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Potential Causes of HDFN among Neonates Delivered in Specialist Hospital Sokoto, Nigeria

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Abstract

Haemolytic disease of the Foetus and New-born (HDFN) is an autoimmune haemolytic disease caused by feto-maternal blood group incompatibility which usually occurs in foetuses and new-borns. The aim of this study was to find out the percentage prevalence of positive DAT among babies' due to IgG antibodies. This descriptive study was carried out among 79 singleton babies born in Specialist Sokoto, North-Western Nigeria to determine invivo sensitization of the foetal red cells to IgG antibodies. Three millilitres of Cord blood were collected, and the red cells were screened for in-vivo sensitization using Ortho Biovue (U.S.A.) Column Agglutination Technology based DAT cassettes. The study showed that four out of seventy-nine singletons, had positive DAT. ABO and Rhesus D blood group was determined on samples from the mothers and the neonates. ABO, Rhesus D and direct antiglobulin (IgG) test was carried out on samples from the mothers and babies using the Column agglutination technique (Ortho Diagnostics, USA). ABO blood group distribution among the mothers indicated that 22(27.8%), 12(15.2%), 1(1.3%) and 44 (55.7%) were blood group A, B, AB and O respectively. ABO blood group distribution among the babies indicated that 21(26.6%), 20(25.3%), 2(2.5%) and 36(45.6%) were ABO group A, B, AB and O respectively. Among the mothers 74(93.7%) were Rh D Positive while 5(6.3%) were Rh D negative. Among the babies, 75(94.9%) were Rh D positive and 4(5.1%) were Rh D negative. Among the 79 babies, the DAT (ABO-IgG) test was positive in 4(5.1%). The positive DAT was due to ABO group incompatibility among 2(2.5%) while Rh D incompatibility (Rh D-IgG) was responsible for the positive DAT in 2(2.5%) of the subjects.

In conclusion, we have observed in this study that HDFN due to ABO incompatibility and Rh D incompatibility are common among babies of African descent delivered in Sokoto, Nigeria. There is need to implement best practices in the prevention, diagnosis and optimal management of HDFN in Sokoto in particular and Nigeria in general.

Introduction

Haemolytic disease of the foetus and new-born (HDFN) is an autoimmune haemolytic disease caused by foeto-maternal blood group incompatibility which usually occurs in foetuses and new-borns (Macher *et al*., 2016). Alloantibodies produced by the mother's immune system as a result of foetal blood containing blood group antigen/s inherited from the father which is incompatible with his mother, flows through the placenta into the mother's blood, have been incriminated in HDNF (Yousuf *et al*., 2012; Velasco *et al*., 2014). The disease occur when this antibody flows through the placenta into the foetus or new-born, and bind to the antigen on the red blood cells of the foetus or new-born, resulting in HDFN (Oseni and Akomolafe, 2011; Cohen *et al.,* 2014). Clinically, most cases of ABO incompatibility -related HDFN occur when the mother is O blood type, and the foetus or infant is A or B blood type or when mother is Group B or A and the foetus is A and B respectively (Kakaiya *et al*., 2010).

HDFN is primarily caused by foetal-maternal ABO blood group incompatibility, accounting for 86% of all HDFN worldwide (Branch *et al*., 2011; Jakobsen *et al*.,2014; Yasuda *et al*.,2014). In contrast, HDFN due to Rh blood group incompatibility occurs when mum is Rh D negative, and baby is Rh D positive and accounts for approximately 15% of HDFN (Riyami *et al*., 2014). Examination of prenatal blood group antibodies is an important clinical method for the prevention of deformed or abnormal birth (Calkins *et al*.,2015; Kumar *et al*.,2016; Macher *et al*.,2016). Currently, the screening of HDFN is mainly performed by checking the ABO and Rh blood group antigens of the couples to detect the incompatibility of their blood type (Özgönenel *et al*., 2015). Also, the presence of Rh antibodies (particularly anti-D and c) in pregnant women is usually determined at booking and at 28 weeks gestation to detect presence of these alloantibodies that can potentially cause HDFN (Houston *et al*.,2015; Li *et al*., 2015). The aim of this study was to investigate the percentage prevalence of HDFN due to IgG and complement among babies with HDFN.

