Ref: Ro Med J. 2022;69(2)
DOI: 10.37897/RMJ.2022.2.1

Hemolytic disease of the newborn, beyond the Rhesus disease

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ABSTRACT

Hemolytic disease of the fetus and newborn is a consequence of maternal immune response to fetal red blood cells antigens inherited from the father, that the mother does not possess, stimulated by antepartum or intrapartum fetomaternal hemorrhage. As a result, fetal erythrocytes are destroyed, leading to various degrees of anemia and hyperbilirubinemia, with high perinatal morbidity and mortality rates. This review's purpose is to reveal the importance of the alloantibodies beyond RhD and ABO system, the updated algorithms used in the diagnosis and management of the disease and the importance of RhD universal immune prophylaxis practice. For this purpose, the database from PubMed and UpToDate was searched for literature reviews, case studies, society guidelines and retrospective studies in English regarding hemolytic disease of the fetus and newborn from 2013 to March 2022. Anti D prophylaxis protocols, early diagnosis and proper intrauterine and postpartum treatment help significantly reduce the disease's burden.

Keywords: hemolytic disease, anemia, fetus, newborn, alloantibodies, RhD, ABO

INTRODUCTION

Hemolytic disease of the newborn, previously known as erythroblastosis fetalis or alloimmune hemolytic disease of the fetus and newborn (HDFN), is characterized by fetal or neonatal red blood cells (RBCs) destruction, mediated by maternal immunoglobulin G (IgG) antibodies. Whenever the fetal RBCs express antigens that are not expressed on the mother's RBCs, and there is a previous maternal exposure to the lacking antigen, specific antibodies are produced which may or may not pass through the placenta and lead to HDFN [1,2].

HDFN is primarily caused by Rhesus incompatibility, ABO blood group system incompatibility but also minor blood group incompatibilities, such as Kell, Duffy, MNS and P systems. The signs and symptoms of the disease can range from mild to severe, depending on the blood group system incompatibility type and on the maternal antibody titer [1,3].

ETIOLOGY AND PATHOPHYSIOLOGY

In USA between 3-80 in 100000 newborns are affected by hemolytic disease of the newborn, annually. The most common causes of HDFN are represented by Rh and ABO system antibodies [4,5].

The Rh blood group system consists of more than 50 antigens on the RBCs surface, of which D, C, c, E, and e are the most common. Rh negative status refers to RhD antigen absence, which is found in 15% of white population, around 4-8% of African population and only 0,1-0,3% of Asians. Lack of expression of RhD antigen is a result of absence of RhD gene, seen in European population or alteration and consecutive inactivation of the RhD gene, most common seen in African population [1,6,7,8].

Weak D, partial D, D(u) and D(el) represent D variants, found in 0,2-1% of White individuals. Weak D phenotype is caused either by a very low number of D antigens on the surface or RBC or by D antigens

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Article History:
Received: 7 June 2022
Accepted: 27 June 2022

lacking epitopes. Genotyping weak D antigen individuals often reveals type 1, 2 and 3 which can be considered Rh D positive. Other types of D variants identified can form anti D antibodies when exposed to D antigen, therefore pregnant patients should be managed as Rh D negative. If genotyping is not available, weak D variant pregnant patients should be considered Rh D negative and receive anti D immune globulin, when indicated [6,7].

Rh G antigen is found on the surface of red blood cells expressing C or D antigen, and antibodies against G appear as anti C with anti D. It is important to distinguish between anti G and real anti C plus anti D in pregnant women, in order to properly administer RhD immune globulin, when indicated, to G immunized patients [6,7].

Anti RhD antibodies can cause from mild hemolytic disease to the most severe forms of HDFN, represented by hydrops fetalis and even fetal demise. Anti D immune globulin prophylactic use reduced the frequency of anti RhD induced HDFN, but it does not prevent alloimmunization to other Rh antigens, such as C, c, E and e [6].

ABO blood group system incompatibility affects almost 15-20% of pregnancies, leading to a low rate of HDFN, of almost 0,6% of all pregnancies, generally less severe than RhD incompatibility related disease. ABO incompatibility is most commonly seen in group O mothers with group A or B infants. Group O individuals who lack A and B antigens, begin to naturally develop mostly IgM but also IgG type anti A and B antibodies in their early life as a result to exposure to the antigens found in food and bacteria, which mimic A and B antigens. Therefore, HDFN related to ABO incompatibility can occur in the first pregnancy, without previous sensitization [1,5,7,9].

Severe hemolysis due to ABO incompatibility is rare and it usually affects the neonate rather than the fetus due to predominantly IgM anti-A and B which are not transported across the placenta, Ig G2 anti-A and B which possess a weaker binding affinity and because A and B antigens are not fully developed on fetal RBCs surface. Furthermore, it appears that A and B antigens are also expressed, besides the RBCs surface, on a variety of fetal tissues among with soluble A and B substances identified in fetal/neonatal plasma which can bind maternal antibodies leaving less antibodies available for binding with RBCs corresponding antigens [5,10,11].

