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HEMOLYTIC DISEASE OF THE NEWBORN: A REVIEW

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ABSTRACT

Haemolytic disease of the newborn (HDN) is a condition in which the lifespan of the fetal/neonatal red cells is shortened due to maternal alloantibodies against red cell antigens inherited from the father. Maternal IgG can cross the placenta, thus IgG red cell alloantibodies can gain access to the fetus. If the fetal red cells contain the corresponding antigen then binding of antibody to red cells will occur. When the antibody is of clinical significance (e.g. anti-D, -c, -E, -K, -Jka), and of sufficient potency, the coated cells will be prematurely removed by the fetal mononuclear phagocytic system. The effects on the fetus/newborn infant may vary according to the characteristics of the maternal alloantibody. The antibodies giving rise to HDN most commonly belong to the Rh or ABO blood group systems. The morbidity of Rh HDN is explained by the great immunogenicity of the D antigen; HDN due to anti-c is also important and its incidence comes second amongst the cases of severe HDN closely followed by the non-Rh antibody, anti-K. (The anaemia caused by anti-K is more properly called *alloimmune anaemia of the fetus and newborn* as it is due to direct inhibition of erythropoiesis by the antibody and haemolysis is not a feature.) Antibodies against antigens in almost all the blood group systems (e.g. Duffy, Kidd, etc.) and against the so-called 'public' and 'private' antigens, have also been responsible for HDN. However, IgM cannot cross the placenta and Lewis and P1 antibodies, which occur frequently during pregnancy, are usually IgM and do not lead to HDN. Furthermore, the Lewis antigens are not fully developed at birth.

Key words: Haemolytic disease of the newborn, Epidemiology, History.

INTRODUCTION

Although the Rh antibody was and still is the most common cause of severe hemolytic disease of the newborn, other alloimmune antibodies belonging to Kell (K and k), Duffy (Fya), Kidd (Jka and Jkb), and MNSs (M, N, S, and s) systems do cause severe hemolytic disease of the newborn [1].

Frequency of Rh negativity is higher in whites (15%) than in blacks (5%) and Hispanics (8%) and is rare in Eskimos, Native Americans, Japanese and Asians, especially in Chinese individuals. The paternal heterozygosity determines the likelihood of an Rh-positive child being born to an Rh-negative mother.

Pathophysiology The exposure of the Rh-negative mother to Rh-positive red cells occurs as a result of asymptomatic fetomaternal hemorrhage during pregnancy. The Kleihauer-Betke acid elution technique that determines the proportion of fetal RBCs in maternal circulation has shown the incidence of fetomaternal hemorrhage to be 75% of all pregnancies. Incidence and degree of such hemorrhage appears to increase with gestation. Fetomaternal hemorrhage has been documented in 7%, 16%, and 29% of mothers during their first, second and third trimesters, respectively. Risk is also increased in pregnancies complicated by placental abruption, spontaneous or therapeutic abortion, and toxemia, as well as after cesarean delivery and ectopic pregnancy.

Procedures such as amniocentesis, chorionic villus sampling, and cordocentesis also increase the risk of alloimmunization. Because the transplacental hemorrhage is less than 0.1 mL in most pregnancies, most women are sensitized as a result of small, undetectable fetomaternal hemorrhage.

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After the initial exposure to a foreign antigen, B-lymphocyte clones that recognize the RBC antigen are established. The maternal immune system initially produces antibodies of the immunoglobulin M (IgM) isotype that do not cross the placenta and later produces antibodies of the IgG isotype that traverse the placental barrier. Predominant antibody subclass appears to be IgG1 in one third of individuals whereas a combination of IgG1 and IgG3 subclasses are found in the remaining individuals.

IgG3 is more efficient in binding to reticuloendothelial cells and causing hemolysis because of its longer hinge region. This is termed the primary response and is dose dependent (documented in 15% of pregnancies with 1 mL of Rh-positive cells in an Rh-negative individual compared with 70% of pregnancies after 250 mL). A repeat exposure to the same antigen rapidly induces the production of IgG. This secondary immune response can be induced with as little as 0.03 mL of Rh-positive RBCs.

The risk of Rh immunization after the delivery of the first child to a nulliparous Rh-negative mother is 16% if the Rh-positive fetus is ABO compatible with its mother, 2% if the fetus is ABO incompatible, and 2-5% after an abortion. The ABO-incompatible RBCs are rapidly destroyed in the maternal circulation, reducing the likelihood of exposure to the immune system. The degree of Rh sensitization of the mother is directly related to the amount of fetomaternal hemorrhage (ie, 3% with < 0.1 mL compared with 22% with >0.1 mL).

