INTRODUCTION

In blood transfusion practice, Haemolytic disease of the Newborn HDN is an important cause of both maternal and fetal morbidity and mortality. In this regard, many antigens on the surface of red blood cells have been implicated such as Rhesus, kell, kidd and MNS. However, Rhesus D antigen (Rh) is still the leading cause of Haemolytic disease of the newborn. The D antigen is the most important and immunogenic antigen in the Rh- Rhesus blood group system. Hence, for most clinical purposes, it is sufficient to classify individuals as Rh D positive or Rh D negative.

Although the Rhesus blood group is the largest blood group system, it is the second most important system after the ABO system. It consists of at least 45 antigens, with the most common being D,c, E, C,e and G. In terms of immunogenicity, the next most immunogenic Rh antigen after D are c and E with most of the Rh antibodies being of the IgG Immunoglobulin class, usually subclass 3. This indicates that most of the Rh antibodies are produced by exposure to foreign antigens via transfusion or pregnancy.

The Rh D antigen is carried by the Rhesus protein which is a complex of variant forms of RhD and RhCE proteins with a glycosylated homolog (RhAG). These Rh proteins are only expressed when RhAG is present on the erythrocyte membrane. The genes encoding the Rh proteins (RhCE and RHD) are highly homologous and found on the short arm of chromosome 1 while the gene encoding RhAG is located on the short arm of chromosome.

In Nigeria, reports of studies in this field are apparently scarce, however, data from there indicate that the incidence of the Rh D antigen in Nigerians and in blacks in the population is high while Rhesus D negativity is low, ranging from about 90-99% for Rhesus D positively, to about 1-9.5% for Rhesus negativity. This figure is at variance with the Caucasian and European data in which Rhesus negativity is high with levels of about 17% and 20-40% in the UK and Basques respectively.

Furthermore, it has been reported that the Rh D antigen exhibit differential clinical expressions that impact upon its immunogenic potential. These variations particularly relate to both qualitative and quantitative characteristics as exemplified by the weak D phenotypes (formerly called D' phenotype) and partial D variants. Partial D antigens are weak variants of Rh D and may be explained as qualitatively altered Rhesus D proteins lacking some epitopes since the D antigens is a collection of conformation dependent epitopes along the entire RhD protein. These alterations maybe caused by RHD/RHCE hybrid alleles. All partial D antigens lack one or more D epitopes and with the exception of category III, can be defined by their epitope profile.

D antigens were classically identified by testing the RBC's with well characterized polyclonal anti-D made by other people with partial D phenotypes and, also, by testing the patient's anti-D against RBCs with known partial D antigens. However, current classification of these RBCs is done by using Human monoclonal antibodies in terms of expressed epitopes. In Europe, anti-D reagents are selected to deliberately type category DVI (partial D) mothers as D-negative and, thus, ensure that such mothers would automatically receive prophylactic Rh immunoglobulin following pregnancy. This is due to the difficulty of properly characterizing category DVI individuals.

The D' phenotype which is frequently mistyped as Rh negative are weak D antigens in which most, if not all, weak D phenotypes carry altered or abnormal RhD protein. The genetic mechanisms responsible for this reduced clinical expression include a severely reduced expression of RHD messenger (RNA (mRNA) suggesting a defect at the level of transcription or pre-in mRNA processing, missense mutation(s) within the predicted transmembrane or cytoplasmic domains of RhD and Amino acid substitutions in the intracellular and transmembrane protein segments. Thus, RBCs with some weak D antigens may not be agglutinated by all monoclonal anti-D and may also not make anti-D.

The literature on D' phenotype in Nigeria is rather scanty. However, the classic studies of Worledge in the mid 1960s and 1970s which was localized in Western Nigeria among the Yorubas reported "low grade" D' to be 7.5% of all persons (donors) thought to be Rh negative and about 0.4% of the general population. In the same vein Nwauche et al working in the Niger Delta region reported the frequency of D' phenotype to be 0.95% amongst Rh-negative adult females in Port Harcourt. These figures are at variance with the Caucasian data in which D' phenotype is reported to be 0.6% in the UK.

