

Minor blood group incompatibility due to blood groups other than Rh(D) leading to hemolytic disease of fetus and newborn: a need for routine antibody screening during pregnancy

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SUMMARY Minor blood group incompatibility due to blood groups other than Rh(D), although an uncommon cause of neonatal hyperbilirubinemia, has the potential to cause severe hyperbilirubinemia and its sequelae in infants, if left undiagnosed and untreated. Here, we describe clinical presentation, diagnosis and treatment of three cases of minor blood group incompatibility due to anti-E and anti-c antibody. All three neonates presented with pallor, icterus and splenomegaly within the first three days of life. Investigations showed indirect hyperbilirubinemia and a positive direct coombs test. Indirect coombs test was positive in the mothers. There was no setting of ABO or Rh(D) incompatibility in any of the neonates. When tested for minor blood group incompatibility, anti E antibody was found to be responsible for hemolysis and hyperbilirubinemia in the first case, and anti c antibody was found in the second case and third case had both anti c and anti E antibodies. While hyperbilirubinemia improved with intensive phototherapy in the first two cases, the third case required a double volume exchange transfusion. On follow up, bilateral sensorineural hearing loss was seen in one of the patients. All three neonates were otherwise healthy, gaining weight and developmentally normal.

Keywords antibody screening, hemolytic disease of newborn, minor blood group, neonatal hyperbilirubinemia, red cell allo-immunization

1. Introduction

The estimated global prevalence of haemolytic disease of the fetus and newborn (HDFN) due to Rh isoimmunisation is 276/100,000 live births per year (1). The prevalence of HDFN for developed countries like United States is estimated to be 3/100,000 to 80/100,000 while in developing regions like Latin America, North Africa/the Middle East, South Asia, sub-Saharan Africa, and Eastern Europe/Central Asia, the prevalence of HDFN due to Rh isoimmunisation is estimated at 252, 278, 385, 386, and 529/100,000 live births, respectively (1,2). The frequency of neonatal hemolytic disease and indirect hyperbilirubinemia due to Rh sensitisation has decreased with the widespread use of anti-D gamma globulin. Hence, the contribution of minor blood groups incompatibility other than Rh(D) antigen, such as Kell, c, C, E, e has gradually increased in HDFN (3,4). The prevalence of red cell antibodies other than anti-D with the potency to induce HDFN is about 1 in 500 pregnancies (5). Anti-c is usually described

as the next most common cause of severe HDFN after anti-D (6). More and more cases of minor blood group incompatibility are now being diagnosed due to advancements in investigation modalities.

Neonates with minor blood group incompatibility may be asymptomatic or the clinical picture may range from mild anemia, reticulocytosis, neonatal hyperbilirubinemia to fetal hydrops (4,7). The clinical presentation, diagnosis and management of three cases of neonatal hyperbilirubinemia due to minor blood group incompatibility and maternal allo-immunisation to anti-E and anti-c antigens is discussed here (Table 1).

2. Patients and Methods

All neonates presenting with icterus were examined for pallor, organomegaly and signs of bilirubin encephalopathy. Investigations including a complete blood count and peripheral smear (for hemolysis, spherocytes, atypical cells and reticulocyte count), serum bilirubin levels, ABO and Rh(D) typing of

Table 1. Result of phenotypic analysis and antibody screening in parents and neonate

Items	Case 1		Case 2		Case 3	
Mother's Blood Group	B+		AB+		A+	
Father's Blood Group	O+		O+		A+	
Baby's Blood Group	O+		B+		A+	
DCT (Baby)	3+		4+		4+	
Antigen detection:	Mother	Father	Mother	Father	Mother	Baby*
D	4+	3+	4+	4+	4+	4+
C	4+	-ve	4+	3+	4+	4+
c	4+	3+	-ve	4+	-ve	4+
E	-ve	3+	4+	4+	-ve	4+
e	4+	2+	4+	4+	4+	-ve
Kell	-ve	-ve	-ve	-ve	-ve	-ve
ICT(Mother)	+ve		+ve		+ve	
Antibodies responsible for hemolysis	Anti E antibody in mother and baby		Anti c antibody in mother and baby		Anti E and anti c antibodies in baby	