After sensitization, maternal anti-D antibodies cross the placenta into fetal circulation and attach to Rh antigen on fetal RBCs, which form rosettes on macrophages in the reticuloendothelial system, especially in the spleen. These antibody-coated RBCs are lysed by lysosomal enzymes released by macrophages and natural killer lymphocytes and are independent of the activation of the complement system.

Reticulocytosis is noted when fetal Hb deficit exceeds 2 gm/dl compared with gestational age norms. Tissue hypoxia develops as fetal anemia becomes severe. When the hemoglobin (Hb) level drops below 8 g/dL, a rise in umbilical arterial lactate occurs. When the Hb level drops below 4g/dL, increased venous lactate is noted. Hydrops fetalis occurs when fetal Hb deficit exceeds 7 g/dL and starts as fetal ascites and evolves into pleural effusions and generalized edema. The various mechanisms responsible for hydrops are hypoalbuminemia secondary to depressed liver function, increased capillary permeability, iron overload secondary to hemolysis, and increased venous pressures due to poor cardiac function [2].

Prolonged hemolysis leads to severe anemia, which stimulates fetal erythropoiesis in the liver, spleen, bone marrow, and extramedullary sites, such as the skin and placenta. In severe cases, this can lead to displacement and destruction of hepatic parenchyma by erythroid cells, resulting in dysfunction and hypoproteinemia. Destruction of RBCs releases heme that is converted to unconjugated bilirubin. Hyperbilirubinemia becomes apparent only in the delivered newborn because the placenta effectively metabolizes bilirubin. Hemolytic disease of the newborn due to Kell sensitization results in hemolysis and suppression of erythropoiesis because the Kell antigen is expressed on the surface of erythroid progenitors. This leads to severe fetal disease at a lower maternal antibody titer than in Rhesus disease.

Hemolysis associated with ABO incompatibility exclusively occurs in type-O mothers with fetuses who have type A or type B blood, although it has rarely been documented in type-A mothers with type-B infants with a high titer of anti-B IgG. In mothers with type A or type B, naturally occurring antibodies are of the IgM class and do not cross the placenta, whereas 1% of type-O mothers have a high titer of the antibodies of IgG class against both A and B. They cross the placenta and cause hemolysis in fetus.

Hemolysis due to anti-A is more common than hemolysis due to anti-B, and affected neonates usually have positive direct Coombs test results. However, hemolysis due to anti-B IgG can be severe and can lead to exchange transfusion. Because A and B antigens are widely expressed in various tissues besides RBCs, only a small portion of antibodies crossing the placenta are available to bind to fetal RBCs. Recent analysis of IgG subclass in ABO incompatible direct coombs positive neonates showed IgG2 was predominent antibody which is poorly transferred across placenta and less efficient in causing hemolysis while IgG1 was noted in 22% of neonates and as a group had similar rate of hemolysis and severity of hyperbilirubinemia [3].

In addition, fetal RBCs appear to have less surface expression of A or B antigen, resulting in few reactive sites; hence the low incidence of significant hemolysis in affected neonates. This results in hyperbilirubinemia as a predominant manifestation of incompatibility (rather than anemia), and peripheral blood film frequently reveals a large number of spherocytes and few erythroblasts, unlike what is seen in Rh incompatibility (erythroblastosis fetalis), in which blood film reveals a large number of nucleated RBCs and few spherocytes [4].

Epidemiology

Frequency: *United States*

Before the establishment of modern therapy, 1% of all pregnant women developed Rh alloimmunization. Since the advent of routine prophylaxis of at-risk women, incidence of Rh sensitization has declined from 45 cases

per 10,000 births to 10.2 cases per 10,000 total births, with less than 10% requiring intrauterine transfusion. Alloimmunization due to Kell antigen accounts for 10% of severely affected fetuses. The most recent data from review of 2001 birth certificates in the United States by the Centers for Disease Control and Prevention (CDC) indicates that Rh sensitization affects 6.7 newborns per 1000 live births [5].

Currently, anti-D is still one of the most common antibodies found in pregnant women, followed by anti-K, anti-c, and anti-E. Of those fetuses who require intrauterine transfusions, 85%, 10%, and 3.5% were due to anti-D, anti-K, and anti-c, respectively [6]. ABO incompatibility frequently occurs during the first pregnancy and is present in approximately 12% of pregnancies, with evidence of fetal sensitization in 3% of live births. Less than 1% of births are associated with significant hemolysis.

