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**Original article****Maternal alloimmunization as a risk factor of haemolytic disease of the foetus and newborn in Owerri metropolis, Nigeria****A.A.O. Chinwe^{a,*}, N. Johnkennedy^b, N. Constance^b, O. Hope^b**^a*Be –Tek Medical Laboratories, Port Harcourt, Rivers State, Nigeria.*^b*Dept of Medical laboratory Science, Faculty of Health Science, Imo State University of Science.*

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ABSTRACT

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Blood group incompatibility between mother and her foetus which usually arises as a result of heterospecific pregnancy causes haemolytic disease of the foetus and newborn (HDN). Five hundred (500) pregnant women attending antenatal clinic at Federal Medical Centre (FMC), Owerri were recruited for this research work. They were aged between 17 and 40 years. All subjects gave informed consent to participate in the study. They were screened for ABO blood groups RhD status and immune alloantibodies. Anti-A and anti-B from group O subjects compared with anti-A from group B women and anti-B from group A women among the trimester. The rate of RhD negativity (5.4%) was significantly lower than the RhD positively (94.6%) ($P < 0.005$). Haemolysin tests were positive in 110 (22.0%) cases and negative in 390 (78.0%) cases. Group O women produced more alpha and / or beta haemolysins than either A or B women ($P < 0.005$). Anti-A titres were higher in the trimesters than anti-B. Indirect antiglobulin test (IAT) was positive in 12(80.0%) cases among ABO group and positive in 3(20.0%) cases among RhD group. There was a higher prevalence of ABO HDN than RhD HDN in the ratio of 3:1 respectively. This study illustrates the presence of immune alloantibodies in the sera of alloimmunized pregnant women and the rise in the antibody titres has helped to identify pregnancies at risk of foetal and neonatal HDN. Anti-D immunoprophylaxis has made HDN caused by sensitization to the D-antigen a preventable disease and prenatal deaths from alloimmunization by immunoprophylaxis has been

primarily responsible for the dramatic reduction in the diseases, although changes in family size and the quality of prenatal care have also contributed.

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1. Introduction

Maternal alloimmunization to Rhesus (Rh) or other red cell antigens, with development of clinically significant immune antibodies causes foetal/ neonatal haemolytic disease, a condition in which the life span of the foetal/ neonatal red cells is shortened (Mollison et al., 1997). In other words, haemolytic disease of the foetus and newborn (HDN) can be caused by any maternal IgG antibody when it crosses the placenta and finds the corresponding antigen on the baby's red cells (Geifman – Holtzman et al., 1997). When the antibody is of clinical significance and of sufficient potency, the coated cells will be prematurely removed by the foetal reticuloendothelial system.

The red cell IgG antibodies giving rise to HDN are classified into three categories – Rh – HDN, ABO – HDN, and HDN due to other all antibodies Rh – HDN occurs in Rhesus negative mothers carrying Rhesus positive babies. (Hoffbrand et al., 2001).

In the first pregnancy, the mother has no anti-D, therefore the baby will not suffer from HDN. During delivery, a few millimetres of baby's red cells may enter the mother's circulation, she has a 10% chance of her immune system recognizing the baby's red cells and producing anti-D. If the mother carries a D-positive baby after the first pregnancy, the maternal antibodies cross the placenta and attach to the baby's red cells, which may result to the destruction of the foetal / neonatal red cells. This type of HDN is the most severe, and may result in foetal death (Geifman-Holtzman, et al., 1997).

In ABO haemolytic disease, group O subjects only make IgG anti-A and -B These IgG antibodies are usually due to transfusion or pregnancy and her first group A or child may have ABO-HDN. However, ABO-HDN does not always occur as expected in most situations where the mother has the IgG ABO-antibody, and the baby has the ABO antigen (Engelfriet et al., 1995).

Issitt (1999) reported that most forms of ABO – HDN are mild because the severity of the anaemia is only mild. ABO-HDN occurs in approximately 1 in 5 births. Severe ABO-HDN, that requires treatment is rare, it is found in approximately 1 in 3,000 births (in America). Therefore, a group O woman may have several A or B children, and any (or none) of her babies may have ABO-HDN (Filbey et al., 1995).

HDN is also caused by other IgG alloantibodies, especially anti-E, anti-c and anti-K. This is due to the immunogenicity of the antigens and their frequencies in the normal population. Most women who have these antibodies produce them after transfusions, not after pregnancy (Bowman, 1999a). Mothers are sensitized through this common routes-blood transfusion or fetomaternal haemorrhage associated with delivery trauma, spontaneous or induced abortion ectopic pregnancy, or invasive obstetrical procedures (Mollison et al., 1997).

