DISTRIBUTION OF MATERNAL RED CELL ANTIBODIES AND THE RISK OF HAEMOLYTIC DISEASE OF THE FOETUS AND NEWBORN IN SOKOTO NIGERIA

ABSTRACT:

Background: The incidence is still of clinical importance because sensitization of the mother may occur through the placenta, blood transfusion, miscarriage, ectopic pregnancy, amniocentesis as well as lack of prophylaxis of alloantibody except RhD.

Aim: The aim of this study is to determine the prevalence of clinically significant red blood cell alloantibodies among pregnant women.

Study Design: This was a cross- sectional study

Duration of Study: The study lasted for a period of one year between January to December, 2021.

Methodology: About 1250 consecutively recruited pregnant women were screened for alloantibody and identification was done to determine the specificity of the antibody using NHSBT reagent with column agglutination card technology.

Results: Among the 1250 apparently healthy pregnant women studied, 73(5.8%) had alloantibodies with specificity as follows: Multiple antibody of C, E 3 (4.1%), D 23 (31.5%), C 6(8.2%), E 5(6.8%), e (1.4%), Jk^a 2 (2.7%), Jkb 2 (2.7), M 1 (1.4%), S 1 (1.4%), Lea 7 (9.6%), Leb 8 (11%), I 4 (5.5%), and Lu 7 (9.6%). When the demographic and obstetric characteristics were compared with the presence of alloantibodies, statistically significant differences were observed in gestational age, past history of pregnancy, preeclampsia, and previous baby delivered with jaundice and previous administration of anti-D prophylaxis.

Conclusion: There is high prevalence of alloantibody among pregnant women that may be linked to lack of premarital testing to know couple at risk and lack of standard protocol of alloantibody testing.

Keywords:

Alloantibodies, Haemolytic disease of the Foetus and Newborn, Pregnant Women, Sokoto.

1.0 INTRODUCTION

"Antibodies are immunoglobulins produced by the B lymphocytes of the adaptive immune system in response to an antigen for which they exhibit specific binding. Depending on the

origin of the antigenic stimulus, antibodies can be termed as; alloantibodies, when produced by an individual against epitopes present in another individual of the same species, autoantibodies when reactive with determinants present on the individual's antigens and xenoantibodies (or heteroantibodies), when produced against antigenic determinants present on the cells of another species" [1]. "The red blood cell (RBC) alloantibodies other than the so-called naturally occurring anti-A or anti-B are called unexpected RBC alloantibodies and can be found in 0.3%–38% of subjects depending on the group of patients or donors studied and the sensitivity of the test method used" [2].

"The prevalence of alloimmunization in pregnant women varies in different parts of the world, with the frequency of incidence that decreases significantly to the range of 0.4 to 2.7% worldwide [3] due to mandatory haematological screening during pregnancy as well as the use of antenatal anti-D prophylaxis" [4]. Based on Fung and colleague recommendations in 2003 for postpartum and antenatal administration there has been a reduction in the number of fatalities caused by Rhesus D and sensitization of pregnant woman have decreased significantly. However, frequencies of alloantibody are still high in Nigeria 3.4% with a specificity of anti-C, anti-E, anti-Jsb, and anti-K although, no anti-D was identified despite 8.6% of the study population being Rhesus D (Rh D) negative [5].

"The incidence is still of clinical importance because sensitization of the mother may occur through the placenta, blood transfusion, miscarriage, ectopic pregnancy, and in procedures such as amniocentesis or may occur naturally from exposure to bacteria or viruses" [6]. "Also, inter-spousal transfusion is an important but often overlooked way of maternal sensitization which is common practice in this part of the world. There are over 50 RBC alloantibodies that have the capability of crossing the placenta and causing HDFN, with anti-D followed by anti-c and anti-K having the highest probability of causing severe HDFN" [7].

"HDFN is caused by maternal IgG antibodies crossing the placenta, binding to the foetal antigen-positive RBCs, and initiating their destruction, thereby causing anaemia" [8]. "Antibody screening in antenatal women aids in the detection and monitoring of patients who are at risk of delivering infants with haemolytic disease of the foetus and newborn (HDFN). Investigations for the presence of irregular antibodies and their titres or quantification during pregnancy are important to calculate the risk of developing HDFN" [9].

2.0 MATERIALS AND METHODS

2.1 Study design

This was a cross-sectional study that involved 1250 pregnant women on their first antenatal visit of pregnancy in Sokoto. Alloantibodies were screened and identified to identify the pregnant women at risk of having foetus with haemolytic disease of the foetus and newborn.