Mortality/Morbidity

Almost 50 different red cell surface antigens have been found to be responsible for hemolytic disease of fetus and newborn. Only 3 antibodies are associated with severe fetal disease: anti-RhD, anti-Rhc, and anti-Kell (K1). Nearly 50% of the affected newborns do not require treatment, have mild anemia and hyperbilirubinemia at birth, and survive and develop normally. Approximately 25% are born near term but become extremely jaundiced without treatment and either die (90%) or become severely affected by kernicterus (10%). The remaining 25% of affected newborns are severely affected in utero and become hydropic; about half of newborns are affected before 34 weeks' gestation, and the other half are affected between 34 weeks' gestation and term [7].

Exchange Transfusion

A study by Smits-Wintjens et al indicated that exchange transfusion in neonates increases the risk of sepsis, severe thrombocytopenia, leukocytopenia, hypernatremia, and hypocalcemia in neonates with hemolytic disease of the newborn (HDN) [8].

Race

Incompatibility involving Rh antigens (anti-D or anti-c) occurs in about 10% of all pregnancies among whites and blacks; in contrast, it is very rare in Asian women.

Sex

Fetal sex plays a significant role in the degree of response to maternal antibodies. An apparent 13-fold increase is observed in fetal hydrops in RhD-positive male fetuses compared with female fetuses in similarly sensitized pregnancies [9].

History

Two usual patterns of Rh isoimmunization severity are noted. The disease may remain at the same degree of severity or may become progressively worst with each pregnancy. A history of hydropic birth increases the risk of fetal hydrops in the next pregnancy to 90%; the fetal hydrops occurs at about the same time or earlier in gestation in the subsequent pregnancy. Women at risk for alloimmunization should undergo an indirect Coombs test and antibody titers at their first prenatal visit. If results are positive, obtain a paternal blood type and genotype with serologic testing for other Rh antigens (C, c, E, e).

Obtaining serial maternal titers is suggested if the father is homozygous. If the father is heterozygous, determine fetal Rh genotype using PCR for the *RHD* gene on fetal cells obtained at amniocentesis [10] or on cell-free DNA in maternal circulation [11]. The sensitivity and specificity of PCR typing on amniotic fluid is 98.7% and 100%, respectively. However, obtaining maternal blood to rule out a maternal *RHD* pseudogene (in a Rh-positive fetus) and obtaining paternal blood to rule out *RHD* gene locus rearrangement (in a Rh-negative fetus) is important to improve the accuracy. Determining fetal Rh genotype is also possible by performing cordocentesis, which is also called fetal blood sampling (FBS). FBS is associated with a more than 4-fold increase in perinatal loss compared with amniocentesis.

Indicators for severe hemolytic disease of the newborn (HDN) include mothers who have had previous children with hemolytic disease, rising maternal antibody titers, rising amniotic fluid bilirubin concentration, and ultrasonographic evidence of fetal hydrops (eg, ascites, edema, pleural and pericardial effusions, decreasing hemoglobin [Hb] levels).

Physical

An infant born to an alloimmunized mother shows clinical signs based on the severity of the disease. The typical diagnostic findings are jaundice, pallor, hepatosplenomegaly, and fetal hydrops in severe cases. The jaundice typically manifests at birth or in the first 24 hours after birth with rapidly rising unconjugated bilirubin level. Occasionally, conjugated hyperbilirubinemia is present because of placental or hepatic dysfunction in those infants with severe hemolytic disease. Anemia is most often due to destruction of antibody-coated RBCs by the reticuloendothelial system, and, in some infants, anemia is due to intravascular destruction. The suppression of erythropoiesis by intravascular transfusion (IVT) of adult Hb to an anemic fetus can also cause anemia. Extramedullary hematopoiesis can lead to hepatosplenomegaly, portal hypertension, and ascites.

Anemia is not the only cause of hydrops. Excessive hepatic extramedullary hematopoiesis causes portal and umbilical venous obstruction and diminished placental perfusion because of edema. Increased placental weight and edema of chorionic villi interfere with placental transport. Fetal hydrops results from fetal hypoxia, anemia, congestive cardiac failure, and hypoproteinemia secondary to hepatic dysfunction. Commonly, hydrops is not observed until the Hb level drops below approximately 4 g/dL (Hct < 15%) . Clinically significant jaundice occurs in as many as 20% of ABO-incompatible infants.

Causes

In the absence of a positive direct Coombs test result, other causes of pathologic jaundice svhould be considered, including intrauterine congenital infections; erythrocyte membrane defects (eg, hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis); RBC enzyme deficiencies (eg, glucose-6-phosphate dehydrogenase [G6PD] deficiency, pyruvate kinase deficiency, triosephosphate isomerase deficiency); and nonhemolytic causes (eg, enclosed hemorrhages, hypothyroidism, GI obstruction, and metabolic diseases).