This indicates that D' phenotype appears to be higher in Blacks than Caucasians.

Rhesus Antibodies

Anti-D is the leading antibody type produced following Rhesus immunization and is typically of the IgG immunoglobulin class. There are reports of involvement of IgG antibodies of all four sub-classes; however, IgG1 and IgG3 are of the greatest clinical significance causing prompt clearance of immunoglobulin coated red cells. There are also reports documenting the involvement of IgA and IgM antibodies. As with most blood group antigen sensitization, IgM antibodies are formed first, and a transition is then made to IgG Rh antibodies often persist in the circulation for long
periods, and anamnestic response is rapidly made on subsequent exposure to the sensitizing antigen. Thus, in the clinical setting, accuracy of Rhd typing is crucial as is the careful checking of the patient's history to determine whether an Rh antibody of any specificity has been previously identified, in which case, antigen negative blood must then be provided. 

Even when the IgG3 component is present, in general, the Rh antibody does not bind complement, thus they cannot mediate intravascular haemolysis although, as with most blood group systems, exceptions to this observation have been made. The haemolysis that then results in a patient with an Rh antibody is extravascular and this classically manifests as a delayed haemolytic transfusion reaction.

Although some Rh antibodies are commonly detected in saline test systems, indicating their IgM nature, anti-D is still the commonest IgM antibody of clinical relevance detected in a routine blood transfusion laboratory despite the prophylaxis of Rh immunization with anti-Rh immunoglobulin (Rhogam), which has however resulted in a significant decrease.

In the context of Rh-negative transfusion recipients, who are generally matched with D negative donors, anti-D is infrequently found in which case, anti-E becomes the most commonly encountered allo-antibody. Thus, in routine screening, pure anti-E is the commonest, followed by anti-c, although anti-c is a commoner cause of HDN.

This is probably because about half the anti-E are weak, naturally occurring IgM antibodies. Anti-e, like anti-C is very rare. About 20-30% of anti-D sera also contain anti-C. Usually, this is actually anti-G (anti-DwC) and the occurrence of anti-C (and anti-G) in the absence of anti-D is uncommon. Furthermore, about 1-2% of anti-D sera also contain anti-E. The incidence of other Rh antibodies is much lower but together, they are commoner than the antibodies against K (Kell), which is the next immunogenic after D.

**CLINICAL ASPECTS**

Rhesus D antibodies, which are mostly IgG readily cross the placenta to cause HDN due to their enhanced ability to traverse the placental barrier, probably due to their small size. The Rh antibodies are well developed on fetal red cells, thus if the father carries an Rh antigen not present on the mother's red cells, the fetus may be at risk once the mother is exposed by transfusion or previous pregnancy to the red cell antigens she lacks.

Clinical complications therefore result from RBC destruction due to the interaction of an allo-antibody with RBC's carrying the corresponding antigen. The RhD antigen being highly immunogenic induces an immune response in 80-85% of D-negative persons when transfused with 200 ml of D-positive blood especially within 2-6 months for this reason in most countries including Nigeria, D typing is performed routinely on every blood donor and transfusion recipient so that D-negative patients receive D-negative RBC products. Consequently, clinical complications such as Rhesus immunization due to mismatched transfusions are infrequent.

In contrast however, D alloimmunization in pregnancy still occurs despite the use of immunosuppressive therapy with anti-D immunoglobulin prophylaxis. However, some workers have reported on the impairment of the development of Rh-antibodies in transplant patients following transfusion with Rh positive blood who were on cyclosporine immunosuppressive therapy while others have observed that the baby of a Rh-D negative multipara with no anti-Rhesus immunoglobulin prophylaxis did not present with HDN following a high titre anti-D sensitization.