Most cases of HDFN related to ABO incompatibility tend to be mild, usually asymptomatic at birth, with absence or mild anemia and development of hyperbilirubinemia in the first 24 hours of life, with a frequent satisfying response to phototherapy. Hydrops fetalis is rare and less than 0,1% of infants with hemolysis will require exchange transfusion, excepting blood group B African American neonates born to group O mothers, because it appears that B antigen is more developed at birth in this particular group, leading to a more severe form of disease [1,7].

Studies show that routine blood typing and testing for every newborn from blood group O mother is not necessary, and whenever a severe hemolytic disease occurs to an infant with ABO incompatibility, further testing should be taken for congenital hemolytic diseases that could be responsible for the severity of the disease [1,11].

ABO mother-fetal incompatibility appears to play a protective role against HDFN related to other alloantibodies because of the rapid clearance of incompatible fetal erythrocytes from maternal circulation mediated by anti A and B antibodies [12].

There are 33 blood groups and over 300 antigens out of which some are responsible for HDFN. Besides Rh system and ABO incompatibilities, antigens such as Kell, Duffy, Diego, Kidd and MNSs are responsible for moderate or even severe forms of hemolysis, leading to hydrops and even fetal demise [1,13].

Kell group of antigens include k, Kp^a, Kp^b, Js^a, Js^b which can cause mild to moderate HDFN while the most antigenic of the group is K, which is responsible for almost 10% of the severe forms of the disease. K antigen is expressed on circulating RBCs surface but also on bone marrow erythroid progenitor cells from early fetal development stage hence anti K antibodies are not only able to cause hemolysis but also erythropoiesis suppression, leading to severe cases of anemia which can develop earlier in pregnancy comparing with the rest of alloantibodies. Another particularity of K antibodies is the fact that their level corelates poorly with the probability of developing fetal anemia and the degree of anemia [3,7,14].

Of the MNSs blood group antibodies anti-M can occur naturally. It is usually an IgM or cold reactive IgG antibody and rarely causes HDFN. Anti-En^a is extremely rare but can cause severe form of disease and anti-Mur is more common among Asian population and can lead from mild to severe HDFN. Anti-S and anti-s antibodies are mostly IgG which cross the placenta and lead to HDFN, rarely severe form, while anti-U can cause severe form of the disease, mostly in African population where U negative phenotype occurs almost exclusively [7,15].

Duffy (Fy^a and Fy^b), Kidd (Jk^a, Jk^b and Jk3) and Diego (Di^a, Di^b, Wr^a and ELO) antibodies are usually responsible for mild forms of HDFN [7].

Hemolytic disease of the newborn represents fetal/neonatal RBCs destruction, mediated by maternal Ig G antibodies produced by the immune system after a sensitizing contact with foreign erythrocyte surface antigens, process called alloimmunization. Primary response to an antigen involves IgM anti-

body formation and takes place from 4 weeks to a 3-month period, while secondary response is more rapid and potent and implies a switch from IgM to IgG class antibody. These IgG antibodies, especially IgG1 and IgG3 subclasses, are transported across the placenta by the Fc receptor into the fetal circulation and lead to hemolysis if the fetus is positive for the corresponding RBCs surface antigens [16,17]. The antibodies opsonize fetal erythrocytes, which are phagocytized by macrophages in fetal spleen, leading to fetal anemia. Subsequent hyperbilirubinemia is only seen in the delivered newborn because bilirubin is metabolized by the placenta in the uterine life. After delivery, with ongoing hemolysis due to persistent maternal antibody in the neonatal circulating system, severe hyperbilirubinemia can occur because of the incapacity of the immature neonatal liver to conjugate bilirubin, resulting in kernicterus if left untreated. With the worsening of hemolysis, fetal erythropoiesis is stimulated in the bone marrow but also in the liver and spleen with subsequent enlargement of these organs. Severe cases with hydrops fetalis are characterized by ascites that evolves to pleural effusion and generalized edema due to portal hypertension, low plasma oncotic pressure caused by reduced hepatic function with subsequent hypoalbuminemia, associated with cardiac failure [4,5,16].

