CASE REPORT

Neonatal haemolytic disease with co-existing Anti-D and Anti-C antibodies: An unusual experience

Hafiza Mehreen Durrani[1], Zubia Zaman[2], Imran Mansoor Raza[3], Munira Jamali[4], Ayesha Saleem[5], Shabana Muzammil Ejaz[6]

Abstract

Neonatal haemolytic disease in the new-born remains of prime importance for paediatricians due to high perinatal morbidity and mortality rates. The Rh antigen family comprises several different antigens, out of which, D antigen incompatibility is well known for causing severe haemolytic disease in the foetus. Although the current literature shows anomalous cases where coexisting non-D-Rh and D-Rh antigens are the causative agents, there is very little information regarding post-natal outcomes in neonates bearing two different incompatibilities simultaneously. Herein, we discuss an unusual case of anti-D as well as anti-C antibodies (non-D-Rh) in a male neonate born to a Rh-negative mother, who developed jaundice and haemolysis in post-natal life. The neonate underwent exchange transfusion and photo therapy due to raised serum bilirubin levels, supplemented with repeated blood transfusions, intravenous immunoglobulin therapy, and immunosuppressive therapy. He responded well to the management and was later discharged from the hospital. Long-term follow-up revealed no side-effects.

Keywords: Maternal Allo-immunisation, Rh incompatibilities, Haemolytic disease of the foetus and new-born (HDFN).

DOI: https://doi.org/10.47391/JPMA.4564

Introduction

D-antigen incompatibility between the mother and new-born can cause sensitisation leading to Haemolytic Disease of the Foetus and New-born (HDFN) which has considerable perinatal morbidity and mortality.[1]

Ninety percent of HDFN cases are due to Rh incompatibility, anti-D being the most common. Anti-C/Anti-c and Anti-E/Anti-e are other variants but remain clinically rare. ABO incompatibility, although more common, causes less severe haemolysis.[1]

According to a Netherland-based study, the frequency of anti-D sensitised women is 1:1,000 and the prevalence of red cell antibodies other than anti-D with the potential to cause HDFN is 1:500 pregnancies.[1,2]

Herein, we report a case encountered at the paediatric department at Dr Ruth K. M. Pfau Civil Hospital, Karachi, Pakistan, in February 2021. We observed anti-D and anti-C alloimmunisation presenting together in a new-born with a Rh-negative mother, causing HDFN. Paediatricians have little experience with managing such a rare clinical scenario, thus highlighting the need for red-cell antibody screening antenatally in all pregnant Rh-negative mothers. The consent of the guardian was taken before writing of the manuscript.

Case Report

A five-day-old male neonate was brought in with the complaint of persistent and widespread jaundice since the second day of life along with reluctance to feed and non-bilious vomiting. His mother denied history of fever, abdominal distension, diarrhoea, constipation, seizures or altered mental activity. The mother was G3 P2+1, with a non-consanguineous marriage, and previous history of early abortion. She reported that her first-born also developed jaundice in neonatal life which later resolved. The mother's blood group was A-negative while the neonate's was O-positive. She was administered RhoGAM (anti-D immunoglobulin) after birth.

The neonate was born at the Paediatric Department at Dr Ruth K.M. Pfau Civil Hospital, Karachi in February 2021 at term via spontaneous vaginal delivery, and breastfeeding was initiated within the first hour. Initial vitals included pulse: 152b/m, respiratory rate: 42 b/m, capillary refill time: >2 sec, temperature: 37.6ºF.

Physical exam revealed generalised icterus. CNS examination showed full non-bulging anterior fontanelles and slightly increased muscle tone with normal reflexes. On palpation, hepatosplenomegaly was appreciated. The rest of the systemic examination was unremarkable.