Similarly, hydrops can occur from nonimmune hematologic disorders that cause anemia, such as hemoglobinopathies (eg, α-thalassemia major), cardiac failure due to dysrhythmia, congenital heart defects, and infections (eg, syphilis, cytomegalovirus [CMV], parvovirus).

- Common causes of hemolytic disease of the newborn
  - Rh system antibodies
  - ABO system antibodies
- Uncommon causes - Kell system antibodies
- Rare causes
  - Duffy system antibodies
  - MNS and s system antibodies
- No occurrence in hemolytic disease of the newborn
  - Lewis system antibodies
  - P system antibodies
- Differential Diagnoses
- Anemia, Acute
- Atrial Flutter
- Cardiac Tumors
- Cytomegalovirus Infection
- Galactose-1-Phosphate Uridyltransferase Deficiency (Galactosemia)
- Hydrops Fetalis
- Hypothyroidism
- Parvovirus B19 Infection
- Syphilis
- Toxoplasmosis
- Tyrosinemia

Laboratory Studies

The following findings may be noted in hemolytic disease of the newborn (HDN):

Hemolytic disease of the newborn is characterized by one or more of the following clinical presentations:

- Rapidly progressive severe hyperbilirubinemia or prolonged hyperbilirubinemia
- Positive maternal antenatal antibody findings and/or diagnosis of anemia or fetal hydrops
- Positive neonatal direct Coombs test (direct antiglobulin test)
- Hemolysis on blood film findings

The severity of hematologic abnormalities is directly proportional to the severity of hemolysis and the extent of hematopoiesis. The following abnormalities are observed on CBC count findings:

- Anemia: Measurements are more accurate using central venous or arterial samples rather than capillary blood.
- Increased nucleated RBCs, reticulocytosis, polychromasia, anisocytosis, spherocytes, and cell fragmentation
  - The reticulocyte count can be as high as 40% in patients without intrauterine intervention.
  - The nucleated RBC count is elevated and falsely elevates the leukocyte count, reflecting a state of erythropoiesis.
  - Spherocytes (< 40%) are more commonly observed in cases of ABO incompatibility. Glucose does not correct the autohemolysis in ABO incompatibility unlike hereditary spherocytosis.
  - In severe hemolytic disease, schistocytes and burr cells may be observed, reflecting ongoing disseminated intravascular coagulation.
  - A low reticulocyte count is observed in fetuses provided with intravascular transfusion in utero and with Kell alloimmunization.
  - Abnormally elevated mean cell hemoglobin concentration (MCHC) and red cell distribution width (RDW) values should prompt a diagnosis of hereditary spherocytosis.
- Neutropenia: This condition seems to be secondary to stimulation of erythropoiesis in favor of myelopoiesis. However, neutrophilia can be observed after intrauterine transfusion because of an increase in circulating cytokines (granulocyte-macrophage colony-stimulating factor).
- Thrombocytopenia: This condition is common, especially after intrauterine or exchange transfusions because of platelet-poor blood product and suppression of platelet production in favor of erythropoiesis.

Hypoglycemia is common and is due to islet cell hyperplasia and hyperinsulinism. The abnormality is thought to be secondary to release of metabolic byproducts such as glutathione from lysed RBCs.

Hypokalemia, hyperkalemia, and hypocalcemia are commonly observed during and after exchange transfusion.

Serologic test findings include the following:

- Indirect Coombs test and direct antibody test results are positive in the mother and affected newborn. Unlike Rh alloimmunization, direct antibody test results are positive in only 20-40% of infants with ABO incompatibility. In a recent study, positive direct antibody test findings have a positive predictive value of only 23% and a sensitivity of only 86% in predicting significant hemolysis and need for phototherapy, unless the findings are strongly positive (4+). This is because fetal RBCs have less surface expression of type-specific antigen compared with adult cells. A prospective study has shown that the titers of maternal immunoglobulin G (IgG) anti-A or anti-B may be more helpful in predicting severe hemolysis and hyperbilirubinemia. The sensitivity and specificity of IgG titers of 512 or higher in predicting need for invasive intervention was 90% and 73%, respectively.
- Although the indirect Coombs test result (neonate's serum with adult A or B RBCs) is more commonly positive in neonates with ABO incompatibility, it also has poor predictive value for hemolysis. This is because of the differences in binding of IgG subtypes to the Fc receptor of phagocytic cells and, in turn, in their ability to cause hemolysis.
- IgG2 is more commonly found in maternal serum but has weak lytic activity, which leads to the observation of little or no hemolysis with a positive direct antibody test result. On the other hand, significant hemolysis is associated with a negative direct antibody test result when IgG1 and IgG3 are predominant antibodies, which are in low concentration but have strong lytic activity, crossing to neonatal circulation.
- In newborns with hemolytic disease due to anti-c or anti-C antibodies, direct antibody test results may be negative, and the diagnosis is established after indirect Coombs testing.