*antigen detection in father could not be tested because father was not available for testing.

neonate and mother, direct coombs test and Glucose 6 phosphate dehydrogenase enzyme levels were done on all patients at admission. In all patients with a positive direct coombs test in the absence of ABO or Rh(D) setting, autoimmune and alloimmune causes were looked for including indirect coombs test, phenotypic analysis for minor blood groups (C, c, Kell, E, e), antibody screening and anti-nuclear antibodies.

Treatment including phototherapy and exchange transfusion was done as per the guidelines; and once the bilirubin was below the cut off and in a decreasing trend, phototherapy was discontinued (8). All patients were monitored for rebound hyperbilirubinemia before discharge. After discharge, patients were kept under follow up for hearing screening, developmental assessment (9) and head circumference monitoring.

2.1. Case 1

A term 40-week gestation, female baby with birth weight 2,860 g was born to a 29-year-old G2P1L1 mother by an uneventful vaginal delivery in hospital. It was a booked pregnancy with regular antenatal visits and normal antenatal ultrasounds. Breast feeding was initiated within first hour of life and continued thereafter. Baby passed urine and stools on the first day of life and was discharged on second day of life. There was no history of jaundice in the first sibling. Jaundice was first noticed by mother on day 3 of life along with highly colored urine. On examination, the baby had icterus up to abdomen and was feeding well. In the follow up visit on day 5 of life, she had icterus up to palms and soles. The baby was admitted and phototherapy started. On examination, the baby was active, alert and accepting breast feeds well. She had hepatomegaly of 1 cm below costal margin and no splenomegaly. There were no signs of bilirubin encephalopathy. All anthropometric measurements were between 3rd and 10th percentile for her gestational age as per Fenton charts.

Investigations showed baby's hemoglobin of 18.5 g/dL, leucocyte count of 10,600/mm³, platelet count of 279,000/mm³ and total serum bilirubin (TSB) of 23.8 mg/dL with a direct component of 0.7 mg/dL. Blood group was O Rh (+), and direct coombs test was positive (+++). Mother and father blood groups were B Rh (+) and O Rh (+) respectively. Glucose 6 phosphate dehydrogenase enzyme levels were normal. Peripheral smear showed normal RBC morphology with no evidence of hemolysis or atypical cells. Phenotype analysis for minor blood group antigens in mother and father showed D(4+), C(4+), c(4+), E(-), e(4+), Kell(-) and D(3+), C(-), c(3+), E(3+), e(2+), Kell(-) respectively. Indirect coombs test was positive in the mother. Antibody screening showed anti-E antibody in the mother and baby. Since antibody screening showed anti-E antibody in the mother and baby, a diagnosis of indirect hyperbilirubinemia due to minor blood group incompatibility as a result of anti-E antibody was established.

The patient received phototherapy for 48 hours and serum bilirubin declined to 13.6mg/dL. On follow up visit, 48 hours after discontinuing phototherapy, there was no rebound hyperbilirubinemia. At 8 weeks follow up, the baby was accepting breast feeds, gaining weight, had normal development and Brainstem Evoked Response Audiometry (BERA) showed normal hearing bilaterally.

2.2. Case 2

A term 38-week gestation, male baby with birth weight 3,500 g was born to a 25-year-old G2P1L0 mother by an uneventful vaginal delivery in hospital. It was a booked pregnancy with regular antenatal visits and normal antenatal ultrasounds. The first baby was a female born at term gestation with no clinical pallor or jaundice and survived for 2 hours after birth. A definite cause of death was not established. Breast feeding was initiated

within the first hour of life. Jaundice was first noticed at life hour 8 with highly colored urine. TSB at 20 hours of life was 23 mg/dL with a direct component of 1.2 mg/dL. At the time of admission at 31 hours of life, baby was active, alert, and accepting breast feeds well. Baby had icterus up to palms and soles, hepatomegaly of 3 cm below costal margin and splenomegaly of 3 cm. There were no signs of bilirubin encephalopathy. All anthropometric measurements were between 50th and 90th percentile for age as per the Fenton charts.

