Rare Antibody Causing Hemolytic Disease of the Fetus and Newborn

Course # DL-006

by

Anya Atenousazar, CLS, BS

Helen Sowers, MA, CLS
Dept. of Biological Science (Retired)
California State University East Bay

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39656 Mowry Ave.              Phone: 510-792-4441
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COURSE # DL-006

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RARE ANTIBODY CAUSING HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN

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OBJECTIVES:
At the end of this course the participant will be able to:
1. Describe the conditions that lead to hemolytic disease of the fetus and newborn (HDFN)
2. Outline the Fisher-Race nomenclature for the Rh system
3. Explain the occurrence of the rare antibody causing HDFN in the case study
4. Describe the treatment used to treat the fetus in the case study
5. State the problems involved in obtaining blood for transfusing the fetus/infant
6. Discuss the consequences in the infant of abnormalities in chemistry results

CASE STUDY
A 22- year-old Hispanic female, gravida 4, para 1-0-2-1* was admitted to Ridgecrest Hospital, Ridgecrest, CA. She had no significant pain, but the tocometer showed that she had contractions. The physician performed a transvaginal ultrasound in order to determine the accurate pregnancy dating. The ultrasound showed that the patient was at 31 weeks and 5 days gestation. Additionally, ultrasound revealed fetal hydrops and anemia characteristic of hemolytic disease of fetus and newborn (HDFN). The doctor ordered some additional prenatal tests to be done on the mother. The test results showed that the HDFN was due to anti–Rh 17 antibody that was being produced by the mother. As a result, intravenous immunoglobulin was given to the mother to inactivate maternal antibodies, preventing them from further crossing the placenta and hemolyzing fetal red blood cells. In addition, the patient received Terbutaline and Procardia in order to prevent preterm delivery. The patient’s history indicated that the two previous miscarriages were also due to the same condition. Of further interest was her sister’s having had a miscarriage due to HDFN caused by the same rare antibody. It was decided that the mother be transferred from Ridgecrest Hospital to the Loma Linda University Medical Center (LLUMC) for more advanced care.

* pregnant 4 times, 1 term birth, 0 pre-term births, 2 miscarriages, 1 living child

DISCUSSION OF Rh BLOOD GROUP SYSTEM
Before 1970 Hemolytic Disease of the Fetus and Newborn was a major cause of death and disability in Caucasian fetuses and newborn infants.

The condition occurs when:
- an infant has a RBC antigen, inherited from the father, that the mother lacks.
- she produces IgG antibodies to this antigen.
• these antibodies in the mother’s blood cross the placenta, attach to, and cause destruction of red blood cells in the fetus.
• the infant develops anemia *in utero* that may lead to hydrops fetalis (severe edema).
• after birth the infant may develop jaundice due to accumulation of bilirubin from continued hemolysis of RBCs and inability of the baby’s liver to conjugate the bilirubin.

In the past, the most common cause of HDFN was Rh (D) incompatibility. About 15% of Caucasians are Rh negative, so the possibility of the incompatibility was significant. It wasn’t until this blood group antigen was identified in 1940 - and then associated with HDFN in 1941 - that the pathogenesis was described. By the late1960s this led to the use of therapeutic antibodies to prevent sensitization of the mother by the infant’s RBCs. Now all Rh-negative mothers at risk of becoming sensitized are given anti-Rh (D) antibodies during pregnancy and at the time of delivery. These anti-D antibodies attach and cause destruction of any of the infant’s RBC that enter the mother’s blood. The most probable time of an Rh negative mother’s becoming sensitized is at the time of delivery of the first Rh positive infant, since there is greater chance of a fetal to maternal bleed at this time.

Other antigens in the Rh system are capable of sensitizing the mother and causing HDFN. They are, in descending order of importance, c, e, C, E. The genotype D-- (Rh 17), possessed by the patient, is very rare. This genotype lacks c, e, C, and E genes.

At present the most common cause of HDFN is ABO incompatibility, with Rh (D) next. Other blood groups are less frequent causes of HDFN.

Laboratory testing plays a crucial role in the prophylaxis of HDFN as well as diagnosis of the disease and identification of the antibody causing the problem.