2.2 Study Area

The study was conducted in Sokoto state. Sokoto State lies between longitude 050 to 130 03' East and latitude 130 06 North and covers an area of 66.33km2 (SSBD, 2007). "It has a land area of about 28,232.37sq kilometers and stands at altitudes of 272m above the sea level. The major indigenous tribes in the state are the Hausa and Fulani and other groups such as Gobirawa, Zabarmawa, Kabawa, Adarawa, Arawa, Nupes, Yorubas, Igbos and so on are also resident there, the town being cosmopolitan. The occupation of city inhabitants include; trading, farming, with a reasonable proportion of the population working in private and public domains. Based on 2006 population census, Sokoto state had a population of 3.5million with Sokoto metropolis having a population of 427,760" [10].

2.3 Study Site

The selected area for this study is Sokoto State and the area covered included Usmanu Danfodiyo University Teaching Hospital (UDUTH), Specialist Hospital Sokoto, Maryam

Abacha Women and Children Hospital, Women and Children Welfare Clinic, General Hospital Yabo and General Hospital Bodinga.

2.4 Study Setting

The study was conducted among the pregnant women that visited various hospitals in Sokoto for their first ante-natal visit. The research laboratory analysis was done in School of Medical Laboratory Science of Usmanu Danfodiyo University in collaboration with Haematology Department of Usmanu Danfodiyo University Teaching Hospital Sokoto.

2.5 Inclusion Criteria and Exclusion Criteria

Eligibility Criteria

All consenting, consecutively recruited pregnant women willing to offer a written or oral informed consent to participate in the study after counselling;

Exclusion Criteria

The pregnant women who do not meet the inclusion criteria were excluded from participating in the study I.e. the Non- pregnant women; Non-consented pregnant women and pregnant women attending hospitals outside the Sokoto metropolis

2.6 Questionnaires

A structured questionnaire were used to obtain a socio-demographic information and obstetric history of each participant such as age, tribe, gestational age, history of previous transfusion, history of bleeding during previous pregnancy etc

2.7 Sampling Methods

Blood samples were collected by venepuncture into ethylene diamine tetracetic acid (EDTA) anticoagulated tubes and used for the screening and identification of red cell alloantibody in

1250 consecutively recruited subjects. Red cell antibody test was carried out using column agglutination technology. The test is based on haemagglutination principle.

.2.8 Laboratory Procedure

Antibody screening was done using column gel technique for alloantibodies screening in the plasma. Each 50ul of suspension of NHSBT reagent red cells (1, 2 and 3) for antibody screening was mixed with 40ul of the patient's serum into the appropriate column gel card the card was incubated for 45 minutes at 37^{o}c. The card was centrifuged for 5 minutes in a card system centrifuge. The result was read macroscopically for agglutination in any of the three (3) reaction chambers which contains the patient's serum, this indicate the presence of an alloantibody that was tested against 11 antibody identification panel of cells.

Antibody identification was done using Column gel card technique for antibodies identification in the plasma. Each 50ul of suspension of NHSBT antibody identification panel of cells (1, 2 …11) for antibody identification was mixed with 40µl of the patients' plasma into the appropriate column agglutination card (AHG/Coombs) 11 reaction chambers. The card was incubated for 45 minutes at 37^{o}c. The card was centrifuged for 5 minutes in a card centrifuge. The result was read macroscopically for agglutination in any of the eleven (1, 2 …11) reaction to effectively identify the specificity of the alloantibody.

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 $p \leq 0.05$

RESULTS

Table 1: Prevalence of red cell alloantibodies among pregnant women study participants (1250)

Number	Antibody specificity	Frequency	% of women with antibody
	All alloantibodies	**73**	**5.8**
1	D	23	31.5
2	C	6	8.2
3	E	5	6.8
4	E	1	1.4
5	C,E	3	4.1
6	Jk^a	2	2.7
7	Jk^b	2	2.7
8	M	1	1.4
9	S	1	1.4
10	Le^a	7	9.6
11	Le^b	8	11
12	I	4	5.5
13	Lu	7	9.6
14	Un-identified pattern	2	2.7
16	Autoantibody	1	1.4

Table 2: Demographic and obstetric characteristics of pregnant women in relation to presence of alloantibodies (n=1250)