Alloimmunization can be caused by fetomaternal hemorrhage (FMH), blood transfusion and needle sharing in intravenous drug users. Fetomaternal hemorrhage occurs in over 75% of pregnancies, most commonly at delivery in 15-50% of births but also along all of the 3 trimesters as follows: 7% in the first trimester, 16% in the second and 29% in the third [18]. Antepartum sensitizing events are represented by spontaneous or induced abortions, threatened abortion, ectopic pregnancies, placental abruption, amniocentesis, chorionic villus sampling, external cephalic version, fetal blood sampling, abdominal trauma, intrauterine fetal death [16,18]. The fetus/ neonate resulting from the first pregnancy is rarely affected by HDFN because significantly exposure to fetal erythrocytes resulting in maternal alloimmunization typically takes place at delivery or in the late third trimester, with primary immune response characterized by IgM antibody formation, which are not able to cross the placenta. HDFN occurs usually during subsequent pregnancies after re-exposure to the same antigen, due to IgG production, excepting, ABO blood group incompatibility and previous alloimmunization caused by transfusion or needle sharing [16].

ANTENATAL DIAGNOSIS AND MANAGEMENT

First prenatal visit should include D typing and screening for RBCs antigens, as well as ABO testing. If maternal antibody screen is positive, the potential of the alloantibody found to determine HDFN should be assessed knowing the alloantibody class (IgM or IgG), the likelihood of the antibody to determine HDFN and its level in maternal plasma [3,6].

For pregnancies at risk for developing HDFN fetal antigen status should be determined non-invasively by testing the paternal antigen status. Because RBC antigens are inherited from both parents a homozygous father implies the fetus will be antigen positive, a heterozygous father implies there is a 50% chance that the fetus will also be positive, while if the father is negative the fetus is also negative with no further monitoring required. When the father is heterozygous for the implicated antigen, fetal antigen testing can be performed by cell-free DNA testing of maternal blood or amniocentesis, reserved only for cases when maternal alloantibody level is at or above critical value (≥4 for Kell and ≥16 for D and other alloantibodies). If the fetus is positive for the concerned antigen, monitoring for fetal anemia implies obtaining serial maternal indirect Coombs titers monthly as long as the titer remains stable and every two weeks if the titer is rising, with additional middle cerebral artery peak systolic velocity (MCA PSV) Doppler measurement, once a critical value is reached. Because there is a low risk of developing severe fetal anemia during early pregnancy there is no need to perform serial titer before 18-20 weeks of gestation, excepting Kell sensitized pregnancies for which MCA PSV Doppler assessment is performed earlier, at around 18 weeks of gestation. MCA PSV≤ 1,5 MoMs for gestational age is corelated with the absence of moderate or severe fetal anemia. Measurements should be taken every two or every week, with increasing the frequency if value approaches 1.5 MoMs. Delivery is recommended between 37 and 38+6 weeks of gestation if the value of MCA PSV remains ≤ 1,5 MoMs, according to Society for Maternal-Fetal Medicine (SMFM) and American College of Obstetricians and Gynecologists (ACOG) guidelines. Otherwise, if MCA PSV> 1,5 MoMs cordocentesis should be taken for fetal hemoglobin determination and subsequent intrauterine transfusion (IUT), if value is more than two standard deviations below the mean value for gestational age or hematocrit is less than 30%. If hemoglobin level is above the limit, another blood sample will be obtained in one or two weeks [3,6,17,18,19].

Intravascular intrauterine transfusion is technically challenging under 18 weeks of gestation because of the small anatomical structures. Patients with severe alloimmunization and history of second trimester perinatal loss could benefit from maternal plasma exchange with or without intravenous immunoglobulin G (IVIG) in order to maintain fetal hematocrit above life-threatening level until IUT is

technically suitable. In pregnancies over 35 weeks of gestation delivery is considered to be safer than IUT. The last IUT is advised to be performed at 30-32 weeks of gestation with subsequent delivery at 32-34 weeks of gestation, after steroid administration for fetal pulmonary maturation [19,20,21,22].

POSTNATAL DIAGNOSIS AND MANAGEMENT

HDFN is suspected when two of the following criteria are accomplished: blood type incompatibility between mother and newborn and presence of at least one sign of hemolysis (decreased number of RBC on peripheral blood smear, macrocytosis, reticulocytosis and polychromasia, indirect hyperbilirubinemia, elevated end-tidal carbon monoxide, microspherocytosis or spherocytosis, commonly encountered in infants with ABO related HDFN). Diagnosis is confirmed through direct or indirect antiglobulin test (DAT or IAT). Negative DAT is a common finding in cases of ABO incompatibility or IUT, with positive IAT. In cases of ABO suspected incompatibility with negative DAT and IAT another cause for hyperbilirubinemia should be looked for such as Gilbert syndrome, erythrocyte enzyme defects (glucose-6-phosphate dehydrogenase and pyruvate kinase deficiencies) and erythrocyte membrane defects (hereditary spherocytosis and elliptocytosis) [1].

Whenever HDFN is known or suspected, cord blood should be tested for blood type, DAT/IAT, bilirubin level, hematocrit, reticulocyte count and cross match in case of transfusion needed [1].