Imaging Studies

High-resolution ultrasonography has been a major advance in detection of early hydrops and has also reduced the fetal trauma and morbidity rate to less than 2% during percutaneous umbilical blood sampling (PUBS) and placental trauma during amniocentesis. High-resolution ultrasonography has been extremely helpful in directing the needle with intraperitoneal transfusion (IPT) and intravascular transfusion (IVT) in fetal location.

Medical Care

Management of Maternal Alloimmunization

As a rule, serial maternal antibody titers are monitored until a critical titer of 1:32, which indicates that a high risk of fetal hydrops has been reached. At this point, the fetus requires very intense monitoring for signs of anemia and fetal hydrops. In Kell alloimmunization, hydrops can occur at low maternal titers because of suppressed erythropoiesis, and, thus, a titer of 1:8 has been suggested as critical. Maternal titers are not useful in predicting the onset of fetal anemia after the first affected gestation. Large differences in titer can be seen in the same patient between different laboratories, and a newer gel technique produces higher titer results than the older tube method. Therefore, standard tube methodology should be used to determine critical titer, and a change of more than 1 dilution represents a true increase in maternal antibody titer. For all the antibodies responsible for hemolytic disease of the newborn (HDN), a 4-fold increase in any antibody titer is typically considered a significant change that requires fetal evaluation.

When indicated, amniocentesis can be performed as early as 15 weeks' gestation (rarely needed in first affected pregnancy before 24 weeks' gestation) to determine fetal genotype and to assess the severity. Maternal and paternal blood samples should be sent to the reference laboratory with amniotic fluid sample to eliminate false-positive results (from maternal pseudogene or *Ccde* gene) and false-negative results (from a rearrangement at the *RHD* gene locus in the father).

Fetal Rh-genotype determination in maternal plasma has become routine in many European countries and is being offered in the United States. Fetal cell-free DNA accounts for 3% of total circulating maternal plasma DNA, is found as early as 38 days of gestation, and is derived from apoptosis of the placental cytotrophoblast layer. It is subjected to real-time PCR for the presence of *RHD* gene–specific sequences and has been found to be accurate in 99.5% of cases. The *SRY* gene (in the male fetus) and DNA polymorphisms in the general population (in the female fetus) are used as internal controls to confirm the fetal origin of the cell-free DNA.A panel of 92 SNPs is compared between maternal sample from buffy coat and plasma. A difference of more than 6 single nucleotide polymorphisms confirms presence of fetal DNA and the validity of the test in a female fetus.

Unfortunately, cell-free fetal DNA testing for determining the genotype for other red blood cell antigens such as E and Kell is not yet available in United States.

Serial amniocentesis is begun at 10-14 day intervals to monitor the severity of the disease in the fetus. All attempts should be made to avoid transplacental passage of needle which can lead to fetomaternal hemorrhage (FMH) and a further rise in antibody titer. Early ultrasonography is performed to establish correct gestational age. Frequent ultrasonographic monitoring is also performed to assess fetal well-being and to detect moderate anemia and early signs of hydrops.

During the period when intrauterine peritoneal transfusion was the only means of treatment, newborns were routinely delivered at 32 weeks' gestation. This approach resulted in a high incidence of hyaline membrane disease and exchange transfusions. With the advent of intravascular transfusion (IVT) in utero, the general approach to the severely affected fetus is to perform IVT as required until 35 weeks' gestation, with delivery planned at term. Establishment of lung maturity is difficult in these fetuses because of contamination of amniotic fluid with residual blood during transfusion; however, if delivery is planned prior to 34 weeks' gestation, maternal steroid administration to enhance fetal lung maturity is indicated.

Recently washed maternal RBCs have been successfully used as a source of antigen-negative RBCs in the event of rare incompatibility but also have been routinely used because of benefits such as decreased risk for sensitization to new red cell antigens, a longer circulating half-life being fresh and decreased risk of transmission of viral agents. Mother can donate a unit of red cells after the first trimester.

Extensive plasmapheresis with partial replacement using 5% albumin and intravenous immunoglobulin (IVIG) or the administration of IVIG at 1 g/kg body weight weekly has been shown to be moderately effective. The mechanism of action appears to be blockage of Fc receptors in the placenta, reducing antibody transport across to the fetus, Fc receptors on the phagocytes in the fetal reticuloendothelial system, and feedback inhibition of maternal antibody synthesis.

