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Case Report

Hemolytic disease of newborn caused by multiple Rh antibodies

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ABSTRACT

Hemolytic disease of Fetus and Newborn (HDFN) usually results due to natural occurring antibodies or alloimmunization in mother but the presence of multiple red cell antibodies increases the risk of development of significant HDFN. Here author reported a case of hemolytic disease of fetus and newborn in a preterm baby caused by multiple maternal antibodies. Direct Antiglobulin Test (DAT) on neonate blood sample was positive (3+) with monospecific DAT showed IgG type which was confirmed by heat elution. Antibody identification of eluate was done using commercial 11-cell panel by gel method showing specificity to anti-D and anti-C antibody which was differentiated from anti-G by sequential adsorption and elution studies. Neonate was treated with double volume exchange transfusion (DVET) using leucoreduced, irradiated O Rh D and C negative PRBC suspended in AB plasma and discharged 6th day in a stable condition. So, all pregnant women should be at least advised for ICT irrespective of Rh D negative status. If ICT is positive, they should be referred to higher center for proper Immunohematological work up, so that proper blood unit for DVET could be identified.

Keywords: Alloimmunization, Anti-C, Anti-D, Antibody identification, Heat Elution, Sequential adsorption

INTRODUCTION

Hemolytic disease of fetus and newborn (HDFN) is the direct effect of immunological breakdown of RBCs in the fetus caused by the IgG type maternal antibodies which can cross placenta. These antibodies are directed against the paternally inherited antigens in the fetus that are absent in the mother. These antibodies are either naturally occurring (Anti-A, Anti-B) or immune mediated formed after a sensitization event like transfusion or pregnancy. In United States, 35 per 10,000 live births are at risk for developing hemolytic disease of the fetus and newborn (HDFN) because of maternal red blood cell alloimmunization, out of which 20% are severely affected.[1,2] Rh blood group system is one of the complex blood group system that is commonly associated with HDFN. Anti-D is one of the most common antibodies encountered in pregnant women in developing countries like India followed by other Rh antibodies.[3,4,5] With introduction of RhIg immune-prophylaxis, maternal D alloimmunization have reduced drastically. There is increased risk of development of significant HDFN with multiple red cell antibodies in mother especially in presence of anti-(Rh) D.[6] Here, we have reported a case of HDFN caused due to multiple maternal antibodies of anti-D and anti-C.

CASE REPORT

A preterm 35wk boy baby with birth weight of 2.1 kg born to a Rh-negative mother, was delivered by LSCS at a peripheral center. He was referred to the neonatal intensive care unit of our hospital with severe jaundice and anemia within 8 hours of delivery for management.

Mother had prior history of one abortion (P1A1), but RhIg was not administered. Indirect Coomb's Test (ICT) was positive during her antenatal check-up at 20th week of gestation, so anti RhIg was not administered. She was advised for regular monitoring of fetus but she presented to the nearby hospital in her locality with decreased fetal movement and leaking PV at 37 weeks of pregnancy where emergency LSCS was conducted.

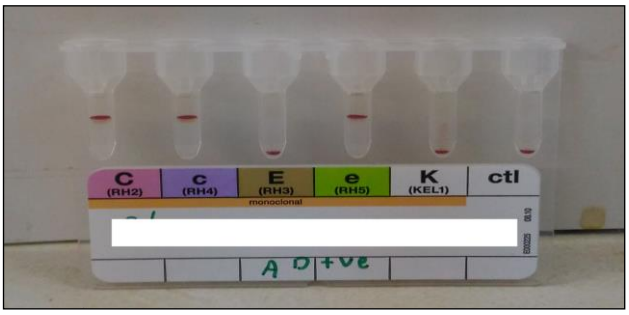

Figure 1: Rh Kell phenotyping of neonate C+c+E-e+K-.