HDN varies in severity and can have a variety of manifestations, which include hyperbilirubinemia and jaundice and when severe can lead to extramedullary haematopoiesis and reticuloendothelial clearance of foetal erythrocytes and This may result in hepatosplenomegaly, decreased liver function, hypoproteinemia, ascites and anasarca. (Belmar et al., 2005). Cases of HDN still lead to kernicterus. The absence of placental clearance and immature fetal bilirubin – conjugating ability can lead to symptoms that manifest several days after delivery and include poor feeding, inactivity, a bulging fontanelle and seizures. The 10% of infants who survive may develop spastic choreoathetosis, deafness and mental retardation. The blood film of a foetus affected by HDN shows polychromasia and increased numbers of nucleated red cells. In most cases, except a few due to ABO antibodies, the direct antiglobulin test on the infant's cells is positive (Mollison et al., 1997).

Treatment of HDN commences either prior to delivery to ensure that the child is born alive or after the birth of an affected child, by exchange blood transfusion, early induction of labour, intra-uterine transfusion, phototherapy and the use of drugs. (Moise, 2002).

2. Materials and methods

2.1. Setting

This study was conducted at Federal Medical Centre (FMC) Owerri, Imo State, Nigeria, a government health care institution. There are various other ethnic groups living in Owerri apart from the indigenes (Uratta, Egbu, Emekukwu, Owerri nchi-ise)

2.2. Study population and enrollment

Five hundred (500) pregnant women visiting the antenatal clinics at their first trimester from January – March 2006 constituted the study population. Their age varies from 17- 40 years and the period of gestation ranged from 2 to 9 months.

2.3. Selection criteria

Informed consent was obtained from all the pregnant women participating in this study. Thereafter, relevant personal biodata (age, parity, educational qualification, duration of pregnancy, place of residence etc) were obtained from the pregnant women. In-depth interview were carried out amongst the pregnant women using a questionnaire. Each pregnant woman underwent a general physical and obstetrical examination by a gynaecologist. Gestational age was accessed from history of last menstrual period and measurement of fundal height by a consultant gynaecologist.

2.4. Exclusion criteria

Pregnant women who were sceptical of the essence of the study and those that could not provide their informed consent were excluded in the study.

2.5. Collection of blood sample

A standard clean venipuncture technique was used to collect 4mls of blood from the pregnant women at booking using sterile needles and syringes. 1ml was dispensed into labelled dipotassium EDTA anticoagulant tubes and mixed for cell grouping. The concentration of the dipotassium salt was 1.5mg/ml of blood. The remaining 3mls of blood was dispensed into clean dry glass tubes and allowed to clot. The clotted blood samples in the tubes were centrifuged at 1500rpm for 5 minutes to separate the serum and the serum used for serum grouping and antibody titration. Pregnant women who are Rhesus D negative were retested at 28 – 34 weeks to detect clinically significant antibodies according to specificity, titre and quantitation.

2.6. Laboratory Reagents

The manufacturers of the reagents and LOT Nos were: Antiserum A,B and D (fortress AA 0704, BB0701, DD 0706). Antihuman Globulin (Biotec, AE 2539). All reagents were commercially purchased and the manufacturer's standard operating procedures strictly followed.

2.7. Methodology

The ABO and Rhesus grouping were done using the standard method as described by Dacie and Lewis (1991). The indirect antiglobulin test was determined using the method of Gorlin (2002). Haemolysin test was done using the method of Dacie and Lewis (1991). Antibody titration was done using the method as described by Judd(1990), while the use of Sulfhydryl reagents to distinguish IgM from IgG antibodies was done by the method of Dacie and Lewis (1991).The result was then statistically analysed.

3. Results

Table 1, shows that the percentage of RhD negativity was 5.4% while that of RhD positivity was 94.6%. The rate of RhD negativity was significantly lower than RhD positivity ($P<0.005$). 287 (57.4%) were of blood group O, 100 (20.0%) were blood group A. 79(15.8%) were group B and 7(3.4%), were group AB. Group O had the highest incidence while group AB had the least.

Table 1

Distribution of Pregnant women into ABO and Rh (D) Blood Groups.