Similar regimens of tests and treatment are used in the management of pregnancies affected by nonRhD alloimmunization, such as anti-Rhc, anti-K (K1), and anti-M. Once the mother is diagnosed with an antibody associated with hemolytic disease, an indirect Coombs titer is performed, along with paternal testing for involved antigen and zygosity. Maternal titers are repeated (monthly until 28 weeks' gestation and then every 2 wk) until a threshold for fetal anemia is reached (1:8 for Kell and 1:32 for rest).

Management of the sensitized neonate

Mild hemolytic disease accounts for 50% of newborns with positive direct antibody test results. Most of these newborns are not anemic (cord hemoglobin [Hb] >14 g/dL) and have minimal hemolysis (cord bilirubin < 4 mg/dL). Apart from early phototherapy, they require no transfusions. However, these newborns are at risk of developing severe late anemia by 3-6 weeks of life. Therefore, monitoring their Hb levels after hospital discharge is important.

Moderate hemolytic disease accounts for approximately 25% of affected neonates. Moderate hemolytic disease of newborn is characterized by moderate anemia and increased cord bilirubin levels. These infants are not clinically jaundiced at birth but rapidly develop unconjugated hyperbilirubinemia in the first 24 hours of life. Peripheral smear shows numerous nucleated RBCs, decreased platelets, and, occasionally, a large number of immature granulocytes. These newborns often have hepatosplenomegaly and are at risk of developing bilirubin encephalopathy without adequate treatment. Early exchange transfusion with type-O Rh-negative fresh RBCs with intensive phototherapy is usually required. Use of IVIG in doses of 0.5-1 g/kg in a single or multiple dose regimen have been able to effectively reduce need for exchange transfusion [12].

Severe hemolytic disease accounts for the remaining 25% of the alloimmunized newborns who are either stillborn or hydropic at birth. The fetal hydrops is predominantly caused by a capillary leak syndrome due to tissue hypoxia, hypoalbuminemia secondary to hepatic dysfunction, and high-output cardiac failure from anemia. About half of these fetuses become hydropic before 34 weeks' gestation and need intensive monitoring and management of alloimmunized gestation as described earlier. Mild hydrops involving ascites reverses with IVTs in only 88% of cases with improved survival but severe hydrops causing scalp edema and severe ascites and pleural effusions reverse in 39% of cases and are associated with poor survival.

Management of ABO Incompatibility

Management of hyperbilirubinemia is a major concern in newborns with ABO incompatibility. The criteria for exchange transfusion and phototherapy are similar to those used in Rh alloimmunization. IVIG has also been very effective when administered early in the course. Tin (Sn) porphyrin a potent inhibitor of heme oxygenase, the enzyme that catalyzes the rate-limiting step in the production of bilirubin from heme, has been shown to reduce the production of bilirubin and reduce the need for exchange transfusion and the duration of phototherapy in neonates with ABO incompatibility.

Tin or zinc protoporphyrin or mesoporphyrins have been studied in newborns. They must be administered intramuscularly in a dose based on body weight, and their effectiveness appears to be dose related in all gestations.Their possible toxic effects include skin photosensitization, iron deficiency, and possible inhibition of carbon monoxide production. Their use in Rh hemolytic disease of newborn has not been reported. Their routine use cannot be recommended yet because of lack of long-term safety data.

Further Inpatient Care

The following may be indicated in patients with hemolytic disease of newborn (HDN):

- The stabilization of a hydropic newborn requires a high level of intensive coordinated management by a neonatal team well prepared for the possibly affected infant.
- In general, immediate intubation followed by draining of pleural effusions and ascites results in immediate improvement in respiratory gas exchange.
- A cautious correction of anemia with packed RBCs or by exchange transfusion is necessary to prevent circulatory overload.
- These neonates have normal blood volume but elevated central venous pressure.
- A close monitoring of metabolic status (eg, watching for hypoglycemia, hypocalcemia, hyperkalemia, acidosis, hyponatremia, renal failure) is absolutely essential to achieve a successful outcome.
- Despite of the first use of phototherapy by Cremer and associates more than 40 years ago, no standard method for delivering phototherapy is yet available.
- Exchange transfusion removes circulating bilirubin and antibody-coated RBCs, replacing them with RBCs compatible with maternal serum and providing albumin with new bilirubin binding sites. The process is time consuming and labor intensive but remains the ultimate treatment to prevent kernicterus. The process involves the placement of a catheter via the umbilical vein into the inferior vena cava and removal and replacement of 5- to 10-mL aliquots of blood sequentially, until about twice the volume of the neonate's circulating blood volume is reached (ie, double-volume exchange).
- This process removes approximately 70-90% of fetal RBCs. The amount of bilirubin removed directly varies with the pretransfusion bilirubin level and amount of blood exchanged. Because most of the bilirubin is in the extravascular space, only about 25% of the total bilirubin is removed by an exchange transfusion. A rapid rebound of serum bilirubin level is common after equilibration and frequently requires additional exchange transfusions.
- The indications for exchange transfusion are controversial, except for the fact that severe anemia and the presence of a rapidly worsening jaundice despite optimal phototherapy in the first 12 hours of life indicate the need for exchange transfusion. In addition, the presence of conditions that increase the risk of bilirubin encephalopathy lowers the threshold of safe bilirubin levels.