Furthermore, other workers have estimated a crude utilization rate of Rhogam in Republic of south Africa for all the indigenes ethnic groups combined to be 14-28% for blacks, 89-94% for whites, 59-64% for Indians and 45-51% for coloureds. Elsewhere several other studies have justified the prophylactic administration of Rhogam on account of a sharp increase in the anti-D titer after delivery. In this study by del- Aquila et al, increments of fifty times or greater were observed.

It has also been suggested that one of the rational ways to initiate prophylaxis of Rh immunization in pregnancy is to go back to first principles and carry out kleihauer's test particularly when neonatal anaemia is found in the child since this test measures the magnitude of the feto-maternal haemorrhage. The other approach may be the recommendation for intensive antenatal prophylaxis particularly in the last trimester based on the findings of the Scottish study which showed that the single commonest identifiable cause for the failure of the Rhesus immunization prevention programme was late immunization in an uncomplicated pregnancy.

In Nigeria, even though there is a paucity of data regarding Rh immunization, the seminal reports by Worledge et al in the Ibadan series and the Lagos series by Odumaya. Both provide a lot of information and insight into this phenomenon in Nigeria. The Ibadan study was conducted in a homogenous ethnic group (Yorubas) living in a malariaous area while the Lagos study was carried out within a cosmopolitan setting that has been influenced by several factors of urbanization including inter tribal marriages. Thus, Worledge et al; in 1968 reported an overall frequency of Rhesus immunization in Nigeria to be 4.5% of which 2.5% was by pregnancy alone while Odumaya in Lagos reported a frequency of 3.8% in 1974.

This indicates a low trend of Rhesus immunization when compared with an overall worldwide figure of 3-11%. Worledge et al also noted that the prognosis for pregnancy induced Rh-180 immunization is worse than for transfusion induced iso-immunization and that the frequency of immunization by pregnancy was not greater in Nigeria than elsewhere in spite of the fact that the chances of a Rh-negative mother carry a Rh- positive foetus is higher in Nigeria than in European countries. In this environment, Rh negatives are immunized mainly by red cells with the K, phenotype, which has 15,000-20,000 D antigen sites per cell less man mest Rr cells but more than Rr cells.

They also predicted the frequency of HDN due to Rh antibodies to be 1:800, which forms a small contribution of 10% of the causes of Neonatal jaundice in this environment. Some of the reasons adduced for the relatively low immunization frequency in Nigeria include the interference in antibody production by a high load of various external stimuli probably less operative interference by obstetric maneuvers such as manual removal of the placenta, amniocenteses, antenatal fetal blood sampling etc which have been reported.
to facilitate passage of foetal red cells into the maternal blood and the possible lower permeability of the placental barrier for foetal red cells or antibody passage. Furthermore, not all D-negative women have D positive children, and not all pregnancies are ABO compatible since ABO incompatibility protects against Rh immunization. D phenotype is a much less effective immunogen than D since it has fewer antigen sites. However, there have been reports of a D infant immunizing a woman and of a case of rhesus disease in a neonate after sensitization of a Rh-positive mother with an incomplete D-antigen.

CONCLUSION

It is quite obvious that it is absolutely necessary that a well coordinated and efficient prevention programme and guidelines are put in place for the management of Rh immunization in Nigeria. It is equally important that these guidelines be properly and regularly evaluated as is the case in advanced centers all over the world. There is also the need to identify individuals who are truly Rh negative since D is a much less effective immunogen which can be easily mistyped as Rh negative especially in areas where D phenotype frequency is high such as in western Nigeria among the Yorubas. This would also prevent the scenario in which Rhogam is erroneously administered to D phenotype individuals who have been mistyped as being Rh negative. We also recommend the urgent need to set up the National Blood Transfusion Service and the training of adequate manpower to run the laboratory and clinical services in this country. Finally, the paucity of data in this important field calls for more studies to generate data that would assist in formulating policies and guidelines that would ultimately elevate the quality of care given to Rh negatives particularly pregnant mothers in this environment.

REFERENCES