**RH SYSTEM NOMENCLATURE**

Below is a review of the Fisher/Race and Weiner nomenclature of the most common antigens and phenotypes:

<table>
<thead>
<tr>
<th>Fisher-Race</th>
<th>Weiner</th>
<th>Rh designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCe</td>
<td>R1</td>
<td>Rh positive</td>
</tr>
<tr>
<td>DeE</td>
<td>R2</td>
<td>Rh positive</td>
</tr>
<tr>
<td>(d)ce</td>
<td>r</td>
<td>Rh negative</td>
</tr>
<tr>
<td>Dce</td>
<td>Ro</td>
<td>Rh positive</td>
</tr>
<tr>
<td>D--</td>
<td>R17</td>
<td>Rh positive</td>
</tr>
</tbody>
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Since anti–Rh antibodies having been the most common causes of HDFN, the diagnosis and the management plan is well established. (4) Anti Rh-17 is a rare alloantibody that can cause moderate to severe HDFN. Individuals who lack C/c and E/e antigens on the red blood cells produce this rare type of Rh antibody after antigenic stimulus. The rare phenotype of such individuals is designated as -D-/D- or D--/D-- and was first discovered by Race and Sanger in 1950. The antibody production occurs only after transfusion or pregnancy cause immune stimulus by one or more of the C/c E/c antigens.

**ANTI-RH 17 AND ITS COMPLICATIONS DURING PREGNANCY**

Individuals who are lacking most or all of the significant Rh antigens normally will not produce Rh antibodies. However, during pregnancies, usually at the time of delivery, mothers
may be antigenically stimulated to develop Rh antibodies against their fetus’s normal Rh antigens that exist on the red blood cells (RBC). In this case, because the fetus inherits some of the C/c/E/e antigens from the father, the fetal cells cause the mother to develop alloantibodies to the antigens that she lacks. In general, the first pregnancy is not affected in terms of complications due to antibody involvement. It is usually only at delivery that enough fetal cells can enter the maternal circulation to stimulate the mother’s immune system to develop antibodies. The alloantibodies that the mother produces are of the IgG class, which, unlike IgM, are capable of crossing the placenta and causing complications in the subsequent pregnancies. When IgG antibodies cross the placenta, they attach to the corresponding antigen on the RBC in the fetus, and eventually will cause hemolysis. Rh–HDFN is more severe than ABO-HDFN because Rh antigens are fully developed at birth and also because Rh antigens are found only on RBC. Thus there are more complications such as fetal anemia and fetal hydrops due to Rh isoimmunization of the fetus.

**DIAGNOSIS**

At the time of admission, various tests were done on the mother as well as on her fetus. In terms of blood banking, the ABO and Rh blood groups as well as an antibody screen were performed on the mother. The mother’s blood group was determined as Group A, Rh positive. A positive antibody screen showed that the mother was producing antibodies. The mother’s Rh phenotype was reported as D--/D-- at LLUMC, and it was confirmed by a reference laboratory. This rare phenotype of the mother was definitely indicative of Rh incompatibility between mother and fetus. That led the physicians to confirm the diagnosis that the mother had anti–Rh 17 antibody.

Based on the mother’s present situation and her history of two previous miscarriages, the doctors were aware that the fetus needed serious medical attention. As a first step, a complete blood count (CBC) was ordered to be done on a cord blood sample that was collected through a special procedure called percutaneous umbilical cord sampling (PUBS). CBC results revealed a very low hemoglobin level, which was indicative of fetal anemia due to maternal antibodies. Considering the severity of the fetal anemia, the doctor suggested intrauterine transfusion (IUT) to treat the anemia. However, the main problem the blood bank faced was finding a compatible blood to use for the intrauterine transfusion. Generally, if the fetus’s blood type is unknown, Group O, Rh negative blood is used for intrauterine transfusion purposes. However, for this fetus, in order for a Group O Rh negative blood to be compatible, it has to lack all antigens that cause the mother to produce antibodies. Otherwise, giving an O negative unit that is positive for any of the C/c/E/e antigens will cause extravascular hemolysis in the fetus. In fact, the chance of finding a compatible blood is extremely rare (<1%) due to the atypical maternal antibodies. Ideally, in rare situations where finding a compatible blood is almost impossible, the mother’s blood would be given since it is known to be negative for the C/c/E/e Rh antigens. In this case, however, the mother was not recommended as a possible donor because her hemoglobin level was too low. The fetus was fortunate since the mother’s sister had the same phenotype. She was used as a direct donor. The doctors decided to use the mother’s sister’s blood even though there was a chance of ABO incompatibility between donor and recipient (anti-A and anti-B are not yet developed in the newborn). Intrauterine transfusions were done twice and the hemoglobin and hematocrit levels were improved each time.
In addition, the infant was transfused three times (both packed red blood cells and plasma) right after she was born. These transfusions had multiple purposes: to treat the severe anemia, and as an exchange transfusion to

1. replace the infant’s circulating RBCs that were coated with maternal antibodies. These coated cells are removed from the infant’s circulation by the infant’s reticuloendothelial system (RE system) and broken down.