Materials and Method

This is a descriptive study carried out on 79 singleton babies to determine in vivo sensitization of the foetal red cells to IgG antibodies. Three millilitres of Cord blood were collected, and the plasma was screened for the presence of alloantibodies by Ortho Biovue system cassettes (AHG/Coombs) technique.

Data analysis:

The data obtained was presented in tabular forms and in proportions and the hypothesis was tested with statistical software (SPSS version 20) at $p<0.05$ significant levels and 95% confidence using the Person Chi-square test.

Results:

Table 1 shows that there were 22 Blood group A Mothers with a Percentage of 27.8%, 12 were of Blood Group B, with a percentage of 15.2%, 1 was AB with a percentage of 1.3% and 44 were of Blood Group O with a percentage of 55.7% respectively. The distribution of Baby's ABO Blood group with group A having a frequency of 21 and Valid Percent of 26.6%, group B with a frequency of 20 and Valid Percent of 25.3%, group AB with the least frequency and group O with the highest frequency of 36 and Valid Percent of 45.6%.

Table 2 shows that 74(93.7%) of the mothers were Rh D Positive Mothers 5(6.3%) were Rh D negative. Among the babies, 75(94.9%) were Rh D positive and 4(5.1%) were Rh D negative. Among the 79 babies, the DAT (ABO-IgG) test was positive in 4(5.1%). The positive DAT was due to ABO group incompatibility among 2(2.5%) while Rh D incompatibility (Rh D-IgG) was responsible for the positive DAT in 2(2.5%) of the subjects.

Table 3 shows prevalence of HDFN due to ABO and Rh D blood group incompatibility between Mum and Babies. The DAT test (IgG) was positive in 4(5.1%) of the babies. The study showed 2(2.5%) of the babies had a positive DAT due to ABO incompatibility between mum and baby. Also, 2(2.5%) had HDFN caused by Rh D incompatibility.

Table 1: ABO Blood group distribution among Mothers and Babies

ABO Group	Mum		Baby	
	N	%	N	%
A	22	27.8	21	26.6
B	12	15.2	20	25.3
AB	1	1.3	2	2.5
O	44	55.7	36	45.6
Total	79	100.0	79	100.0

Table 2: Rh D Blood group distribution among Mothers and Babies

Rh D Group	Mum		Baby	
	N	%	N	%
Positive	74	93.7	75	94.9
Negative	5	6.3	4	5.1
Total	79	100.0	79	100.0

Table 3: Prevalence of HDFN due to ABO and Rh D blood group incompatibility between Mum and Babies.

DAT Test	N (%) Due to ABO Incompatibility	N (%) due to ABO Incompatibility
Positive	2(2.5%)	2(2.5%)

Discussion

We observed that of the 79 singleton babies born 4 (5.1%) had HDFN indicated by a positive DAT (IgG) test. HDFN is caused by maternal alloimmunization through exposure to incompatible red blood cell antigens of the foetus or through incompatible blood transfusion (Jackson *et al.,* 2021). The immunoglobulin G (IgG) antibodies formed are actively transported across the placenta and can cause foetal haemolysis and anaemia. When untreated, progressive foetal anaemia resulting in hydrops foetalis and ultimately death. In foetuses that survives, there is persistent haemolysis resulting in neonatal anaemia and hyperbilirubinemia, which when untreated can leads to kernicterus. The direct antiglobulin test (DAT) is a highly sensitive clinically significant laboratory test that detects immunoglobulin and/or complement on the surface of red blood cells. The DAT is valuable in the diagnosis of haemolytic disease of the foetus/newborn (Khantisitthiporn, 2024). False reactions may occur with improper technique, including improper washing, centrifugation, specimen agitation at the time of result interpretation as well as patient-related factors including rouleaux and spontaneous red blood cell agglutination contribute to false results (Zantek *et al.,* 2012).

Our finding of a prevalence of ABO -related HDFN is consistent with a previous report which indicated that the incidence of ABO HDFN is 0.33–2.2% (Dufour and Monoghan 1980). Similarly, a previous report (Akanmu *et al.,* 2015) in Lagos, Nigeria estimated that 14.3% of deliveries will result in a blood group O woman giving birth to a child who is non-group-O. Approximately 4.3% of deliveries are likely to suffer ABO HDFN. It could be that the ABO antibody formed because of ABO incompatibility are IgG antibodies subclass that does not fix compliment since no complement activation occurs, therefore the immune mechanisms of red cell destruction may be in the absence of complement component coating of the infant red cells. Also, erythrocytes sphering is a common finding in ABO haemolytic disease and IgG anti-A has been shown to be capable of mediating ADC (Chen *et al.,* 2017). Again, it had earlier been demonstrated that destruction of red cells in vitro was antibody-dependent, cell-mediated and was suggested that this mechanism may account for much of the haemolysis due to antibody of the IgG class observed in vivo (Flegel, 2015).

