic anaemia that is mild in nature [3].

During pregnancy and also delivery, foetal RBCs may enter into the maternal circulation through the placenta. Some foetal cells may contain some antigens (C, D, and E) which may be lacking in the mother. These antigens may probably have been inherited from the father, and they are capable of stimulating the mother to produce alloantibodies. The type of RBC alloantibodies that can be produced by the mother depends totally on the combination of antigens on the foetal RBCs. Rh (D) is the most potent among all of the Rh antigens. Research has shown that, if an Rh negative mother should be exposed to a single drop of Rh positive blood, it can lead to the stimulation of the body to produce anti-D-antibodies.

Full Rh phenotypes of pregnant women are not routinely done in our setting. This puts pregnant women potentially at risk of producing Rh antibodies against Rh antigens (C, D and E) present in the donor unit but which the pregnant woman lacks. This alloantibody developed puts the woman potentially at risk for HDFN if she is pregnant and carrying a baby that is positive for the antigen for which the maternal alloantibody is specific.

HDFN is caused by maternal IgG antibody crossing the placenta, binding to the foetal antigen-positive RBCs, and initiating their destruction, thereby causing anaemia. Prior to the use of prophylactic Rh immunoglobulin, anti-D frequently caused foetal brain damage due to increased levels of bilirubin (kernicterus) and even death

(erythroblastosis foetalis). Despite the fact that widespread use of prophylactic Rh immunoglobulin in the developed world has brought about a significant reduction of alloimmunization of Rh negative pregnant women and a reduction in HDFN resulting from Rh blood group incompatibility between mother and baby, a significant number of women in our environment still become alloimmunized during pregnancy. Also the risk of HDFN resulting from Rh blood group incompatibility between mother and baby remains a huge challenge in our environment for a variety of reasons; lack of universal access to prophylactic Rh immunoglobulin, lack of provision of prophylactic immunoglobulin D following potentially sensitizing events during a Rh negative pregnancy, lack of testing of blood group and provision of prophylactic immunoglobulin D to Rh negative women in which termination of pregnancy is indicated, unrecognized miscarriage, and lack of Kleihauer [5] testing for foetal maternal haemorrhage (FMH) in Rh negative women following the delivery of Rh positive baby [6]. Therefore the investigation of pregnant women for these Rh antigens is very important for the better and evidenced-based management of these patients (facilitate the prevention of alloimmunization, reduce the risk of HDFN and reduce the risk of transfusion reaction particularly in multiply transfused patients). The determination of the prevalence of clinically significant red cell antigens is important to justify the need for the 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 immunized to produce Rh antibodies and prevent the risk of HDFN. The aim of this present study is to determine the prevalence and ethnic distribution of Rhesus D antigen among pregnant women attending Antenatal Clinic (ANC) in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria.

2. Materials and Method

2.1. 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 60.33Km². 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) [7]. The residents are mainly Hausa/Fulani and other non-indigenous ethnic groups like Yoruba, Igbo, and Zarma tribe from neighbouring Niger Republic. The

main occupation of the people is trading, farming with few numbers of civil servants.

2.1.1. Study Design

Subjects included in this case study to investigate the prevalence of Rhesus D antigen included 155 consecutively- recruited pregnant women visiting the antenatal clinic of Usmanu Danfodiyo University Teaching Hospital in Sokoto, North Western, Nigeria.

2.1.2. Sampling Method

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

2.1.3. 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 $p \leq 0.05$.

2.1.4. 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.

2.1.5. 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.

2.2. 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.

2.2.1. 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.

2.2.2. 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 (D). Samples

that were not tested the same day were stored at $2-8^{\circ}\text{C}$ till the following day.

2.2.3. Method

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

3. 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. Out of 155 subjects tested for their Rhesus D phenotype, 144 (92.9%) were Rh (D) positive while 11(7.1%) were Rhesus (D) negative. Table 1 show the prevalence of Rh (D) among subjects. The prevalence of Rhesus D positive phenotype was compared based on the ethnicity. The prevalence of Rh D positive phenotype was highest among the Yoruba ethnic group (100%) and lowest among the Ibo ethnic group (83.3%). Table 2 show the distribution of Rhesus D positive phenotype based on ethnic group of subjects. The prevalence of Rh D negative phenotype was highest among the Hausa ethnic group (42.5%) and lowest among the Yoruba ethnic group (0%). Table 3 show the distribution of Rhesus D negative phenotype based on ethnic group of subjects. Pregnant subjects were stratified based of their age groups. Majority of the subjects were in the 26-35 years age group (49%) followed by the 15-25 years age group (45.2%) while the least were in the 36-45 years age group (5.8%). Table 4 show the distribution of the subjects based on age groups. Subjects were categorized based on their highest level of educational attainment. Majority of subjects were more educated (42.6% and 31.6%) respectively for subjects educated to secondary and tertiary level respectively compared to (21.9% and 3.9%) for those with no formal education and primary education respectively. Table 5 show the distribution of subjects based on level of educational attainment.