ABO blood group	Rh (D) positive	Percent of positive	Rh(D) negative	Percent of negative	Total	Percent of Total
A	100	20.0	5	1.0	105	21.0
B	79	15.8	3	0.6	82	16.4
AB	7	3.4	1	0.2	8	1.6
O	287	57.4	18	3.6	305	61.0
Total	473	94.6	27	5.4	500	100.0

Table 2

The distribution of Indirect Antiglobulin Test (IAT) among ABO and Rhesus blood groups.

ABO blood group	Number of subjects (%)	(%) positive	(%) negative
A	100 (20.0)	1(1.0)	99(99.0)
B	79(15.8)	1(1.3)	78(98.7)
AB	7(1.4)	0(0)	7(100.0)
O	287(57.4)	10(3.5)	277(96.5)
ABO Total	473 (94.6)	12(80.0)	461(95.1)
Rhesus			
RhD	27(5.4)	3(20.0)	24(4.9)
Total	500(100.0)	15(3.0)	485(97.0)

Table 2 shows that out of the 500 samples collected, 15(3.0%) were positive using IAT, 12(80.0%) out of the 15 cases positive with indirect antihuman globulin tests were due to immune ABO antibodies, thus representing a prevalence of 1 in 42 or 2.4% in the whole study group and 1 in 29 or 3.5% in group O mothers and 3(20.0%) were positive among Rh(D) negative group. Table 3 shows that among the women with positive IAT, 10 (77.0%) were of ABO group, while 3 (23.0%) were of Rh (D) group, and there was increase in antibody titres from the first to the last trimester.

3.1. Key

IAT – Indirect Antiglobulin Test

G – Gravidae

P – Parity

G2P1 – Multigravidae carrying 2nd pregnancy. 1 live child

G3P2 - Multigravidae carrying 3rd pregnancy. 2 live children

G4P1 -2 - Multigravidae carrying 4th pregnancy. Only 1 child alive 2 dead

G4P3 - Multigravidae carrying 4th pregnancy. 3 live children

4. Discussion

Blood group incompatibility between mother and her foetus which usually arises as a result of heterospecific pregnancy causes haemolytic disease of the foetus and new born (HDN). There is usually seepage of the foetal red cells (RBCs) to the maternal circulation to cause alloimmunization when there is trauma during child birth in such pregnancies (Mollison et al., 1997). These immune antibodies cross the placenta to the foetal circulation to cause haemolytic disease of the foetus and newborn (Moise, 2005).

In this study, out of five hundred (500) pregnant women attending antenatal clinics at Federal Medical Centre, Owerri (FMC), Imo State, Nigeria, 27 (5.4%) were Rhesus negative (Table 1). This is in agreement with Onwukeme (1990) who stated that the rate of Rhesus negativity in Nigeria was 2.9-6.6%. Mba (2003), stated that the rate of Rhesus (D) negativity was 4%. The prevalence of Rhesus (D) haemolytic disease in any given population

depends largely on the proportion of women of child bearing age that are Rhesus (D) negative in that population and on whether the Rhesus (D) negative women are responders or non-responders Azubuike,1989 and Bowman, (1999b). The Rhesus positive women were 473 (94:6%) positive. This is similar to the work reported by Cheesbrough (2000), who stated that the frequency distribution of Rhesus (D) antigen in Africa was 94-95%. Blood group O was the commonest blood group in the whole population and the frequency was significantly higher than other blood groups ($P<0.005$). This was followed by group A,B and finally, blood AB. The rate of RhD negativity was significantly lower than RhD positivity ($P<0.005$).

Table 2 shows that 12(80.0%) pregnant women had ABO antibodies while 3(20.0%) cases were due to anti-D. This suggests a higher prevalence of ABO disease than Rh(D) haemolytic disease in the ratio of 4:1. This disagrees with Mba (2003)), who stated a higher prevalence of ABO disease than RH(D) disease in the ratio of between 2:3:1. Although ABO disease was more prevalent, Howard et al., (1998) stated that the commonest cause of fetomaternal blood group incompatibility is anti-A or anti –B in group O mothers with group A or B babies, but this rarely results in severe HDN requiring exchange transfusion. Hoffbrand et al., 2001), stated that a high titre of immune antibody will not necessarily cause problems in utero as A and B antigens are present on cells of all other tissues and body fluids and not only on red cells. According to cariani et al., (1995), in ABO incompatibility, hydrops fetals is very rare because anti-A and anti-B antibodies that develop during the first few months of life are usually IgM immunoglobulins that cannot cross the placenta.

