A case report on non-D HDFN: Highlighting the role of antibody screening in RhD positive antenatal women

Bharat Singh, Rajendra Chaudhary, Preeti Elhence, Jyoti Kala Bharati, Anubha Srivastava

Abstract:
The widespread use of anti-D immunoglobulin has resulted in a relative increase in the importance of non-D alloimmunization as a cause of hemolytic disease of the fetus and newborn (HDFN). Non-D alloantibodies that are capable of causing severe HDFN include anti-K, anti-E, and anti-c. Anti-c is clinically the most important Rh system antibody after anti-D. Here, we report three cases of neonates presenting with anemia and hyperbilirubinemia with strongly positive direct antiglobulin test who required phototherapy and neonatal exchange transfusion due to non-D antibody in RhD positive antenatal women. Anti-c was common in all the three cases while two cases have one additional non-D antibody. Due to faulty practices, antenatal antibody screening was not done for any case considering the mother’s RhD positive status. Hence, antenatal antibody screening should be performed routinely, in all RhD positive pregnant women to reduce the delay in diagnosis and the management of HDFN occurring due to non-D antibodies.

Keywords:
Anti-c antibody, exchange transfusion, hemolytic disease of the fetus and newborn, maternal alloimmunization, phototherapy

Introduction

Hemolytic disease of the fetus and newborn (HDFN) is characterized by the development of maternal immunoglobulin G (IgG) antibodies directed against a paternally derived fetal red cell antigen. This antigen-antibody interaction causes fetal hemolysis by crossing the placenta and sensitizing red cells for destruction by the fetal reticuloendothelial system. Serious fetal anemia usually occurs in pregnancies complicated by anti-D, anti-c, and anti-K antibodies.[1] Of these, alloimmunization against RhD antigen remains the most common and most severe form of HDFN.[2] Clinically, relevant non-D alloantibodies occur in 1:300 pregnancies, primarily of the specificities anti-E, -K, and -c, while the risk of HDFN caused by these antibodies is 1:500.[3] Disease spectrum for non-D alloimmunization depends primarily on antibody specificity and its titer. Properly formulated protocols to screen both RhD positive and negative antenatal women for red cell alloantibodies need to be imposed to prevent perinatal mortality and morbidity due to HDFN.[4] We report three cases with severe hyperbilirubinemia and persistent anemia due to maternal alloimmunization against non-D antigens in RhD positive women. All the three newborns required exchange transfusion and intensive phototherapy (PT).
Case Report (Study of the Three Cases)

The study group consisted of three neonates who were admitted to the neonatology unit of our hospital from 2016 to 2020. Two of them were referred from other hospitals and were in early neonatal age (4th and 6th day of life) while one case got admitted directly to our hospital in late neonatal age (10th day of life). The clinical details of patients are elaborated in Table 1. Indirect hyperbilirubinemia along with anemia was the most common presentation. All the mothers were multigravida with no history of blood transfusion. Routine antenatal antibody screening was not performed for any case. Antenatal ultrasound (USG) had shown fetal growth restriction (FGR) in case number 2 and 3 while scan was completely normal in case number 1.

Blood samples for blood grouping and direct antiglobulin test (DAT) were sent to the immunohematology laboratory. As the serum bilirubin level crossed the threshold value in all the cases, a decision of double volume exchange transfusion (DVET) was taken, and PT was started. Laboratory investigations demonstrated moderate-to-severe anemia, reticulocytosis, peripheral smear suggestive of hemolysis, and a strongly positive DAT. Due to the absence of apparent cause of the immune hemolysis such as RhD or ABO incompatibilities and DAT being positive, further immunohematological investigations for minor blood groups incompatibility were done as given in Table 2. Immunohematological work-up revealed one or more irregular red cell antibodies in both maternal serum and neonatal red cell eluate. Anti-c was the common antibody in all the three cases. In addition, case 2 and case 3 showed anti-E and anti-Jka activity, respectively. Minor blood group phenotyping was done to establish the presence or absence of the relevant antigens in the neonates and their parents. On phenotyping, the fathers and the neonates were found to be positive while the mothers were negative for the corresponding antigens in all three cases. Glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase deficiency were excluded in all the neonates. This confirmed that an immune type of hemolytic disease of newborn caused by maternal alloimmunization against paternally derived minor red cell antigens was the etiology for the hemolysis.

All the three neonates responded well to DVET using specific antigen-negative red cell units. DVET was required twice in case 2 while only once in the rest two cases. Post-DVET, PT was continued; and once the bilirubin was beneath the threshold, PT was stopped. Jaundice reduced gradually in all the three neonates. The patients were discharged from the hospital after 6–11 days of treatment. All the three neonates are in regular follow-up in the neonatology outpatient department.

Table 1: Obstetric history and clinical details of three cases

| Case 1 | Case 2 | Case 3 |
|--------|--------|--------|
| Antenatal clinical history | | |
| Obstetric history | G2L1A0 | G2L1A0 | G3L2A0 |
| History of neonatal jaundice in previous pregnancy | No | No | No |
| History of blood transfusion | No | No | No |
| Antenatal antibody screen | No | No | No |
| Length of gestation (weeks) | 39 | 37 | 39 |
| Birth weight (kg) | 2.8 | 1.9 | 2.2 |
| Antenatal USG findings | Normal scan | Signs of FGR | Signs of FGR |
| Neonatal clinical details | | |
| DOL at presentation (days) | 6 | 4 | 10 |
| TSB/ISB on admission (mg/dl) | 27.5/21.4 | 33.7/27.2 | 30.4/25.7 |
| Hematocrit on admission | 27.3 | 18.3 | 25.1 |
| Hepatosplenomegaly | No | Yes | Yes |
| Intervention | | |
| PT | Yes | Yes | Yes |
| DVET (number) | Yes (1) | Yes (2) | (1) |
| Top-up transfusion (PRBC) | No | Yes | No |
| LOS at hospital (days) | 6 | 11 | 9 |
| TSB (mg/dl) at discharge | 6.2 | 5.8 | 7.5 |
| Hematocrit at discharge | 35.8 | 39.1 | 38.5 |