Table 1. Prevalence of Rh (D) among Subjects

Rh (D) Antigen Status	Number	%
Positive	144	92.9
Negative	11	7.1
Total	155	100%

Table 2. Distribution of Rhesus D positive phenotype based on ethnicity

Ethnic groups	Number Tested	Number Rh D Positive	% D Positive
Hausa	94	90	95.8
Fulani	19	17	89.5
Yoruba	9	9	100
Igbo	18	15	83.3
Others	15	13	86.7

Table 3. Distribution of Rhesus D negative phenotype based on ethnicity

Ethnic groups	Number Tested	Number Rh D Negative	% D Negative
Hausa	94	4	42.5
Fulani	19	2	10.5
Yoruba	9	0	0
Igbo	18	3	16.7
Others	15	2	13.3

Table 4. 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 5. 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

4. Discussion

The Rhesus blood group system is the second most clinically significant red cell antigen system after the ABO blood group system [8]. The Rh system is involved in haemolytic disease of the foetus and new-born, haemolytic transfusion reaction and in forensic work [9]. In this study, we observed the prevalence of Rhesus (D) positive and negative prevalence of 92.9% and 7.1% respectively among our cohort of pregnant women tested. Our finding is consistent with previous reports obtained among non-Caucasians by Erhabor and colleagues in the Niger Delta of Nigeria [10] and in Gusau in North Western Nigeria [11] (93% and 7%) and (98.8% and 1.2%) respectively. Our finding is also consistent with previous report by Egesie and co-workers [12] who observed Rh-D positive and negative prevalence of 98% and 2% respectively among their cohort of undergraduate students in the Niger Delta of Nigeria. Similarly, 96.7% Rh (D) positive prevalence was

recorded among the Ibos ethnic group of Eastern Nigeria by Ukaejiofor and colleagues [13]. Other documented Rh-D positive rates includes; 95% by Jeremiah and coworkers [14] in Port Harcourt, 97.1% by Gwaram and Abdulahi [15] in Kano, 96.6% by Pramanik and colleagues [16] in Nepal, 94% by Mwangi [17] in Kenya, 93% by Bashwari and colleagues [18] in the Eastern region of Saudi Arabia, 97.7% in West Bengal India [19], 95.94% in Guinea [20], 84.35% in South Gujarat by Kahar and Patel [21], 99.67% in Taiwan [21], 92.7% among Maldivian donors [22], 92.8% by Sarhan and colleagues [23] in Southwest of Saudi Arabia, 95.4% by Nwauche and Ejele [24] in the Niger Delta of Nigeria and 96.5% by M'baya and colleagues [25] among Malawians. In the Orient, almost 100% of the population are Rh (D) positive [26]. Similarly, a previous report that investigated the frequency and ethnic distribution of Rh (D) blood groups in Mauritania showed that Rhesus D positive phenotype was prevalent in 94.23% while Rh (D) negative accounted for only in 5.77% of the total population [27]. In the same vein, a study carried out to provide gene frequency values for the ABO and Rh (D) alleles among a total of 4,748 healthy infant population in South Nigeria indicated that 4520 (95.2%) were Rh-positive while 228 (4.8%) were Rh-negative [28]. Similarly, phenotype and gene frequencies of ABO and RH (D) systems were studied in 37,846 random blood donors in five zone of Nigeria (South West) (Yoruba)-Zone A, North West (Hausa-Fulani)-Zone B, Plateau (Birom)-Zone C, South East (Igbo)-Zone D and North East (Kanuri)-Zone E) indicated an Rh (D) gene frequency of 81.5% [29].

In this study we observed a lower prevalence of Rh (D) negative status of 7.1% among the pregnant women studied compared to previous report among Caucasians ($\geq 14\%$) [30-32]. Our finding is consistent with previous report among Africans and Asians which obtained Rh (D) prevalence of $\leq 7.2\%$ [12-18,24]. Our study is also consistent with a previous report by Nwauche and Ejele [25] who studied 65 subject made up of 35 pregnant women and 30 blood donors in the Niger Delta of Nigeria and obtained Rh (D) prevalence of 95.38% and Rhesus D negative phenotype of 4.6%. Similarly, a previous report [28] indicated that approximately 96.5 and 3.5% of Malawians are D positive and D negative respectively [26]. The incidence of Rhesus disease of the new-born is mathematically related to the frequency of D negative individuals in a population, so Rhesus disease is rare among populations in Africa and the Eastern half of Asia, and among the indigenous peoples of Oceania and the Americas, but more common in other genetic groups, most especially Western Europeans.