2. lower bilirubin levels. Indirect bilirubin is the by-product of hemoglobin catabolism, which can put the baby at risk of kernicterus if it is left untreated after birth. Kernicterus, bilirubin deposits in the basal ganglia and other areas of the brain, is a potentially fatal condition that leaves permanent neurological damage in the infants that survive. The use of exchange transfusions on infants with hyperbilirubinemia reduces the chances of neurological damage. The level of bilirubin that produces kernicterus is approximately 20 mg/dl, so it is the accepted practice to perform these transfusions to reduce the level of bilirubin when it approaches this level. (In utero excess bilirubin produced by hemolysis of RBCs is taken up by the placenta, transferred to the mother’s circulatory system, and degraded by the mother’s liver.) After birth the neonate’s immature liver is unable to metabolize the increased amount of bilirubin and it accumulates in the blood. Two exchange transfusions were performed on the infant to remove sensitized RBCs along with accumulated bilirubin. In addition, platelet products were transfused to the baby in order to treat thrombocytopenia, which had occurred secondary to alloimmunization.

A complete blood count (CBC) was done on the baby on a regular basis before and after her birth. Percutaneous umbilical blood sampling (PUBS) before birth and regular venous draws after birth were used for hematology testing. These confirmed the fetus’s severe anemia secondary to HDFN. As a consequence of the severe anemia, the fetus’s bone marrow, as well as the liver and spleen, tried to repair the loss of RBCs by overproduction and premature release of immature red cells precursors - nucleated red blood cells (nRBCs) - into the peripheral blood, thus the hematology test results showed an increased number of nRBCs and reticulocytes. In fact, in this baby the reticulocyte count was increased to 23% (reference range: 3-6%) a few days after the birth, which was indicative of severe blood loss and also extramedially hematopoiesis. Generally, existence of nucleated red blood cells along with markedly elevated polychromasia and anisopoikilocytosis on a fetus’s blood smear is characteristic of HDFN. The baby’s urine analysis results showed a positive ictotest, which was consistent with the hemolytic disease.

Chemistry tests including bilirubin level (total and direct bilirubin), liver function panel, and blood urea nitrogen (BUN) levels were routinely done to evaluate the patient’s condition in terms of severity of hemolysis. Due to patient’s HDFN a high level of bilirubin is expected. Additionally, hemolytic anemia and cholestasis were the cause of liver problems, which resulted in high levels of indirect bilirubin. Elevation in the hepatic enzyme level was indicative of patient’s liver damage, which resulted in decreased production of serum proteins. The liver damage was caused by stressors of bilirubin conjugation demand, ascites, and immaturity of the organ. The patient’s BUN was raised as the kidneys were trying to clear the high amount of by-products of protein synthesis. Additionally, ascites and insufficient surfactant both contributed to respiratory distress, leading to a buildup of CO2 and respiratory acidosis. Hemolysis also leads to the occurrence of electrolyte imbalances in patients. Increased potassium and ionized calcium levels occur due to the acidic state and low protein level. Serum potassium elevation can be explained by hemolysis in which potassium leaks out of the RBCs during their destruction.
Decreased protein level will cause more calcium to be replaced by hydrogen ions, which will lead to increased ionized (free) calcium in the system.

In terms of microbiology, a prenatal workup including TORCH (Toxoplasmosis, other, rubella, CMV, HSV1 &2), group B streptococcus (GBS), rectal/vaginal culture, and MRSA (methicillin resistant Staphylococcus aureus) was done on the mother. All test results except for urine culture were negative. Urine culture results indicated that the mother is positive for non-hemolytic staphylococcus, which ultimately was treated with vancomycin and cefatoxime after a week.

**TREATMENT**

At the time of admission, the doctor was primarily focused on prolonging the pregnancy, thus doses of Terbutaline and Procardia were given to the mother in an attempt to stop the contractions. Immediately after the reference laboratory confirmed anti-Rh 17, a series of treatments were started for the fetus. Initially, intravenous immunoglobin was given to prevent maternal antibodies from further hemolyzing fetal red blood cells. However, due to the severity of the fetal anemia, the doctor realized the necessity of intrauterine transfusions (IUT) for the patient. As a result, two IUTs were scheduled and performed, which led to improvement of hemoglobin and hematocrit each time. Phototherapy was done immediately after birth as a treatment for hyperbilirubinemia; however it was discontinued after 12 hours due to the severity of HDFN. Instead, an exchange transfusion was performed on the patient in order to wash away sensitized red blood cells from the baby’s system. In addition, the patient was transfused three times with packed red blood cells and plasma. Furthermore, multiple platelet products were transfused to the baby in order to raise the platelet count caused by thrombocytopenia secondary to alloimmunization. Total parenteral nutrition (TPN) was given to the baby for 41 days in order to treat cholestasis. Additionally, InfaSurf and supplemental oxygen were given to the baby to treat respiratory acidosis.