On investigation, serum total bilirubin was 10.4 mg/dl at 8hrs of life. Hemoglobin was 8.6 gm/dl with corrected reticulocyte count of 6.7%. G6PD estimation showed 28.4 units/gm Hb (within normal limit). Forward blood grouping of neonate was performed using anti A, anti B and anti D antisera (ERYCLONE, Tulip diagnostics, Goa) in standard tube technique as per AABB technical manual and revealed A Rh D positive with extended Rh and Kell phenotype (Ccee) K- (Figure 1).(IH-Card Rh-Phenotype + K, Biorad).Direct antiglobulin test (DAT) (AHG gel card, biorad) on neonate blood sample was positive (3+) with monospecific DAT (IgG and C3d coombs gel card , Tulip) showed IgG type. Heat elution was performed to confirm the antibody specificity in the DAT positive cells. Red cell antibody screening of eluate using commercial cell panel (Diacell I-II-III, Biorad, Switzerland) showed positive with auto control being negative. Antibody identification was done using commercial 11-cell panel by gel method (ID-Diapanel, Biorad, Switzerland) showing specificity to anti-D and anti-C antibody (Table 1, Figure 2).

Table 1: Antibody identification panel for eluate of neonate showing anti-D + anti-C specificity.

	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xga	GEL, IAT
	D	C	E	c	e	CW	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	JKa	JKb	Lea	Leb	P1	M	N	S	s	Lua	Lub		
CCC^{W}D.ee $R_1{}^{W}R_1$	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	0	+	0	+	+	+	0	0	+	0	+	+	4+
CCD.ee R_1R_1	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	+	0	+	0	+	0	+	0	+	0	+	+	4+
ccD.EE R_2R^2	+	0	+	+	0	0	0	+	0	+	nt	nt	0	+	+	+	0	0	0	+	0	+	0	0	+	+	4+
Ccddee r'r	0	+	0	+	+	0	0	+	0	+	nt	nt	+	+	0	+	0	+	+	+	+	0	+	0	+	+	2+
ccddEe r"r	0	0	+	+	+	0	0	+	0	+	nt	nt	+	+	+	+	+	0	+	+	0	+	0	0	+	+	0
ccddee rr	0	0	0	+	+	0	+	+	0	+	nt	nt	+	0	0	+	0	0	+	+	0	+	+	+	+	nt	0
ccddee rr	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	0	0	+	0	0	+	0	+	0	+	+	0
ccD.ee R_0r	+	0	0	+	+	0	0	+	0	+	+	nt	0	0	+	0	0	+	+	+	+	+	0	0	+	+	4+
ccddee rr	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0
ccddee rr	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	+	+	0	+	+	+	+	+	0	0
ccddee rr	0	0	0	+	+	0	0	+	+	+	nt	nt	+	+	0	+	+	0	0	+	0	+	+	0	+	+	0

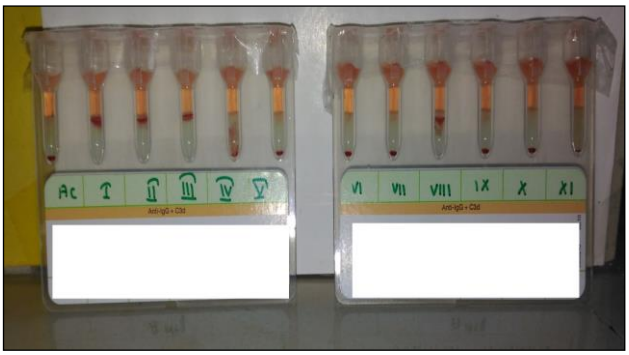

Figure 2: Antibody Identification of neonate's eluate showing anti D + anti C specificity.

Blood grouping of mother was A Rh D negative with extended Rh and Kell phenotype (ccee) K- (Figure 3) and father being O Rh D positive with (CCee) K- (Figure 4).Red cell antibody screening on mother serum showed positive with auto control negative and antibody identification demonstrated specificity to anti-D and anti-C antibody. Presence of anti-G in mother's serum and baby's eluate was ruled out by sequential adsorption and elution studies using r'r (Cde) and R0r (cDe) red blood cells which confirmed the presence of anti-D and anti-C, not anti-G (Table 2).

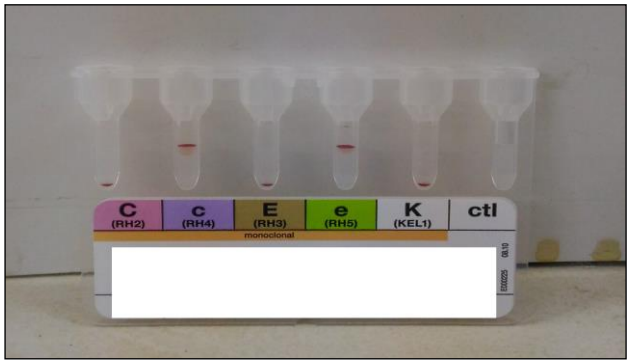

Figure 3: Rh Kell phenotyping of mother C-c+E-e+K-.

