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Letter to Editor

Transfusion Medicine Section

Importance of Maternal Red Cell Antibody Screening: A Spectrum of Two Neonatal Cases with a Positive Direct Antiglobulin Test

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Dear Editor,

The Rh blood group system is highly polymorphic, immunogenic and complex human blood group system, the major antigens being the D, C, c, E, and e antigens [1]. Its role is well-established in the Haemolytic Disease of the foetus and the Newborn (HDFN), wherein anti-D alloantibody, primarily of IgG type, reacting optimally at 37°C, cross the placenta and induces a haemolytic process [2]. Amongst other Rh antigens, 'c' antigen is quite immunogenic and is associated with severe HDFN and is present in almost 80% of the Indians [3]. Anti-c alloantibody, is also primarily of IgG type, and has the potential to cause HDFN [4]. Maternal Antibody Screening (ABS) is undertaken to detect such clinically significant Immunoglobulin G (IgG) type of Red Blood Cell (RBC) alloantibodies. The prevalence of such alloantibodies may vary due to ethnic differences; however, it is present in approximately 1% of pregnant women [5].

Two cases of HDFN due to anti-c alloantibody are being reported here. The Direct Antiglobulin Test (DAT), compatibility testing, ABS (3-cell panel) and identification (11-cell panel) were done by Column Agglutination Technique (CAT) (Bio-Rad, Switzerland) [6]. Antibody identification was also performed on Fully Automated Immunohaematology Analyser (FAIHA) (Neo, Immucor Inc., USA) which uses a 14-cell panel and is based on solid red phase cell adherence assay [7]. Heat elution at 56°C was also performed on DAT positive neonatal RBCs [8].

First report was of a neonate, Appropriate for Gestational Age (AGA) (2.75 kg), born to a G3P2002 mother at 37 weeks gestation, who was admitted on day 9 of life for anaemia {haemoglobin (Hb)- 6.5 gm/dL} after referral from a hospital to the institute. Investigations revealed that the neonate was glucose-6-phosphate dehydrogenase deficient. The mother had a transfusion history in her first pregnancy five years back. The peripheral smear showed acanthocytes, echinocytes and few tear-drop RBCs. Two A RhD positive Packed RBC (PRBC) pedibags of 100 mL each, negative for 'c' antigen, extended Rh (C,E,c,e) and K antigen matched with mother and crossmatch compatible with mother's serum, were transfused uneventfully. Immunohaematology findings of the neonate and mother are presented in [Table/Fig-1].

In another case, a neonate, AGA (3.25 kg), born to a G4P2012 mother at 37 weeks gestation, was admitted on day 10 of life in view of increasing pallor and Hb of 3.4 gm/dL. The peripheral smear showed anisopoikilocytosis, reduced RBC density, tear drop RBCs and polychromasia. On checking blood requisition records, it was found that the neonate was issued one O RhD positive PRBC unit three days back. This compatible top-up transfusion was retrospectively phenotyped (C+E-c-e+K-) from the preserved tube segment of the PRBC unit and was found to be identical to that of the mother's phenotype. This neonate was transfused uneventfully with PRBC pedibag (90 mL), extended Rh (C,E,c,e) and K antigen matched and crossmatch compatible with mother. The immunohaematology findings of the neonate and mother are presented in [Table/Fig-1].

In both the cases, the neonates had HDFN due to anti-c alloantibody. The standard treatment modalities in these neonates are phototherapy and Exchange Transfusion (ET) [9]. In a large prospective study by Koelewijn JM et al., on 298000 pregnant women to study the risk of HDFN caused by non-D alloantibodies, it was found that anti-K lead to severe HDFN in 26%, anti-c in 10%, anti-E in 2% [10]. Phototherapy was required in 33%. Wenk RE et al., reported 70 cases of anti-c antibody in mother with c+ neonates, where 29% of the neonates had moderate HDFN and required transfusion therapy [9]. In one more series by Kozlowski CL et al., (100 cases) [11], 14% of the affected neonates required ET, while Hackney DN et al., in their study found that 25% of the c+ foetuses had severe HDFN and 17% required intrauterine transfusions [12]. In a study from our centre by Sankaralingam P et al., on 1000 RhD positive pregnant women, 0.7% were alloimmunised, anti-E was the most common (85.7%), followed by anti-c (71.4%), anti-Cw (14.3%) and anti-S (14.3%) alloantibodies [13]. Exchange transfusion was

Case	Blood group (ABO and RhD)	Alloantibody specificity		Extended Rh and Kell phenotype					Neonatal DAT	Eluate yield*	At admission		At discharge (Post-transfusion)	
		By CAT	By FAIHA	C	E	c	e	K			TSB (mg/dL)	Hb (g/dL)/ Hct (%)	TSB (mg/dL)	Hb (g/dL)/Hct (%)
Case 1														
Neonate	A RhD positive	Anti-c, Anti-E	Anti-c	N	N	+	+	N	4+	Anti-c	14.8 (DB-1.6)	6.5 /15%	9.2	40%
Mother	A RhD positive	Anti-c, Anti-E	Anti-c	+	N	N	+	N	-	-	-	-	-	-
Case 2														
Neonate	O RhD positive	Anti-c, Anti-E	Anti-c	+	+	+	+	N	4+	Anti-c	2.5 (DB- 1.2)	3.4	1.1	7.5 g/dL
Mother	O RhD positive	Anti-c, Anti-E	Anti-c	+	N	N	+	N	-	-	-	-	-	-

[Table/Fig-1]: Laboratory and immunohaematology findings of the neonates and their mothers.
CAT: Column agglutination technique; FAIHA: Fully automated immunohematology analyser based on solid phase red cell adherence (SPRCA); DAT: Direct antiglobulin test; RBCs: Red blood cells; +: positive; N: negative; TSB: Total serum bilirubin; Hb: Haemoglobin; Hct: Haematocrit; DB: Direct (=conjugated) bilirubin (mg/dL); *Heat elution (at 56°C) of DAT positive neonatal RBCs

required in two of the four (50%) DAT positive neonates and the mean duration of phototherapy were also higher in the four DAT positive neonates (p-value <0.01).

From India, Sheeladevi CS et al., reported the first case of HDN due to anti-c alloantibody in a RhD positive mother (2007), where the diagnosis was established retrospectively [14]. In both of the present cases, the neonates did not require ET and were treated with top-up transfusions and phototherapy and responded well. This could possibly be attributed due to lower maternal anti-c titer, however, in the present cases the maternal titer could not be determined. Usually, a critical titer of 16 or 32 and more requires closer monitoring of the foetus during the pregnancy.

In the present cases, the possible alloantibodies using CAT method were anti-c and anti-E. As there was no 'select-cell' (E+c-) in the 11-cell identification panel, anti-E alloantibody could not be ruled out. Thus, a 14-cell panel of was used on FAIHA which established the presence of only the anti-c alloantibody and excluded anti-E, as one of the panel cells which was E+c- gave a 'negative' result. Thus, 'select-cell' process turned out to be very useful in ruling out anti-E alloantibody.

Screening for non RhD alloantibodies in pregnant women is still not routinely performed in the country due to various limiting factors including cost, lack of resources, expertise and awareness. Based on the above mentioned studies and our present case report (of HDFN due to anti-c), it is recommended that all antenatal mother should be screened for red cell alloantibodies. However, due to limited resource availability in blood centres across the country it may not be feasible to perform antibody screen in all pregnant women. Nevertheless, it may be done at those tertiary care centres where antibody screen facility is available. Thus, present cases re-emphasise the need for a protocol for antibody screen in antenatal women to identify and monitor the foetus at risk of developing HDFN.

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