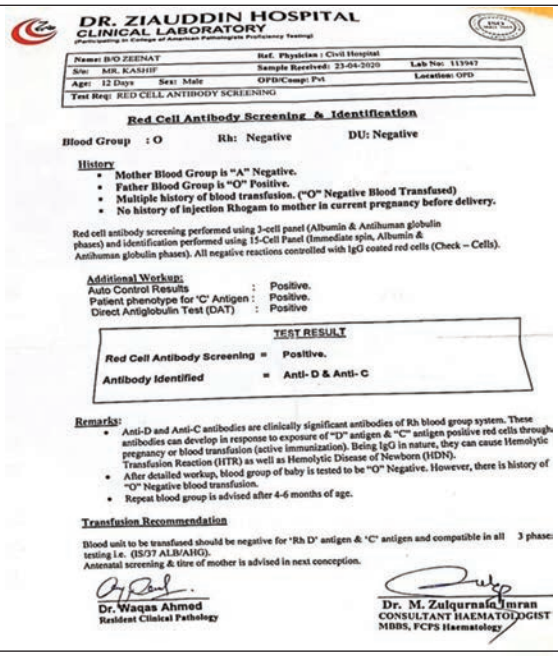
Relevant workup has been summarised in Table. Moreover, his blood chemistry results were BUN 9 mg/dl, Creatinine 0.8 mg/dl, Na 133 mEq/L, K 4.0 mEq/L, Cl 89 mEq/L, and Albumin 4.8 mg/dl. Peripheral smear demonstrated spherocytes and a few target cells. Reticulocyte count was 3.6% and Direct Coombs test was positive.

[1,5,6]Department of Pediatrics, Dr. Ruth KM Pfau Civil Hospital, Karachi, Pakistan;
[2,3]4th Year MBBS Student, DOW University of Health Sciences, Karachi, Pakistan;
[4]Dow University of Health Sciences, Karachi, Pakistan.
Correspondence: Hafiza Mehreen Durrani. e-mail: mehreendurrani2016@gmail.com

Table: Laboratory investigation of the patient.

Lab Parameters	On admission	On subsequent days during stay in hospital		On discharge
Haemoglobin(g/dl)	11.9	11.3	12.8	15.6
Haematocrit (%)	34.1	33.5	37	44.6
Mean corpuscle volume(fl)	92.1	86.6	81.7	82.7
Total leucocyte count (10^9/L)	12.9	7.2	9.3	13.7
Neutrophil (%)	66	75	69	51
Lymphocyte (%)	27	22	28	42
Platelet count (10^9/L)	187	48	31	266
Bilirubin (mg/dl)	35	14.4	10.2	6.6
Retic count (%)	3.6			0.5
Peripheral smear findings	Anisopoikilocytosis, polychromasia, Dimorphic picture of RBCs, spherocytes seen, few target cells.			

Direct Antiglobulin test positive and antibodies identified are Anti-D and Anti-C.



DR. ZIAUDDIN HOSPITAL
CLINICAL LABORATORY

Name: B/O ZEENAT | Ref. Physician : Civil Hospital
S/o: MR. KASHIF | Sample Received: 23-04-2020 | Lab No: 113947
Age: 12 Days | Sex: Male | OPD/Comp: Pvt | Location: OPD
Test Req: RED CELL ANTIBODY SCREENING

Red Cell Antibody Screening & Identification

Blood Group : O Rh: Negative DU: Negative

History
- Mother Blood Group is "A" Negative.
- Father Blood Group is "O" Positive.
- Multiple history of blood transfusion. ("O" Negative Blood Transfused)
- No history of injection Rhogam to mother in current pregnancy before delivery.

Red cell antibody screening performed using 3-cell panel (Albumin & Antihuman globulin phases) and identification performed using 15-Cell Panel (Immediate spin, Albumin & Antihuman globulin phases). All negative reactions controlled with IgG coated red cells (Check – Cells).

Additional Workup:
Auto Control Results : Positive.
Patient phenotype for 'C' Antigen : Positive.
Direct Antiglobulin Test (DAT) : Positive

TEST RESULT
Red Cell Antibody Screening = Positive.
Antibody Identified = Anti- D & Anti- C

Remarks:
- Anti-D and Anti-C antibodies are clinically significant antibodies of Rh blood group system. These antibodies can develop in response to exposure of "D" antigen & "C" antigen positive red cells through pregnancy or blood transfusion (active immunization). Being IgG in nature, they can cause Hemolytic Transfusion Reaction (HTR) as well as Hemolytic Disease of Newborn (HDN).
- After detailed workup, blood group of baby is tested to be "O" Negative. However, there is history of "O" Negative blood transfusion.
- Repeat blood group is advised after 4-6 months of age.

Transfusion Recommendation
Blood unit to be transfused should be negative for 'Rh D' antigen & 'C' antigen and compatible in all 3 phase testing i.e. (IS/37 ALB/AHG).
Antenatal screening & titre of mother is advised in next conception.

Dr. Waqas Ahmed
Resident Clinical Pathology

Dr. M. Zulqurnain Imran
CONSULTANT HAEMATOLOGIST
MBBS, FCPS Haematology

Figure.