Exchange transfusion should be considered in newborns born at more than 38 weeks' gestation with a bilirubin-to-albumin ratio of 7.2 and in newborns born at 35-37 weeks' gestation with a bilirubin-to-albumin ratio of 6.8. Exchange transfusion is not free of risk, with the estimated morbidity rate at 5% and the mortality rate as high as 0.5%. Apnea, bradycardia, cyanosis, vasospasm, and hypothermia with metabolic abnormalities (eg, hypoglycemia, hypocalcemia) are the most common adverse effects.

- IVIG has been shown to reduce the need for exchange transfusion in hemolytic disease of the newborn due to Rh or ABO incompatibility. The number needed to treat to prevent one exchange transfusion was noted to be 2.7 and was estimated to be 10, if all the infants with strongly positive direct Coombs test were to receive the medication [13,14]. In addition, it also reduced the duration of hospital stay and phototherapy. Although it was very effective as a single dose, multiple doses were more effective in stopping the ongoing hemolysis and reducing the incidence of late anemia.
- Tin-mesoporphyrin in a dose of 4.5 mg/kg (6 µmole/kg) was used in an infant with persistent hemolysis due to Rh alloimmunization to prevent need for further phototherapy, without any adverse effects.

Prevention

Consider the following in patients with hemolytic disease of the newborn:

- Rh immune globin (RhIG) was licensed in 1968 in North America after several studies demonstrated its effectiveness in preventing Rh alloimmunization when administered to the mother within 72 hours of delivery. The current standard is to administer RhIG to all unsensitized Rh-negative women at 28 weeks' gestation with an additional dose administered soon after birth if the infant is Rh-positive, irrespective of the ABO status of the baby. RhIG is not indicated for mothers with weak or partial D status because most are not at risk for alloimmunization.
- The standard dose of RhIG is 300 mcg and is increased (300 mcg for every 25 mL of fetal blood in maternal circulation) based on the amount of fetomaternal hemorrhage, which can be quantified using the Kleihauer-Betke technique. Because only 50% of pregnancies with excess fetomaternal hemorrhage can be identified by clinical risk factors, routine screen for excess fetomaternal hemorrhage (FMH) is undertaken in all Rh negative women. However, if the incidence of excess FMH is 0.6%, the maximum risk of sensitization is 0.1%, suggesting routine assessment for excess FMH may not be justified.
- Also administer RhIG to unsensitized Rh-negative women after any event known to be associated with transplacental hemorrhage such as spontaneous or elective abortion, ectopic pregnancy, amniocentesis, chorionic villous sampling, fetal blood sampling (FBS), hydatiform mole, fetal death in late gestation, blunt abdominal trauma, and external cephalic version. The indications for first trimester threatened abortion and ectopic pregnancy with no cardiac activity are not cost effective and are left to the clinician.

- No more than 5 units of RhIG should be given by intramuscular route in 24-hour period. An intravenous preparation is now available for administration of large doses. If RhIG was inadvertently omitted after delivery, the protection can still be offered if given within first 4 weeks. A repeat dose is not needed if delivery occurs within 3 weeks after administration of RhIG during antenatal period. The current incidence of Rh immunization stands at 0.1% with the above recommendations.
- Most RhIG is derived from human plasma obtained from sensitized women or male donors sensitized with RhD positive cells. Because it is a blood product, it has risks of transmission of viral infections such as hepatitis C and may not be acceptable in some religious denomination. Hence 2 monoclonal anti-D antibodies derived from recombinant technology, BRAD-1 and BRAD-3, are being tested in clinical trials. A new novel polyclonal recombinant antibody, rozrolimupab has also been tested in phase I and II clinical trials with no adverse effects.

Complications

The 2 major complications of hemolytic disease of the newborn are bilirubin encephalopathy (kernicterus) and late anaemia of infancy.

- Bilirubin encephalopathy

○ Before the advent of exchange transfusion, kernicterus affected 15% of infants born with erythroblastosis. Approximately 75% of these neonates died within 1 week of life, and a small remainder died during the first year of life. Survivors had permanent neurologic sequelae and were thought to have accounted for 10% of all patients with cerebral palsy (CP).