We also observed that 2(2.5%) had HDFN due to Rh D incompatibility. Our finding is consistent with previous reports (Roberts, 2008; Basu *et al.,* 2011; Hendrickson and Delaney, 2016) which indicated that most severe cases of HDFN were attributed to Rh(D) incompatibility between an Rh(D)-negative woman and her Rh(D)-positive foetus, with Rh(D) alloimmunization having occurred during a previous pregnancy. However, with the introduction of routine Rh IG

immunoprophylaxis; alloantibodies of other specificities other than anti D have emerged as an important cause of HDFN and are now responsible for greater proportion of these cases (Liumbruno *et al*., 2010). HDFN typically occurs when there is an ABO or Rh incompatibility between the mother and foetus. ABO incompatibility-related HDFN tend to be milder for several reasons compared to that due to Rh incompatibility that tend to be more severe requiring more monitoring and treatment. ABO HDFN are often associated with minor degrees of red cell destruction evident by incidence of neonatal jaundice and slight lowering of the haemoglobin. Also, the A and B antigens tend to be fully developed at birth coupled with the fact that they are not confined to the red cells only. A small fraction of anti-A/anti-B IgG crossing placenta tend to be partly IgG2 which only cause red cell destruction in infants whose macrophages carry the high-affinity FcRIIa receptor. Fcγ receptors in placental tissue that tend to bind IgG1 with higher affinity compared to IgG2 (Kumawat *et al*.,2018).

There are other situations apart from ABO and Rh D when the mother has an HDFN causing alloantibodies (anti-Kell, anti-C, anti-c, anti-E, anti-e, anti-Duffy, anti-Kidd, anti-M, anti-N, and anti-U) that are directed to the group specific antigen located on the baby red cells (Li et al.,2020). The pathophysiology of the disease has to do with low molecular weight immunoglobulin G (IgG) antibodies produced in previous pregnancy against foreign foetal red blood cell antigens often inherited from the father and which the mother lacks. In subsequent pregnancies if the foetus is carrying the group specific antigen to which the maternal alloantibody is specific, the low molecular weight IgG antibodies can enter the foetus through the placenta and bind with the antigens on foetal erythrocytes. Sensitized foetal erythrocytes are excessively damaged and taken off the circulation by the reticuloendothelial system predominantly the spleen. This often results in hyperbilirubinaemia, foetal or neonatal anaemia, oedema, hepatosplenomegaly, and other symptoms. with a potential for miscarriage or stillbirth in severe cases (De Haas *et al*.,2015). There is need for the implementation of the following best practices to facilitate the prevention, diagnosis and management of HDFN in Nigeria; routine testing of ABO, Rh D group and alloantibody screening of mothers at 1st antenatal booking, foetal genotype testing of foetus for mum that has Rh D and Kell genotype using non-invasive techniques in mum that have the group specific alloantibodies, fetomaternal haemorrhage testing in Rh D negative pregnant women who are carrying Rh D positive babies from Cell Foetal DNA testing following a potentially sensitising event, Implementation of Routine Antenatal Anti-D Prophylaxis for non-sensitized Rh D negative women who are carrying a Rh D positive baby, provision of facility for intrauterine blood transfusion for anaemic babies inutero that are severely affected by HDFN, provision of donor red cell that meet the requirement for transfusion to neonates that are severe affected and need exchange and top up blood transfison to manage their HDFN. These implementations are likely to help optimize the care offered to babies affected by HDFB in Nigeria (Erhabor *et al*.,2023).

Conclusion

In conclusion, we have observed in this study that HDFN due to ABO incompatibility and Rh D incompatibility are common among babies of African descent delivered in Sokoto, Nigeria. There is need to implement best practices in the prevention, diagnosis and optimal management of HDFN in Sokoto in particular and Nigeria in general

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