Variable	Total Number	Presence of alloantibody		T-test
	n(%)	Yes [n(%)]	No [n(%)]	
Age (Years)				
<20	101(8.1)	9(12.3)	92(92.2)	0.002
20-30	766(61.3)	48(6.3)	718(93.7)	
30-40	366(29.1)	16(4.4)	350(95.6)	
>40	17(1.4)	0(0)	17(100)	
Ethnicity				
Hausa	1125(90)	66(5.9)	1059(94.1)	0.909
Igbo	64(5.1)	0(0)	64(100)	
Yoruba	43(3.4)	4(9.3)	39(90.7)	
Others	18(1.4)	3(16.7)	15(83.3)	
Gestational Age				
First	85(6.8)	7(8.2)	78(91.8)	0.002
Second	747(59.8)	59(7.9)	688(92.1)	
Third	418(33.4)	7(1.7)	411(98.3)	
Blood transfusion in the past				
Yes	140(11.2)	11(7.9)	129(92.1)	0.170
No	1110(88.8)	62(5.6)	1048(94.4)	
Past history of Pregnancy				
Spontaneous abortion	411(32.9)	28(6.8)	383(93.2)	0.000
Stillbirth	101(8.1)	4(4)	97(96.0)	
Termination of pregnancy	13(1.0)	1(7.7)	12(92.3)	
Life birth	725(58)	40(5.5)	685(94.5)	
Pregnancy induced hypertension				
Yes	104(8.3)	10(9.6)	94(90.4)	0.935
No	1146(91.7)	63(5.5)	1083(94.5)	
Preeclampsia				
Yes	41(3.3)	6(14.6)	35(85.4)	0.001
No	1209(96.7)	67(5.5)	1142(94.5)	
Antepartum Haemorrhage				
Yes	79(6.3)	4(5.1)	75(94.9)	0.385
No	1171(93.7)	69(5.9)	1102(95	
Postpartum Haemorrhage				
Yes	79(6.3)	2(2.5)	77(97.5)	0.192
No	1171(93.7)	71(6.1)	1100(93.9)	
Previous jaundiced baby				
Yes	64 (5.1)	25(39.1)	39(60.9)	0.002
No	1186(94.9)	48(4.0)	1138(96)	
Anti -D immunoglobulin				
Yes	27(2.2)	9(33.3)	18(66.7)	0.000
No	1223(97.8)	64(5.2	1159(94.8)	
Mode of Previous delivery				
Vaginal delivery	1202(96.2)	57(4.7)	1145(95.3)	0.002
Caesarean section	20(1.6)	1(5)	19(95)	

Both	28(2.2)	15(53.6)	13(46.4)

4. DISCUSSION

This study screened one thousand, two hundred and fifty (1250) pregnant subjects for alloantibody among the women attending antenatal clinic in different hospitals in Sokoto state (Usmanu Danfodiyo University Teaching Hospital, Specialist Hospital Sokoto, Women and Children Welfare Clinic, Maryam Abacha Women and Children Hospital, General Hospital Bodinga and General Hospital Yabo) at their first booking.

Table 1 shows the prevalence of red cell antibodies among pregnant women study participants. The study recorded frequency of alloantibodies among pregnant women as 5.8% of all alloantibodies with specificity as follows: Multiple antibody of C, E 3 (4.1%), D 23 (31.5%), C 6(8.2%), E 5(6.8%), e (1.4%), Jk^a 2 (2.7%), Jkb 2 (2.7), M 1 (1.4%), S 1 (1.4%), Lea 7 (9.6%), Leb 8 (11%), I 4 (5.5%), and Lu 7 (9.6%). Other blood group antibodies that the screening cell showed positive but reaction pattern is not indicative any in the antigram (most probably low-frequency antigen of unknown specificity) recorded 2 (2.7%) while 1 (1.4%) of the screening cell shows positive while the specificity showed negative. This findings is in agreement with previous reports of Gothwal and colleagues reported that (3.119%) of pregnant women were alloimmunized with specificity of anti-M (9.23%), anti-c (3.076%), anti-E (1.538%), anti-e (1.538%), anti-Lewis (a) (1.538%), unspecified antibodies (1.538%), multiple antibodies anti-D and anti-C (3.076%), anti-e and anti-c (1.538%), and anti-D and anti-G (1.538%) in tertiary care centre of Western India [11] and that of Karim and colleagues in Pakistan reported the frequency of maternal alloimmunization among pregnant women was 1.8% of with specificity of non-anti-D (1.6%), anti-M (15%), anti-Lewis(a) (15%), anti-c (5%), anti-E (5%), anti-e (5%), anti-Lewis(b) (5%) and nonspecific antibodies (30%) and the prevalence of anti-D 2.9% in D negative blood type [12] as well as

the report of [13] Ugandan who reported that 2·2% of pregnant women were alloimmunized to RBC antigens with specificity including anti-S, 12; anti-M, 11; anti-Lea, 6; anti-D, 4 and 1 each of anti-K, anti-Fyb, anti-Jka, anti-Lua and anti-Kpa. [14] in isreal also reported that 5.8% of the pregnant women had antibody with specificity of anti-E (23%), anti-K (16%), and anti-c (10.8%) and multiple alloantibodies were observed in 15% of women and severe HDFN developed in 6.8% of these pregnancies