○ The mechanism by which unconjugated bilirubin enters the brain and damages it is unclear. Bilirubin enters the brain as lipophilic free bilirubin unbound to albumin, as supersaturated bilirubin acid that precipitates on lipid membrane in low pH, or as a bilirubin-albumin complex that transfers bilirubin to tissue by direct contact with cellular surface. The blood-brain barrier is comprised of ATP-dependent transport proteins and pumps free bilirubin from the brain back into plasma and maintains the concentration gradient of unconjugated bilirubin. A damaged blood-brain barrier enhances the entry and fails to remove all forms of bilirubin into the brain, which is especially important in preterm neonates with respiratory acidosis and vascular injury.

○ Bilirubin has been postulated to cause neurotoxicity via 4 distinct mechanisms [15]. (1) interruption of normal neurotransmission (inhibits phosphorylation of enzymes critical in release of neurotransmitters), (2) mitochondrial dysfunction, (3) cellular and intracellular membrane impairment (bilirubin acid affects membrane ion channels and precipitates on phospholipid membranes of mitochondria), and (4) interference with enzyme activity (binds to specific bilirubin receptor sites on enzymes).

○ The pathologic findings include characteristic staining and neuronal necrosis in basal ganglia , hippocampal cortex , brainstem nuclei , and cerebellum . The cerebral cortex is generally spared. About half of these neonates also have extraneuronal lesions, such as necrosis of renal tubular, intestinal mucosal, and pancreatic cells.

○ Clinical signs of bilirubin encephalopathy typically evolve in 3 phases. Phase 1 is marked by poor suck, hypotonia, and depressed sensorium. Fever and hypertonia are observed in phase 2, and, at times, the condition progresses to opisthotonus. Phase 3 is characterized by high-pitched cry, hearing and visual abnormalities, poor feeding, and athetosis.

○ Long-term sequelae include choreoathetoid CP, upward gaze palsy, sensorineural hearing loss, dental enamel hypoplasia of the deciduous teeth, and, less often, mental retardation. The abnormal or reduced auditory brainstem response of wave I (auditory nerve) and wave II and V (auditory brainstem nuclei), depicted as decreased amplitudes, and increased interval I-III and I-V characterize phase I of early, but reversible, encephalopathy. Subtle bilirubin encephalopathy that consists of either cognitive dysfunction, isolated hearing loss, or movement disorder has been described in absence of kernicterus and better correlates with free bilirubin levels.

○ Currently, the mortality rate stands at 50% in term newborns, but mortality is nearly universal in the preterm population who may simply appear ill without signs specific for kernicterus. Research has indicated that bilirubin production rates may be the critical piece of information identifying jaundiced infants at risk of neurotoxicity. A high bilirubin production rate is thought to result in rapid transfer of bilirubin to tissue, causing high tissue load, in which case any small further increase has great potential to enter the brain. Because the total serum bilirubin represents not only bilirubin production but also distribution and elimination, it is not an absolute indicator of risk of kernicterus. Techniques have been developed to measure the bilirubin production ratesaccurately and noninvasively using end-tidal carbon monoxide measurement and percutaneous measurement of carboxyhemoglobin.

Late anemia of infancy

○ Infants with significant hemolytic disease often develop anemia in the first month of life and frequently (50%) require packed RBC transfusion. The anemia appears to be due to several factors including suppression of fetal erythropoiesis from transfusion of adult Hb during intrauterine or exchange transfusion, resulting in low erythropoietin levels and reticulocyte count.

o Continued destruction of neonatal RBCs by high titers of circulating maternal antibodies also contributes the development of anemia. Weekly Hcts and reticulocyte count need to be monitored after discharge until renewed erythropoiesis is noted. Administration of recombinant. Human erythropoietin (rh-EPO) has been shown to minimize the need for transfusion in these newborns.

Potential complications of exchange transfusion include the following

- Cardiac - Arrhythmia, volume overload, congestive failure, and arrest
- Hematologic – Over heparinization, neutropenia, thrombocytopenia, and graft versus host disease
- Infectious - Bacterial, viral (cytomegalovirus [CMV], human immunodeficiency virus [HIV], hepatitis), and malarial
- Metabolic - Acidosis, hypocalcemia, hypoglycemia, hyperkalemia, and hypernatremia
- Vascular - Embolization, thrombosis, necrotizing enterocolitis, and perforation of umbilical vessel
- Systemic – Hypothermia

CONCLUSION

Haemolytic Disease of the New Born(HDN) is caused by alloimmune antibodies from the mother to the foetus if there is incompatibility in the blood group of the foetus inherited from the father and that of the mother.This can be prevented by early premarital counselling and proper antenatal care and management.The titre of the antibodies should be monitored.

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