In the delivery room beside assessing cardiovascular and respiratory system, the severity of hemolysis should also be estimated. Symptomatic anemia is defined by tachycardia, pallor, and tachypnea, which may be aggravated by pleural effusion or pulmonary hypoplasia in hydropic infants. Such newborns may present at delivery with shock or near shock, requiring immediate transfusion with only 10ml/kg group O Rhesus D negative RBCs, in order not to compromise the cardiovascular system by fluid overload. Thoracentesis and paracentesis may be needed for respiratory and cardiovascular stabilization. Afterwards for reducing hemolysis and correcting the anemia early exchange transfusion is performed. Newborns with severe anemia (hematocrit <25%), no signs of shock and severe hyperbilirubinemia would benefit from exchange transfusion by replacing fetal RBCs coated in maternal antibodies with donor RBC which lack the sensitizing antigen, removing serum bilirubin and a part of unbound maternal antibodies. For moderate to severe anemia (hematocrit between 25-35%) and non-severe hyperbilirubinemia, simple transfusion is usually performed while infants with mild or no anemia (hematocrit >35%) and non-severe hyperbilirubinemia which are at risk for late anemia could benefit from erythropoietin stimulating agents and iron supplementation in order to avoid subsequent transfusion [1,23,24].

The treatment of unconjugated neonatal hyperbilirubinemia is mainly represented by phototherapy and oral hydration. If oral hydration is not proper, IV hydration should be considered. Ongoing monitoring of serum bilirubin levels is required until safe range levels are acquired without ongoing treatment. Exchange transfusion is used to treat severe hyperbilirubinemia if the infant presents signs of acute bilirubin encephalopathy such as hypotonia, high-pitched cry, lethargy, or poor sucking, at bilirubin levels of 4,5 mg/dL or in case or rising levels of bilirubin greater than 0,5 mg/dL per hour, despite intensive phototherapy. Neonates with TSB levels rising despite intensive phototherapy or between 2-3 mg/dL could benefit from IVIG therapy, and maybe avoid transfusion [1,25].

Breastfeeding and delayed cord clamping are not contraindicated in alloimmunized pregnancies [1].

PREVENTION OF HDFN

The use of RhD immune globulin reduced the rate of alloimmunization in high-risk pregnancies from 13-16% to 0,14-0,2% [26]. RhD negative pregnant patients with a negative antibody screening test in the first trimester and at 28 weeks of gestation, should receive 300 μg anti D immune globulin at 28 weeks of gestation and another dose in the first 72 hours after the delivery of an RhD positive infant, according to ACOG. The dose of 300 μg of anti D immune globulin is suitable for maternal exposure to 15 ml of fetal RBCs. The afterbirth dose can be augmented for larger FMH, which occurs in 2-3 in 1000 deliveries. Therefore, every RhD negative women who delivered an RhD positive newborn should be assessed for the volume of FMH, in order to determine the amount of RhD immune globulin necessary to prevent alloimmunization. A positive qualitative rosette fetal red blood cell assay is followed by a Kleihauer-Betke test in order to establish the percentage of fetal RBCs in maternal circulation. FIGO as well as Canadian, British and Australian and New Zealand guidelines recommend besides a single dose regimen, at 28 weeks of gestation, a double dose regimen at 28 and 34 weeks of gestation using various doses. Administration of RhD immune globulin is advised after every potential sensitizing event including molar pregnancies. Some guidelines suggest not to use RhD immune globulin for under 8 weeks pregnancy termination, but there is no consensus regarding this matter [26-29]. RhD alloimmunized patients do not benefit from RhD immune

globulin administration but can avoid HDFN by obtaining a pregnancy using RhD negative donor sperm, in vitro fertilization with selected RhD negative embryo, for heterozygous fathers or by using a non alloimmunized gestational surrogate [27].

CONCLUSIONS

RhD alloimmunization often leads to severe HDFN, but RhD immune globulin prophylaxis administration at 28 weeks of gestation and after birth, significantly decreased the rate of the disease among RhD negative pregnant patients. Although HDFN due to minor blood groups antigens in rarely encountered, Kell antigen is responsible for earlier and more severe alloimmunization, leading, besides anemia, to suppression of erythropoiesis. ABO incompatibility can lead to HDFN during the first pregnancy, but it usually causes a mild form of the disease. Therefore, when a severe form is encountered another cause for neonatal anemia should be searched for.

Intrauterine Doppler assessment of MCA PSV has a high sensitivity in predicting a moderate to severe anemia, hence a value ≥ 1.5 MoMs should be followed by invasive assessment of the severity of anemia with subsequent intrauterine transfusion if needed.

The management of newborn alloimmune hemolysis varies with the severity of the anemia, with exchange transfusions being performed in severe cases.

Breastfeeding and delayed cord clamping are not contraindicated in alloimmunized pregnancies.

Conflict of interest: none declared
Financial support: none declared

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