"Although the subjects are not the same, the finding are also in agreement with another previous report that reported 6.5% antibody screening positive with 13 RBC antibodies against antigens in the Rh system some had multiple antibodies, M antigen, and one against a low frequency antigen of unknown specificity" [15]. "The finding is lower and at variance with previous findings that reported frequency of allo-immunization to be 18.7 % among the previously transfused and 5 % in all sickle cell disease patients with a specificity of 46.7 % Rhesus, 40 % Kell, while Lutheran and Duffy 13.3 %, each as well as auto-antibodies in 1.25 %" [16]. It is also at variance with the report of El Fetouh and colleagues who reported prevalence of different alloantibodies as 9.16% with some having more than 1 antibody detected in a patient with haematological malignancies at Egypt with f alloantibodies specificity as the Rh system [45.3% with specificity of anti-E (21.3%) and anti-D (6.4%), anti-C (9.4%) and anti-c (8.2%)], followed by Kell (25.1%), Duffy (13.9%), MNS (7.5%), Kidd (7.1%), and Lewis (1.1%) [17]. It is also at variance with the report of Hussein and colleagues who recorded alloimmunization incidence as 22.8% with 37.4% Rh-related alloantibody followed by 26% anti-Kell, 8.9% anti-MNS 8.9% anti-Kidd, 8.1% anti-Duffy, 5.7% anti-Le, 2.4% anti-Lu and 1.6% anti-P1 [18].

Table 2 shows demographic and obstetric characteristics of pregnant women in relation to presence of alloantibodies. When the demographic and obstetric characteristics were compared with the presence of alloantibodies, statistically significant differences were

observed in gestational age, past history of pregnancy, preeclampsia, previous baby(ies) delivered with jaundice and previous administration of anti-D prophylaxis. Gestational age within the second trimester recorded highest number of pregnant women with the alloantibodies which may be associated with the fact that at first low level of antibodies that may not be detected at the first trimester and also lost of pregnancies towards the end of pregnancy (last trimester). Pregnant women that recorded either miscarriages or stillbirth at one or more time of previous pregnancies shows statistical significant alloantibodies compared with those that had no record of such which may be due to the subject used were mostly multigravidae not nulligravidae or primigravidae. Our study also recorded that those pregnant women with previous baby(ies) delivered with jaundice had statistically significant alloantibodies compared to those with no history of jaundiced baby and also pregnant women with history of previous anti D prophylaxis administration had no alloantibody compared with those that have no record of anti D prophylaxis administration which is an indication that anti-D antibody is the most common alloantibody in this area of study followed by non-clinically significant alloantibody. This is in agreement with previously reported significant correlation between presence of alloantibody and vaginal bleeding, gravida and previous history of newborn with neonatal jaundice but reported no significant correlation between alloimmunization and age, gestational week, and previous pregnancy loss [19]. It is however in agreement with previous report of Lieberman and colleague that reported significant correlation between alloantibody with age and jaundice but no significant correlation between gravida, parity, blood transfusion and gestational age [20].

5. CONCLUSION

There is an alarmingly high prevalence of alloantibody among pregnant women of 5.8% with specificity of both clinically significant antibody (antibody to Rhesus, MNS and Kidd blood

group), non-clinically significant antibody (Lewis and Lutheran blood group) as well as non-specific antibodies.

There is a statistically significant correlation between the subject age, gestational age, history of pregnancy, pre-eclampsia, history of baby delivered with jaundice, mode of delivery and use of anti-D prophylaxis with the development of alloantibody as most of the subjects with alloantibody were within the reproductive age of >20 and <40, were within the second trimester of pregnancy, had the previous history of either miscarriages or stillbirth and have not had anti-D prophylaxis in the sensitization episode of last pregnancy.

RECOMMENDATIONS

1. Incorporation and mandatory adaptation of blood group phenotyping as premarital screening as well as prior to transfusion to reduce the rate of antibody formation and haemolytic disease of the foetus and newborn
2. There is a need to routinely screen all pregnant women for alloantibodies at antenatal booking to identify women at risk of developing HDFN

Ethical Approval and Consent

Written and written informed consent was obtained from all participants using a standard protocol while ethical clearance was obtained from the Ethical Committee of Ministry of Health, Sokoto as well as the study site in accordance with Helsinki declaration.

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