During admission, he was given intensive phototherapy, but the serum bilirubin levels remained elevated, which necessitated exchange transfusion. O-negative whole blood of 390 ml was used, and post-procedure bilirubin levels lowered to 14.4 g/dl.

However, the neonate had persistent haematuria, with low Hb and platelet count, suggesting ongoing haemolysis. Detailed antibody screening revealed Anti-D and Anti-C antibodies, as shown in Figure.

IVIG was administered at 1.0 g/kg dose over an eight-hour period. Prednisolone was also started per oral from 1mg/kg and continued for two months. The neonate was continuously monitored and responded well to therapy. The resolution of haemolysis with a rise in haemoglobin and platelet count was noted, with education regarding transfusion of anti-D and anti-C blood products if required. He was discharged in late February 2021 and made a full recovery with an unremarkable follow-up.

Discussion

HDFN is the consequence of maternal antibodies attaching to red cell antigens present in the foetus resulting in haemolysis and foetal anaemia. Out of all the 29 blood groups identified, the Rh group consists of heterogeneous antigens, of which, five are commonly responsible for HDFN as documented by the International Society of Blood Transfusion (ISBT).[3] Individuals who are positive for Rh-D antigen contain the RHD gene which has dominant inheritance whereas Rh-d negative variant is due to the absence of both genes. In the RHCE antigen, the gene encoding C/c combine with E/e antigen and are transmitted en bloc with different point mutations being accountable for C/c and E/e pairs.[1,3]

It was earlier thought that allo-sensitisation of D antigen is mainly responsible for HDFN, which manifests variably ranging from haemolytic anaemia and pathological jaundice to severe hydrops foetalis. However, in the 1960s, this surveillance led to the implementation of anti-Rh immune globulin (RhoGAM) injected into the Rh-negative mother, both intrapartum and immediately postpartum of each Rh-positive infant, which substantially reduced the burden of morbidity and mortality caused by anti-D alloimmunisation.[1-3]

Haemolytic anaemias in new-borns is associated with irreversible neurotoxicity due to the deposition of excess unconjugated bilirubin in basal ganglia and brain stem nuclei. This leads to long-term morbidity including athetoid cerebral palsy, hearing problems, and psychomotor disabilities.[1] In our case, the neonate showed symptoms since the second day of life, but severe icterus was brought into attention on the fifth day of life when he showed reluctance to feed and irritability.

The usual measures taken to treat indirect hyperbilirubinaemia include intensive phototherapy, and modalities such as IVIG and exchange transfusion. Although these interventions reduce indirect bilirubin levels, as well as removing excess antibodies from the body, they also carry substantial risks.[4]

In our case, keeping early signs of kernicterus in mind, the neonate was kept in bilisphere until exchange transfusion was arranged. After transfusion, serum bilirubin dropped but showed low Hb and thrombocytopenia. Thus, IVIG and oral steroids were administered.[5]

Under prenatal testing, the diagnosis of HDFN can be anticipated early in foetal life. Use of paternal molecular

RHD zygosity testing, and non-invasive foetal RHD genotyping utilising cff-DNA and maternal plasma along with combining doppler studies of middle cerebral arteries to detect foetal anaemia is a recent approach for targeted anti-D prophylaxis in Rh-negative mothers and to recognise the probability of HDFN in their foetuses.[2]

In developing countries, the concept of foetal genetic testing is premature, and reliance is mostly based on doppler studies. After extrauterine life, a decrease in haemoglobin with an increase in reticulocyte count and positive Coombs test is confirmatory for establishing the diagnosis of HDFN.[6] In our case, the neonate had a positive Coombs test and red cell antibody screening-detected anti-D and anti-C antibodies (Figure). These antibodies probably developed in the mother due to exposure to Rh (D) and Rh (C) antigens in previous pregnancies. Being a rare combination, our literature search reported a study from South India which was based on screening 5,347 pregnant women antenatally, out of 79 allo-sensitized mothers, three women possessed both anti-D and anti-C. Moreover, the study did not emphasise on the postnatal consequences of these antibodies on the babies.[7] In the past, a few studies have reported the incidence of non-D antibodies in causing HDFN with a scarcity of data on their coexistence with D antibodies and enhancing haemolytic transfusion reaction.[8]