Maximum serum total bilirubin reached during the hospital stay was 14.2 mg/dl at 12hrs of life with unconjugated bilirubin being 13.7 mg/dl. Neonate was treated with double volume exchange transfusion using leucoreduced, irradiated O Rh D and C negative PRBC suspended in AB plasma. He was under phototherapy till post-natal day 5 and was discharged on day 6. There was no rebound hyperbilirubinemia after stoppage of Phototherapy.

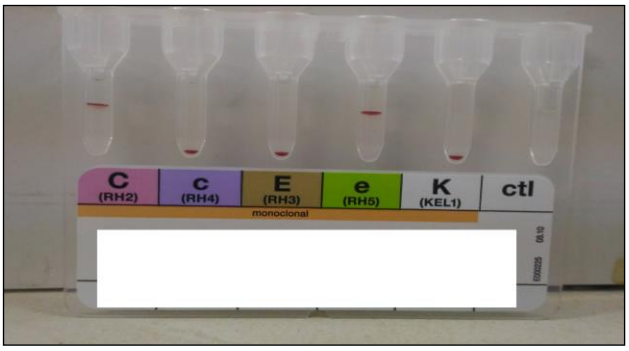

Figure 4: Rh Kell phenotyping of father C+c-E-e+K-.

Table 2: Results of Sequential adsorption of maternal serum and neonatal eluate with Cde and Dce cells.

Adsorption of the mother serum and neonate eluate by Cde cell	Adsorbed serum tested with Cde cell Dce cell	Second adsorption of the adsorbed serum with Dce cell	Adsorbed serum tested with Cde cell Dce cell
	Negative Positive		Negative Negative

DISCUSSION

Here author have reported a case of Hemolytic disease of newborn due to multiple Rh antibody (anti-Rh D and anti-C). Nordvall et al, reported 27 % of cases in pregnancies complicated with alloantibodies have multiple antibodies of which anti D (67%) is most commonly being implicated.[7,8]

Here, the neonate with multiple antibody specificity i.e. anti-D and anti-C presented with features of severe HDFN requiring double volume exchange transfusion (DVET) with phototherapy prolonging hospital stay. This observation is supported by the study of Howard et al, that showed anti-D in combination with other antibodies, especially with anti C results in more severe form of HDFN.[9]

Anti-G should be ruled out when antegram of antibody screening and identification is suggestive of anti-D and anti-C. The G antigen belonging to the Rh blood group system is encoded by RHD and RHCE genes which is present in almost all D-positive or/and C-positive red blood cells (RBCs) but can also be rarely found on D-C- cells. Because of such unique co-distribution with either the C or D antigen, Anti-G antibody mimics the pattern of anti-D + anti-C in the antegram. Its differentiation from anti D and anti C is very important in case of antenatal mothers as it implicates false presence of anti D depriving them from receiving Rh Ig prophylaxis putting their future pregnancies at risk of developing anti D and its harmful effect on the fetus. Anti D and anti C are implicated in causing HDFN to various severity while there is controversies regarding the status of anti G.[10,11] Studies have advocated anti-G with high titers to be associated with moderate to severe degree of HDFN.[12,13]

In view of transfusion support, it is not required to differentiate between the 3 antibodies because these patients will receive D and C antigen negative PRBCs, irrespective of the presence of anti-G.

Here though the mother had ICT positive, further immunohematology work-up was not performed. In resource poor countries like India, very limited institutions have the provision of antibody screening and identification. Many cases of HDFN come from peripheral health services without any complete diagnostic evaluation endangering the fetus to develop severe form of HDFN. Hence it is relevant to refer the patient as early as possible for Immuno hematological work up to find all the implicated antibodies in a tertiary care center if ICT is positive. It influences the transfusion medicine support with corresponding antigen negative blood products affecting the final clinical outcome.

CONCLUSION

ICT/antibody screening has a clinical importance in prognosis of HDFN. With limited facilities of antibody screening, all pregnant women should be at least advised for ICT irrespective of Rh D negative status. If ICT is positive, it should not be interpreted as only anti-D. Cases should be referred to higher center for antibody screening and identification so that complete antibody and antigen profile could be determined and also proper blood unit for DVET could be identified.

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