| Table 2: Immunohematological investigations |
|-------------------------------------------|
| Maternal sample | Case 1 | Case 2 | Case 3 |
| ABO and Rh | A RhD positive | O RhD positive | AB RhD positive |
| Antibodies in serum | Anti-c | Anti-c and Anti-E | Anti-c and Anti-Jk |
| Titer | Anti-c-32 | Anti-c-64 | Anti-c-16 |
| Minor red cell phenotype | “C” | “C” and “E” | “C” and “JK” |
| Patalernal sample | | |
| ABO and Rh | O RhD positive | B RhD positive | B RhD positive |
| Minor red cell phenotype | “C” | “C” and “E” | “C” and “JK” |
| Neonate sample | | |
| ABO and Rh | A RhD positive | O RhD positive | B RhD positive |
| DAT | 3+ (IgG) | 4+ (IgG + C3d) | 3+ (IgG) |
| Antibody in sera and eluate | Anti-c | Anti-c and Anti-E | Anti-c and Anti-Jk |
| Minor red cell phenotype | “C” | “C” and “E” | “C” and “JK” |

DOL=Day of life, FGR=Fetal growth restriction, TSB=Total serum bilirubin, ISB=Indirect serum bilirubin, DVET=Double volume exchange transfusion, LOS=Length of stay, PT=Phototherapy, USG=Ultrasound, PRBC=Packed red blood cells

DAT=Direct antiglobulin test
the last follow-up visit, normal growth and development, with no signs and symptoms of anemia, was seen in all the three cases.

Discussion

Although the Directorate General of Health Services, India, recommends antibody screening for both RhD positive and RhD negative antenatal women, it is being done primarily for RhD negative women or those presenting with bad obstetric history. The unavailability of advanced immunohematological services at many centers in India further complicates the situation. Due to all these reasons, many clinically significant red cell antibodies are missed in RhD positive antenatal women along with failure to provide antigen-negative unit to the fetus or newborn. This leads to delay in transfusion of compatible blood unit to the neonate presenting with hyperbilirubinemia and anemia. In this case series also, as all the mothers were RhD positive, no routine antenatal antibody screening was done for any of the cases. This strongly suggests that antenatal antibody screening of all women irrespective of RhD status coupled with upgradation of other laboratory services, maternal alloimmunization can be identified at an early stage. This would ensure timely referral of these cases to higher center for further management including USG monitoring, intrauterine transfusions (IUTs), and exchange transfusions.

A variety of non-RhD red cell alloantibodies can cause HDFN with varying severity ranging from mild-to-severe disease. In this case series, anti-c antibody was the common antibody and all the neonates required DVET. Koelewijn et al. demonstrated anti-c as the third most common (after anti-K and anti-E) and second most severe (after anti-K) non-D antibody in antenatal women. Approximately 40% of the Indian population is negative for Rhc antigen and therefore, is at risk of alloimmunization against this antigen. Phenotypic frequency of Rhc antigen and its ability to cause fetal hemolysis is responsible for frequently encountered cases of anti-c HDFN.

The prevalence of anti-D-induced HDFN has decreased sharply as an aftermath of the common use of Rh immune globulin in Western countries. As a result, non-D antibodies have become more important. In developing countries like India, guidelines for antenatal antibody screening in both RhD negative and RhD positive women have to be updated and followed in clinical practice to reduce the occurrence of non-D HDFN. Pregnancies sensitized with non-D antibodies should be monitored closely to ensure timely therapeutic intervention such as IUTs and exchange transfusions.

Acknowledgments

The authors would like to acknowledge the contributions of each author as under.

Bharat Singh - Original draft preparation
Rajendra Chaudhary - Literature search and supervision
Jyoti Kala Bharti - Data curation
Preeti Elhence and Anubha Srivastava - Manuscript review and editing.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. Transfus Med Rev 2007;21:58-71.
2. Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. Blood Transfus 2011;9:388-93.
3. Koelewijn JM, Vrijkotte TG, Vander Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-RhD, to detect haemolytic disease of the foetus and newborn: A population study in the Netherlands. Transfusion 2008;48:941-52.
4. White J, Qureshi H, Massey E, Needs M, Byrne G, Daniels G, et al. Guideline for blood grouping and red cell antibody testing in pregnancy. Transfus Med 2016;26:246-63.
5. Saran RK. Transfusion Medicine Technical Manual. 2nd ed. New Delhi: National Government publication; Directorate General of Health Services, Govt. of India; 2003. p. 175-9.
6. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med 2008;13:207-14.
7. Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd and MNs blood group antigens in the Indian blood donor population. Indian J Med Res 2013;137:521-6.
8. Chavez GF, Mulinare J, Edmonds LD. Epidemiology of Rh haemolytic disease of the newborn in the United States. J Am Med Assoc 1991;265:3270-4.
9. Kornstad L. New cases of irregular blood group antibodies other than anti-D in pregnancy. Frequency and clinical significance. Acta Obstet Gynecol Scand 1983;62:431-6.