Necessity of Improvised Antenatal Testing for Rh Isoimmunization – Exploring From a Case Series

Rhesus (Rh) isoimmunization is a preventable cause of neonatal morbidity and mortality. Sequential antenatal indirect antiglobulin testing (IAT) and maternal Rh immunoglobulin (RhIg, anti-D) prophylaxis are known preventive interventions. Around 3.7 lakh cases of Rh isoimmunization are recorded worldwide per year, and out of them, 56,672 cases per year are from India [1]. Though there is a decline in such cases in low- and middle-income countries (LMICs), including India, there is still a lot of scope for improvement. In this case series, Rh isoimmunization was surprisingly diagnosed in five neonates delivered to Rh-negative mothers during the postnatal period, despite antenatal negative IAT and RhIg prophylaxis at peripheral health care facilities, raising questions about the efficacy ofIAT methodology in the LMIC setup. Additionally, failure of RhIg as a preventive therapy is concerning.

The case details were retrieved from the records of the immunohematological (IH) work-up performed for the neonatal exchange transfusion blood requests at a tertiary centre in India. Maternal samples for blood grouping were analyzed by forward and reverse typing using the column agglutination technique (CAT-Fwd-rvs gel card, Tulip Diagnostics Limited, India) and antibody screening using ReaCell I-II-III (Tulip Diagnostics Limited, Hungary). The discrepancies between forward and reverse blood results were resolved as per standard methods [2]. The implicated antibody was identified using ReaCell ID-Panel (Tulip Diagnostics Limited. 2023, Hungary) through an IAT for positive antibody screening results. In the presence of an anti-D and anti-C pattern, sequential adsorption-elution studies using select cells were performed to differentiate it from anti-G [3]. In conventional tube technique (CTT), the maternal serum IgM titres were determined at room temperature and IgG titres at anti-human globulin (AHG) phase after dithiothreitol (DTT) treatment. Neonatal samples were analysed for forward blood typing, the direct antiglobulin test (DAT), and an elution study on DAT-positive cells.

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Four singleton late preterm neonates and one term neonate had clinical features of hemolytic disease of the newborn (HDN). All mothers had a single time negative IAT result at 28–30 wk of gestation and had received antenatal RhIg prophylaxis during the current as well as previous conceptions. There was no history of preceding antepartum hemorrhage or blood transfusions in any affected pregnancies or hydrops fetalis. Maternal and neonatal characteristics are depicted in **Table I**.

High anti-D IgG titers were found in all the five maternal samples. Multiple antibodies against the Rh system were detected in two maternal samples (case 4 and case 5). Maternal blood group discrepancies found in cases 1, 2, and 4 led to identification of IgM phase anti-D.

Two neonates (case 1 and case 4) required exchange transfusion, intensive phototherapy, and intravenous immunoglobulin; one neonate (case 3) was treated with intensive phototherapy, and two newborns (case 2 and case 5) succumbed.

The process of selecting and preparing reconstituted blood components took approximately 4 h for the first-time request of exchange transfusion, in the presence of multiple antibodies, and 3-3.5 h for cases involving a single antibody. The procedure involved coordinated efforts of three skilled experienced staff working simultaneously in IH and component preparation unit. Requirement of blood products for the second cycle was addressed within 15 minutes of receiving the request.

The presence of high titer of IgG anti-D in the maternal sample, grade 4+ direct antiglobulin test (DAT) in the neonatal sample and clinical features of HDN, suggested Rh isoimmunisation in all cases. Surprisingly, all mothers had received RhIg prophylaxis. The increased strength of DAT reflects the severity of hemolysis and need for a therapeutic intervention [5]. In contrast, lower IgG titer (d" 8) in maternal sample are observed with passive RhIg prophylaxis. Rh isoimmunization in clinical practice, may be attributed to various perinatal factors such as prior cesarean section, packed red blood cell transfusion [6], inadequate RhIg prophylaxis, potential genetic variations

Table I Maternal and Neonatal Demographic Characteristics, Immunohematological Result and Neonatal Outcome of Rh Isoimmunization