Investigations showed hemoglobin of 10.7 g/dL, leucocyte count of 13,900/mm³, platelet count of 266,000/mm³ and total bilirubin of 25.9 mg/dL with a direct component of 1.6 mg/dL at 31 hours of life. Baby's blood group was B Rh (+) and direct coombs test was positive (++++). Mother's and father's blood group were AB Rh (+) and O Rh (+) respectively. Glucose 6 phosphate dehydrogenase enzyme level was normal. Peripheral smear showed hemolysis with a reticulocyte count 3.5%. Phenotype analysis for minor blood group antigens in mother and father showed D(4+), C(4+), c(-), E(4+), e(4+), Kell(-) and D(4+), C(3+), c(4+), E(4+), e(4+), Kell(-) respectively. Indirect coombs test was positive in the mother. Antibody screening showed anti-c antibody in the mother and baby. A diagnosis of indirect hyperbilirubinemia due to minor blood group incompatibility as a result of anti-c antibody was made.

Intensive phototherapy was initiated at 31 hours of life and continued. TSB at 67 hours of life was 22.7 mg/dL with a direct component 2.4 mg/dL. A double volume exchange transfusion was performed at 78 hours of life at a TSB of 25 mg/dL with a direct component of 2.9 mg/dL. Following exchange transfusion, intensive phototherapy was continued and serial TSB values showed a decreasing trend. Phototherapy was discontinued at 103 hours of life at a TSB of 13 mg/dL. There was no rebound hyperbilirubinemia. The baby was accepting breast feeds and gaining weight at discharge. At 8 weeks follow up, the baby was accepting breast feeds, gaining weight, had normal development and BERA showed bilateral sensorineural hearing loss.

2.3. Case 3

A term 38-week gestation, male baby with birth weight 2,920 g was born to a 22-year-old primigravida mother by caesarean delivery for fetal distress. It was a booked pregnancy with regular antenatal visits and normal antenatal ultrasounds. Breast feeding was initiated at 3 hours of life and continued thereafter. Baby passed urine and stools on the first day of life. Jaundice was noticed by mother on the second day of life. The baby was admitted to a peripheral health center and phototherapy was started. The investigations showed a maximum TSB of 25.2 mg/dL with direct fraction of 1.2 mg/dL at 110 hours of life. The blood group of the baby and both the parents was A Rh (+). Direct coombs test of the baby

was positive (++++). On the 8th day of life the baby was referred in view of persistent hyperbilirubinemia despite receiving phototherapy. At presentation, the baby had icterus up to palms and soles. The baby was active, alert and accepting breast feeds well. He had hepatomegaly of 1.5 cm below costal margin and spleen was not enlarged. There were no signs of bilirubin encephalopathy. All anthropometric measurements were between 10th and 50th percentile for his gestational age as per Fenton charts.

Investigations showed hemoglobin of 10.2 g/dL, leucocyte count of 9,500/mm³, platelet count of 318,000/mm³ and TSB of 23 mg/dL with a direct component of 1.7 mg/dL. Glucose 6 phosphate dehydrogenase enzyme levels were normal. Peripheral smear showed evidence of hemolysis with reticulocyte count of 8.5%. Phenotype analysis for minor blood group antigens in both parents was planned but the father was not available for testing. The mother and baby showed an antigen profile of D(4+), C(4+), c(-), E(-), e(4+), Kell(-) and D(4+), C(4+), c(4+), E(4+), e(-), Kell(-) respectively. Indirect coombs test was positive in the mother. Since antibody screening showed anti-E and anti-c antibodies in the mother and baby, a diagnosis of indirect hyperbilirubinemia due to minor blood group incompatibility as a result of anti-E and anti-c antibodies was considered.