The patient was discharged on 7/31/12 and at that time only passive anti-e was present and the patient was stable, even though the hemoglobin level was on a decreasing trend. Based on the follow-up information collected in August from the mother, the jaundice seemed to be improving, the baby had a good appetite, and other findings were unremarkable. Subsequent examination data showed that the baby had poor growth and weight gain. She was still jaundiced and had hepatosplenomegaly. Additional tests, an ultrasound for hepatosplenomegaly and a liver panel, were ordered. The physicians continued treating the cholestasis and recommended increasing calorie intake. Overall, there are some lingering problems from the HDFN and hydrops but the baby is stable and no new problems are arising.

**CONCLUSION**

A twenty-two year old female was admitted to the hospital in early May 2012 due to a high concern for her pregnancy. The infant was delivered on 5/17/12 and suffered from a variety of conditions due to the hemolytic disease of the newborn. The antibody causing this HDFN was identified as anti-Rh 17, which is produced by individuals who lack C/c and E/e antigens on RBCs. Of interest is that her sister recently experienced a fetal demise while pregnant due to the same antibody.

Hemolysis can cause a variety of problems including: hydrops fetalis, anemia, liver, and respiratory problems. In the case of the pregnant woman, the most important thing to do was to first identify the offending antibody. The patient’s history was extremely significant because this
antibody was previously identified in this patient by reference laboratories, so less time was spent on trying to identify the antibody. Knowing the antibody greatly helped with the selection of blood so clinical laboratory scientists (CLS) in the blood bank could ensure that the least incompatible blood was given. As a result the mother’s sister’s blood (which was Cc, Ee negative) was selected for IUT. Multiple transfusions were given to the baby to address the hemolysis and anemia. Hematology results were used to follow the patient’s status and the results correlated with the condition. Chemistry was also used to follow the patient’s status and the most important finding was hyperbilirubinemia, primarily a result of cholestasis. A number of factors that may contribute to cholestasis are hemolysis due to Rh antibodies, intrauterine transfusion, and increased demand on the liver. Additionally ascites and insufficient surfactant both contribute to respiratory distress, leading to a build-up of CO₂ and respiratory acidosis. Electrolyte imbalance occurred due to hemolysis, which increased the potassium level, and increased ionized calcium, which was due to the acidotic state and low protein levels. After several months the infant had some lingering problems from the HDFN and hydrops but was stable and no new problems developed.

REFERENCES
REVIEW QUESTIONS
Course #DL-006
Choose the one best answer

1. Individuals with which phenotype may develop anti-Rh17 antibody?
   a. those who lack E/e
   b. those who lack D
   c. those who lack C/c
   d. both a and c are correct

2. Historically the most common cause of HDFN was
   a. ABO incompatibility
   b. anti-c
   c. anti-d
   d. anti-D

3. Hyperbilirubinemia associated with HDFN is not usually found in the fetus because
   a. the fetal liver breaks it down
   b. bilirubin is not produced in the fetus
   c. it is removed by the mother’s system
   d. it is removed by ultraviolet radiation

4. Gravida 3, para 2-0-1-2 indicates the woman has been pregnant
   a. 3 times, no pre-term births, one miscarriage, 2 live births
   b. 5 times, 3 term births, 2 miscarriages, 1 living child
   c. 3 times, 1 pre-term birth, 0 miscarriages, 2 living children
   d. 3 times, 2 pre-term births, two miscarriages, 1 living child

5. Which of the following conditions does not cause HDFN?
   a. the father possesses an antigen the mother lacks
   b. the mother produces anti-IgM antibodies
   c. the antibodies produced by the mother cross the placenta
   d. the antibodies attach to the infant's RBC and cause destruction of the RBCs

6. All but which of the following is the ultimate reason for exchange transfusion?
   a. increase the number of leukocytes
   b. remove the sensitized red blood cells from the baby's system
   c. reduce the effects of hyperbilirubinemia
   d. replace the patient's blood with healthy donor cells

7. What are the consequences of overproduction of RBCs by a fetus's liver and spleen?
   a. increased white blood cells
   b. increased nRBCs and reticulocytes
   c. increased platelet count
   d. decreased hematocrit
8. Cholestasis in the infant was due to
   a. insufficient surfactant
   b. increased potassium
   c. increase in indirect bilirubin
   d. increased BUN

9. Intravenous immunoglobulin was given the mother in order to
   a. prevent sensitization of the mother to anti-c, e, C, and E
   b. combine with the antibodies in the mother’s blood and inactivate them
   c. cross the placenta
   d. protect the mother from infection

10. What is the consequence of ascites and insufficient surfactant in the newborn?
    a. respiratory alkalosis
    b. metabolic acidosis
    c. metabolic alkalosis
    d. respiratory acidosis