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*
Maternal characteristics					
Age (years)	27	26	30	33	29
Blood group	B negative	B negative	AB negative	O negative	O negative
Blood group discrepancy	Yes (due to anti-D)	Yes (due to anti-D)	No	Yes (due to anti-D)	No
Obstetric History	G2P1A1L0	G3P2A1L0	G3P2A1L1	G2P2L1	G2P1A1
Outcome of previous pregnancy	G1 MTP at 16 wk	G1: Male child delivered by vaginal route; Died at one month of age, cause unknown; BG: B Rh D positive G2: Spontaneous abortion at 8 wk	G1: Male child delivered by caesarean section; no neonatal morbidity; BG: B Rh D positive G2: Ectopic pregnancy	G1: Term male child delivered by caesarean section, no neonatal mor-BG: O Rh D positive	G1: Spontaneous abortion at 8 wk
Anti-D prophylaxis	G1 One dose RhIg post-termination G2 One dose RhIg at 28 week	G1 Two dose RhIg at 28 week and within 72h delivery G2 One dose RhIg post abortion G3 One dose RhIg at 28 week	G1 Two doses RhIg at 28 week and within 72 h of delivery G2 One dose RhIg post termination G3 One dose RhIg at 28 week	G1 One dose RhIg within 72 h of delivery G2 Two doses of RhIg at 28th and 32 week	G1 One dose RhIg post-bortion G2 One dose RhIg at 30 week
IAT in current pregnancy	Negative at 28th week	Negative at 28th week	Negative at 28th week	Negative at 28th week	Negative at 30th week
Antibody screening in current pregnancy	Anti-D detected at RT and IAT phase	Anti-D detected at RT and IAT phase	Ant-D detected in IAT phase	Anti-D detected at RT phase and Anti-D, Anti-C, Anti-G detected at IAT phase	Anti-D and Anti-C detected in IAT phase
IgM titres	Anti-D: 2	Anti-D: 256	Anti-D: Nil	Anti-D: 4 Anti-C: Nil Anti-G: Nil	Anti-D: Nil Anti-C: Nil
IgG titres	Anti-D: 512	Anti-D: 1024	Anti-D: 32	Anti-D: 64 Anti-C: 8 Anti-G: 8	Anti-D: 1024 Anti-C: 8
Neonatal characteristics					
Gestational age	36 wk	34 wk	36 wk	38 wk	34 wk
Mode of delivery	Cesarean	Cesarean	Cesarean	Cesarean	Cesarean
Birth weight(kg)	3.1	1.5	2.7	3.1	1.6
Blood group	B positive	O Positive	B positive	O positive	O positive
Monospecific DAT	IgG, Grade:4+	IgG, Grade:4+	IgG, Grade:4+	IgG, Grade:4+	IgGGrade:4+
Neonatal management	Jaundice at 4h of age Phototherapy, 2 cycles of DVET, IVIG	Jaundice at 1h of age Phototherapy, DVET	Jaundice on day 2 Phototherapy	Jaundice at 2h of age Phototherapy, 2 cycles of DVET, IVIG	Cardiorespiratory support
Neonatal outcome	Discharged on day 6	Died at 6 h of age	Discharged on day 8	Discharged on day 20	Died at 2 h of age

A Abortion, Ab Antibody, DAT Direct antiglobulin test, DVET Double volume exchange transfusion, G Gravida, IAT Indirect antiglobulin testing, IVIG Intravenous immunoglobulin, L Living, MTP Medical termination of pregnancy, P Para, RhIg Rh immunoglobulin, RT Room temperature, Wk Week

in the Fc gamma receptor (FCGR) gene, or increased expression of the functional inhibitory receptor Fc^3RIIc [7].

The presence of an unexpected antibody was perceived while resolving blood group discrepancy in our three cases, which had been missed in prior antenatal testing. As most centers of LMIC depends on forward blood group typing by slide/tile method to detect A, B antigen present over the RBC. Reverse typing with O cells aids in identifying unexpected antibodies to other blood group systems. Using identification panel cells at room temperature, the antibody was identified as IgM anti-D. It indicates either recent maternal exposure to fetal D+ red cells or a delay in the class switching from IgM to IgG.

In case 4 and case 5, alloimmunization to other antigens of Rh system apart from D antigen, i.e., anti-C and anti-G were noticed. The presence of multiple antibodies could have explained the disease severity [8], i.e., requiring two-times exchange transfusion, intravenous immunoglobulin and phototherapy, with prolonged hospitalization in case 4 and immediate neonatal death in case 5.

The major challenges in appropriate detection of isoimmunization include a lack of uniformity in testing methods, skilled technicians, advanced semi-automated or automated systems, accessibility to commercial cell panels, quality assurance of laboratory facilities, and legislative restrictions on performing tests in LMIC setup [9]. Most centers rely on conventional tube testing (CTT) using in-house pooled O-positive cells or reagent O-cells for IAT testing. Moreover, CTT has lower sensitivity than column agglutination technique (CAT) in antibody detection and identification, i.e., anti-E and anti-C, and thus a limited role in diagnosing non-D Rh isoimmunization [10].

A comprehensive IH work-up during pregnancy is needed considering that the presence of Rh isoimmunization might be concealed when using the existing laboratory facilities. This may also facilitate timely provision of blood components when needed. Though, we could not establish the exact cause of RhIg failure, this report highlights the critical importance of having advanced IH work-up, i.e., the CAT method and panel cells, and their appropriate interpretation in the management of Rh isoimmunization.

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