The patient received phototherapy for 70 hours and serum bilirubin declined to 10.1 mg/dL. On follow up visit, 48 hours after discontinuing phototherapy, there was no rebound hyperbilirubinemia. At 8 weeks follow up, the baby was accepting breast feeds, gaining weight, had normal development and BERA showed normal hearing bilaterally.

3. Results and Discussion

HDFN, also known as *erythroblastosis fetalis*, occurs when fetal red blood cells, containing paternally inherited antigens that the mother lacks, cross the placenta and stimulate antibody production. The antibodies return to the fetal circulation and result in hemolysis of fetal RBC. The hemolysis may be subclinical or the clinical presentation may vary from *hydrops fetalis*, anemia with reticulocytosis, or hyperbilirubinemia, requiring phototherapy or even exchange transfusion (3,4). Historically, Rh D alloimmunization had been the most common cause of HDFN. The introduction of antenatal and postnatal immuno-prophylaxis with anti-D immunoglobulins has reduced the incidence of Rh D allo-immunisation from 14% to 1-2% worldwide (7). Subsequently, ABO incompatibility has emerged as the single largest cause of HDFN. However, 3-5% cases of HDFN are reportedly caused by non-ABO, non-Rh(D) antigens including C, c, E, e, Kell, Duffy, Kidd and other antigen systems (10).

There are multiple case reports of minor blood group

Table 2. Guidelines in developed countries recommending routine antibody screening during pregnancy

Countries	Prevalence of red cell antibodies/ alloantibodies other than anti-D (Ref.)	Recommendation (Ref.)
Netherlands	1.23%/0.32% (5)	ABO, Rh(D) and Rh(c) blood group antigen typing and screening for RBC antibodies at the booking visit and again at week 27 (16).
United Kingdom	1%/0.3% (17)	ABO, Rh(D) and Rh(c) blood group antigen typing and screening for RBC antibodies at the booking visit and again at week 28 (14).
Australia & New Zealand	0.73%/0.65% (18)	ABO, Rh(D) blood group antigen typing and screening for RBC antibodies at the booking visit (19).
United States	0.3-3.4%/0.74% (20)	ABO, Rh(D) blood group antigen typing and screening for RBC antibodies at the booking visit (21).

incompatibility reported from the Asian subcontinent, including reports from India describing severe hemolytic disease due to anti C and anti E in two Rh D positive women, both detected postnatally (11). There are case reports from Korea, Taiwan and China describing hemolytic disease due to anti C anti E, anti M, anti Jk and anti Mi (12).

Newborns with evidence of hemolysis including positive direct agglutination test and hyperbilirubinemia where no evidence of Rh and ABO incompatibility is found, as in the three cases discussed above, a possibility of minor blood group incompatibility should be considered. These case reports show that HDFN caused by minor blood group incompatibility other than D antigen, may vary from mild to severe in its presentation. Since Rh(D) positive women are as likely to form alloantibodies as Rh(D) negative women, screening for antibodies during pregnancy is needed (13). This brings to attention the necessity of introducing antibody screening for pregnant women as part of antenatal care to look for significant alloantibodies other than antiD. Antenatal antibody screening should be done in all pregnant women irrespective of the Rh (D) antigen status to ensure timely availability of antigen negative blood and reduce effects of severe hyperbilirubinemia on the newborn (13-15).

Many developed nations have implemented regular screening of all pregnant women and even have national screening programs (Table 2) (5,14,16-21). Although universal screening seems justified, the cost and infrastructure required would be immense. Developing countries and under resourced nations need to consider universal antenatal screening and frame guidelines accordingly.

4. Conclusion

Minor blood group incompatibility other than anti-D remains an underreported and frequently misdiagnosed entity. Its presentation ranges from being asymptomatic to significant hyperbilirubinemia and kernicterus. Antenatal detection of significant antibody titre in the

mother can ensure timely management and prevent significant morbidity and mortality in the neonate at risk.