Rai et al reported a case of HDFN where an Rh-negative mother tested positive for anti-C and anti-D antibodies. At 34 weeks of gestation, the patient underwent spontaneous preterm delivery of a low birth weight baby. The new-born had severe pallor and mild icterus. Despite persistent phototherapy and transfusions with O Rh (D) negative C, c, E and Kell negative fresh whole blood, the new-born developed sepsis and died of multiple organ failure on day four.[9]

According to Healsmith et al, 20% of the pregnancies which had isolated anti-C antibodies reached the critical titre to cause haemolysis compared to 84.6% when anti-C and anti-D antibodies occurred simultaneously. The article also highlighted that rates of Caesarean section, induction of labour, direct antiglobulin positivity, and treatments for hyperbilirubinaemia and anaemia were all more common when anti-D accompanied anti-C.[10] Similarly, Özköse et al reported a significantly higher rate of severe hydrops foetalis in the anti-D combined non-D group (3/25, 12%) than in the anti-D group (1/128, 0.08%, $p<0.001$).[11]

We share this study to highlight the idea of how the co-existence of both anti-D and anti-C antibodies can lead to severe haemolysis in postnatal life. This calls for the need of proper guidelines for red cell antibodies screening in antenatal women to ensure safer and better outcomes.

Conclusion

There is differential immunogenicity of red cell antigen with RhD being the most immunogenic among all yet there is potential to learn more about this phenomenon and discover factors and other red cell antigens capable of producing allo-sensitisation in pregnant females leading to HDFN. The paradigms of management of haemolytic anaemias due to the irregular red cell antibodies might remain similar but knowing them could help the paediatrician prepare in advance and implement useful strategies to deal with worse consequences.

Disclaimer: None.

Conflict of interest: None.

Funding disclosure: None.

References

1. de Haas M, Thurik F, Koelewijn J, van der Schoot C. Haemolytic disease of the fetus and newborn. Vox Sang 2015;109:99-113. doi: 10.1182/asheducation-2015.1.146.
2. Sørensen K, Kjeldsen-Kragh J, Husby H, Akkök ÇA. Determination of fetal RHD type in plasma of RhD negative pregnant women. Scand J Clin Lab Invest 2018;78:411-6. doi: 10.1080/00365513.2018.1475681.
3. Avent N, Reid M. The Rh blood group system: a review. Blood 2000;95:375-87.
4. Smits-Wintjens VE, Rath ME, van Zwet EW, Oepkes D, Brand A, Walther FJ, et al. Neonatal morbidity after exchange transfusion for red cell alloimmune hemolytic disease. Neonatology 2013;103:141-7. doi: 10.1159/000343261.
5. van Klink J, van Veen S, Smits-Wintjens V, Lindenburg I, Rijken M, Oepkes D, et al. Immunoglobulins in Neonates with Rhesus Haemolytic Disease of the Foetus and Newborn: Long-Term Outcome in a Randomised Trial. Fetal Diagn Ther 2015;39:209-13. doi: 10.1159/000434718
6. ACOG Practice Bulletin No. 192: Management of Alloimmunization During Pregnancy. Obstet Gynecol 2018;131:e82-90. doi: 10.1097/AOG.0000000000002528.
7. Dholakiya SK, Bharadva S, Vachhani JH, Upadhyay BS. Red cell alloimmunization among antenatal women attending tertiary care center in Jamnagar, Gujarat, India. Asian J Transfus Sci 2021;15:52-6. doi: 10.4103/ajts.AJTS_72_17.
8. Zwiers C, Oepkes D, Lopriore E, Klumper FJ, de Haas M, van Kamp IL. The near disappearance of fetal hydrops in relation to current state-of-the-art management of red cell alloimmunization. Prenat Diagn 2018;38:943-90. doi: 10.1002/pd.5355.
9. Rai P, Sharma G, Singh D, Garg J. Approach to a Pregnant Woman with Anti D + Anti C Reactivity Pattern: A Diagnostic Conundrum. J Clin Diagn Res 2017;11:ED06-8. doi: 10.7860/JCDR/2017/27902.10550.
10. Healsmith S, Savoia H, Kane SC. How clinically important are non-D Rh antibodies? Acta Obstet Gynecol Scand 2019;98:877-84. doi: 10.1111/aogs.13555.
11. Gedik Özköse Z, Oğlak SC. The combined effect of anti-D and non-D Rh antibodies in maternal alloimmunization. Turk J Obstet Gynecol 2021;18:181-9. doi: 10.4274/tjod.galenos.2021.68822.