There are several obstetric advantages associated with a low prevalence of D-negative status among pregnant women in Sokoto. The risk of Rh (D) alloimmunization will be of a much smaller magnitude than it is in most Western Countries where a significant proportion of the population lacks the major Rh (D) antigen. In such individuals, the chances of becoming sensitized to the D

antigen following exposure either by transfusion of Rh(D) positive red cells or during pregnancy involving a Rhesus positive foetus is very high. Alloantibody D produced as a result of such immunization has serious clinical significance including haemolytic disease in the newborn and/or transfusion reactions. Despite the fact that the prevalence of Rh-negative phenotype is significantly lower among Africans compared to Caucasians, Rh alloimmunization remains a major factor responsible for perinatal morbidity in most developing countries for several reasons; lack of universal access and unavailability of anti-D immunoglobulin, lack of anti-D prophylaxis for Rhesus negative women who have a potentially sensitizing events during pregnancy (amniocentesis, cordocentesis, antepartum haemorrhage, vaginal bleeding during pregnancy, external cephalic version, abdominal trauma, intrauterine death and stillbirth, in utero therapeutic interventions, miscarriage, and therapeutic termination of pregnancy), unavailability of prophylactic immunoglobulin D following termination of pregnancy among Rhesus negative women and unavailability of Feto Maternal Haemorrhage (FMH) testing following potentially sensitizing events during pregnancy. The haemolytic condition occurs when there is Rh incompatibility between the mother and the foetus. The disorder in the foetus due to Rh D incompatibility is known as erythroblastosis foetalis. Haemolytic (blood lysis or destruction) or breaking down of red blood cells. Erythroblastosis refers to the release of immature red blood cells into the peripheral blood to compensate for HDFN-associated anaemia. When the condition is caused by the Rh D antigen-antibody incompatibility; it is called Rh (D) haemolytic disease of the new-born (often called Rhesus disease). The sensitization to Rh D antigens usually result from feto-maternal transfusion during pregnancy or during delivery and often lead to the production of maternal immune (IgG) anti-D antibodies which have a low molecular weight and thus can pass through the placenta barrier. The first baby is usually unaffected. This is of particular importance to D negative females at or below childbearing age, because any subsequent pregnancy may be affected by Rhesus (D) haemolytic disease of the new-born if subsequent babies are D positive. The sensitization is preventable by injections of prophylactic IgG anti-D antibodies (Rho (D) Immune Globulin) given following any potentially sensitizing events during pregnancy and within 72 hours after delivery of an Rh positive baby. Symptoms and signs of HDFN in the foetus include: enlarged liver, spleen, or heart and fluid build-up in the foetus' abdomen. Commonly observed signs in the new-born include; anemia that creates the newborn's pallor, jaundice or yellow discoloration of the newborn's skin, sclera or mucous membrane, enlargement of the newborn's liver and spleen, severe oedema of the entire body and dyspnea or difficulty in breathing. These symptoms may be evident right after birth or after 24–48 hours after birth.

5. Conclusion

This study indicated Rhesus D positive and negative prevalence of 92.9% and 7.1% respectively.

Recommendations

The Rh system play a significant role in the incidence of HDFN and HTR. Rhesus antigens can cause HDFN and HTR. There is need for routinely screening of all pregnant women in the area for their Rh D phenotype. Pregnant women found to be Rhesus D negative should be offered anti-D immunoglobulin to prevent Rhesus D haemolytic disease of the newborn. It is also important that such women are under the care of a qualified Obstetrics and Gynaecologist to allow for their evidenced- based management and to prevent HDFN. We recommend that prophylactic D immunoglobulin of 500-1500IU be offered routinely to pregnant women who are Rh (D) negative at 28 weeks gestation and following potentially sensitizing events during pregnancy. Pregnant women should be tested routinely for the presence of clinically significant alloantibodies and antigens during pregnancy. Those positive for alloantibodies should be given antigen red cells that are negative for red cell antigens to which the alloantibody is specific. The Rh blood group of all babies delivered by Rh (D) negative mothers should be determined at delivery and if found to be Rh positive, Feto Maternal Haemorrhage testing (Kleihauer or flow cytometry test) should be carried out to estimate the quantity of foetal red cells that may have entered the maternal circulation to facilitate the administration of optimal quantity of anti-D immunoglobulin to prevent HDFN. Facilities for the demonstration of FMH should be made available in laboratories in Nigeria. Healthcare staff looking after Rh (D) negative women should be trained on anti-D prophylaxis. Universal access to prophylactic anti-D should be provided for Rh (D) negative women. Rh (D) negative women who are of child bearing age and who are to have a medical termination of pregnancy and those who suffer from miscarriages or any other potentially sensitizing events during pregnancy should be offered prophylactic immunoglobulin D to prevent alloimmunization.

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