References

1. Bhutani VK, Zipursky A, Blencowe H, *et al.* Neonatal hyperbilirubinemia and Rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. *Pediatr Res.* 2013; 74 Suppl 1:86-100.
2. Geaghan SM. Diagnostic laboratory technologies for the fetus and neonate with isoimmunization. *SeminPerinatol.* 2011; 35:148-154.
3. Özcan M, Sevinç S, Erkan VB, Yurdugül Y, Sarıcı SÜ. Hyperbilirubinemia due to minor blood group (anti-E) incompatibility in a newborn: a case report. *Turk PediatriArs.* 2017; 52:162-164.
4. Eder AF. Update on HDFN: new information on long-standing controversies. *Immunohematology.* 2006; 22:188-195.
5. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. *Transfusion.* 2008; 48:941-952.
6. Geoff D, Imelda B. The Rh blood group system. In: *Essential guide to blood groups.* Blackwell Publishing, Amsterdam, the Netherlands, 2007; pp 33-44.
7. Roberts IA. The changing face of haemolytic disease of the newborn. *Early Hum Dev.* 2008; 84:515-523.
8. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* 2004; 114:297-316.
9. Frankenburg WK, Dodds J, Archer P, Shapiro H, Bresnick B. The Denver II: a major revision and restandardization of the Denver Developmental Screening Test. *Pediatrics.* 1992; 89:91-97.
10. Kornstad L. New cases of irregular blood group antibodies other than anti Rh D in pregnancy: frequency and clinical significance. *ActaObstetGynecol Scand.* 1983; 62:431-436.
11. Thakral B, Agrawal SK, Dhawan HK, Saluja K, Dutta S, Marwaha N. First report from India of haemolytic disease of the newborn by anti c and anti E in Rh (D) positive mothers. *Haematology.* 2007; 12:377-380.
12. Wu KH, Chu SL, Chang JG, Shih MC, Peng CT.

- Haemolytic disease of the newborn due to maternal irregular antibodies in the Chinese population in Taiwan. *Transfus Med.* 2003; 13:311-314.
13. Basu S, Kaur R, Kaur G. Hemolytic disease of the fetus and newborn: current trends and perspectives. *Asian J Transfus Sci.* 2011; 5:3-7.
 14. White J, Qureshi H, Massey E, Needs M, Byrne G, Daniels G, Allard S, British Committee for Standards in Haematology. Guideline for blood grouping and red cell antibody testing in pregnancy. *Transfus Med.* 2016; 26:246-263.
 15. Azuonwu O, Nnenna I, Douglass SA, Ntaa BN. Consequences of haemolytic disease of the fetus and newborn (HDFN) and the clinical significance of antibody screening in prenatal diagnosis: a study of multigravida and primigravida women in Port Harcourt, Niger Delta. *Journal of Clinical and Laboratory Medicine.* 2016; doi <http://dx.doi.org/10.16966/2572-9578.106>
 16. Slootweg YM, Koelewijn JM, Van Kamp IL, Van der Bom JG, Oepkes D, De Haas M. Third trimester screening for alloimmunisation in Rh(c) negative pregnant women: evaluation of the Dutch national screening programme. *BJOG.* 2016; 123:955-963.
 17. Smith HM, Shirey RS, Thoman SK, Jackson JB. Prevalence of clinically significant red blood cell alloantibodies in pregnant women at a large tertiary-care facility. *Immunohematology.* 2013; 29:127-130.
 18. Pal M, Williams B. Prevalence of maternal red cell alloimmunisation: a population study from Queensland, Australia. *Pathology.* 2015; 47:151-155.
 19. Guidelines for Blood Grouping & Antibody Screening in the Antenatal and Perinatal Setting. 2nd Edition 2004, Prepared by Scientific Subcommittee of the Australian and New Zealand Society of Blood Transfusion Inc.
 20. Moinuddin I, Fletcher C, Millward P. Prevalence and specificity of clinically significant red cell alloantibodies in pregnant women - a study from a tertiary care hospital in Southeast Michigan. *J Blood Med.* 2019; 10:283-289.
 21. ACOG Practice Bulletin No. 192: Management of alloimmunization during pregnancy. *Obstet Gynecol.* 2018; 131:e82-e90.

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