Table 3

Comparison of Group O women with positive IAT.

No of subjects	Age	Parity	Blood group	IgG antibody titre in trimesters		
				1st	2nd	3rd
1	34	G3P2	O RhD Neg.	4	32	128
2	35	G4P3	O RhD Neg.	8	64	128
3	28	G2P0 - 1	O RhD Neg.	-	32	64
4	22	G4P1-3	O RhD Pos.	8	64	128
5	31	G3P2	O RhD Pos.	8	128	128
6	33	G4P3-1	O RhD Pos.	-	64	128
7	26	G2P1	O RhD Pos.	8	32	64
8	25	G4P3	O RhD Pos.	4	64	128
9	30	G2P1	O RhD Pos.	-	64	128
10	32	G4P2	O RhD Pos.	8	64	128
11	28	G4P3	O RhD Pos.	4	64	128
12	26	G4P1	O RhD Pos.	-	32	64
13	31	G3P2	O RhD Pos.	8	64	128
Total 13	No of positive ABO titre 10 (77.0%)		No of negative titre RH 3 (23.0%)			

Cheesbrough (2000), reported that anti-A in group O mothers may be both IgM and IgG and these antibodies may be present in the woman most of her life, hence the increase in titre.

McDonnell et al., (1998) observed that the protective effect conferred by ABO incompatibility is believed to be due to maternal destruction and subsequent clearance of the ABO incompatible foetal erythrocytes before sensitization can occur. The commonest cause of feto – maternal blood group incompatibility is anti-A or anti-B in group O mothers with group A or B babies, but this rarely results in severe HDN requiring exchange transfusion (Howard et al., 1998).

This work reveals that the pregnant women produced IgG immune allo-antibodies of low titres in the first trimester and the highest titre reached was 8 and varying concentrations of immune antibodies in the third trimester. The highest titre being 128. This shows a significant increase in antibody titre from the first trimester to the third trimester and it agrees with the work done by Clarke and Hussey (1994), who stated that there is a rise in

anti-A or anti-B titres during the last months of pregnancy. Also, among O Rh(D) negative women, 3(16.7%) multigravidae had immune anti-D antibodies. Among the RhD-negative multigravidae, 1(33.3%) was carrying a second pregnancy (G2P0-1) had an increase in antibody titre in the third trimester. The reason for the increased titre might be due to the fact that she was carrying a Rh-D positive foetus in the first pregnancy and there was foeto-maternal haemorrhage, of which she was not administered anti-Rh prophylaxis. This might have primed her and this second pregnancy is also likely to be a Rh-D positive foetus in which she mounted an immune response against the foetal erythrocytes. This agrees with the work of Bowman, (1999a) who showed that sensitization can occur when an Rh-negative mother is exposed to positive foetal erythrocytes. Secondary to foeto-maternal haemorrhage. This work agrees with the works of Jackson and Branch (1996) and Mollison et al., (2000) who showed that the risk and severity of alloimmune response increase with subsequent pregnancy involving a foetus with Rh-positive blood and that in women who are prone to Rh-immunization, the second pregnancy with an Rh-positive foetus often produces mildly anaemic infant.

The remaining 2(66.7%) Rh-D negative multigravidae, that were carrying their third or fourth pregnancies (G3P2 and G4P3) had significant increase in antibody titres in the third trimester. The reason for the increased titres recorded might probably because immunization had occurred in the previous pregnancies and this agrees with the work of Belmar et al., (2005) who observed that succeeding pregnancies produce more seriously affected infants who ultimately may die in utero from massive antibody induced haemolytic anaemia. This finding also agrees with the works of Bowman (1992) and Mollison et al., (2000) who state that once immunization has occurred, successive D-positive pregnancies often manifest HDN of increasing severity.

5. Conclusion

This study has shown that immune antibodies are present in the sera of alloimmunized pregnant women and that there is a rise in maternal antibody titres as pregnancy advances. Although there is a rise in antibody titre, it does not necessarily mean that HDN will occur as A and B antigens are present on cell of all other tissues and body fluids and not only on red cells. This study has also shown a higher prevalence of ABO antibodies than anti-D. Anti-D immuno prophylaxis had made HDN caused by sensitization to the D-antigen a preventable disease and prenatal deaths from alloimmunization by immunoprophylaxis has been primarily responsible for the dramatic reduction in the diseases, although changes in family size and the quality of prenatal care have also contributed.

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