

## Case Report:

### Haemolytic disease of the newborn due to multiple maternal antibodies

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#### ABSTRACT

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Haemolytic disease of the foetus and newborn (HDFN) is a condition in which the lifespan of an infant's red blood cells (RBCs) is shortened by the action of specific maternal immunoglobulin G (IgG) antibody. Rhesus (Rh)- D haemolytic disease of the newborn is a prototype of maternal isoimmunization and foetal haemolytic disease. Although rare, the other blood group antigens capable of causing alloimmunization and haemolytic disease are c, C, E, Kell and Duffy. We report a case of HDFN due to anti-D and anti-C in the maternal serum as a result of anamnestic response to Rh-D and C antigens. This report highlights the importance of antibody screening in antenatal women which could assist in diagnosing and successfully treating the foetus and newborn with appropriate antigen negative cross-matched compatible blood.

**Key words:** Alloimmunization, Anti D, Anti C, Antenatal Screening, haemolytic disease of newborn

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#### INTRODUCTION

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Haemolytic disease of the newborn (HDFN) occurs due to the premature destruction of the red blood cells (RBCs) of the foetus in uterus and in neonates in early neo-natal period after delivery. The RBC destruction is brought about most commonly by immunoglobulin G (IgG) antibodies produced by the mother due to the blood group incompatibility between mother and foetus. The mother can be stimulated to form IgG antibodies by previous pregnancy or transfusion and in small number during the pregnancy itself.

The most common cause of HDFN sufficiently severe to require treatment is incompatibility of Rh system and in 95% of these cases, Rh D antigen is responsible. Rarely other antigens of Rh system like c, C and E have been implicated.<sup>1</sup> The wide spread adoption of antenatal and postnatal Rhesus

immunoglobulin (RhIg) prophylaxis has resulted in marked decrease in the prevalence of alloimmunization due to Rh D antigen to less than 0.1%.<sup>2</sup> Maternal alloimmunization to other Rh, Kell and Duffy red cell antigens still remains as the cause of foetal disease since no prophylactic immunoglobulins are available to prevent the formation of these antibodies.<sup>3</sup>

It is recommended that routine red cell antibody screening in pregnant women be done at the first visit and, if no antibodies are detected, to be repeated in the third trimester between 28 and 36 weeks.<sup>4</sup> The guidelines<sup>4</sup> state that further testing is unnecessary, since immunization during late pregnancy is unlikely to result in an antibody concentration that would be sufficient to cause severe haemolytic disease of the neonate. Timely detection and close follow up of this condition is necessary to reduce harmful effects on the newborn. Transfusion services play a vital role in the antenatal detection,

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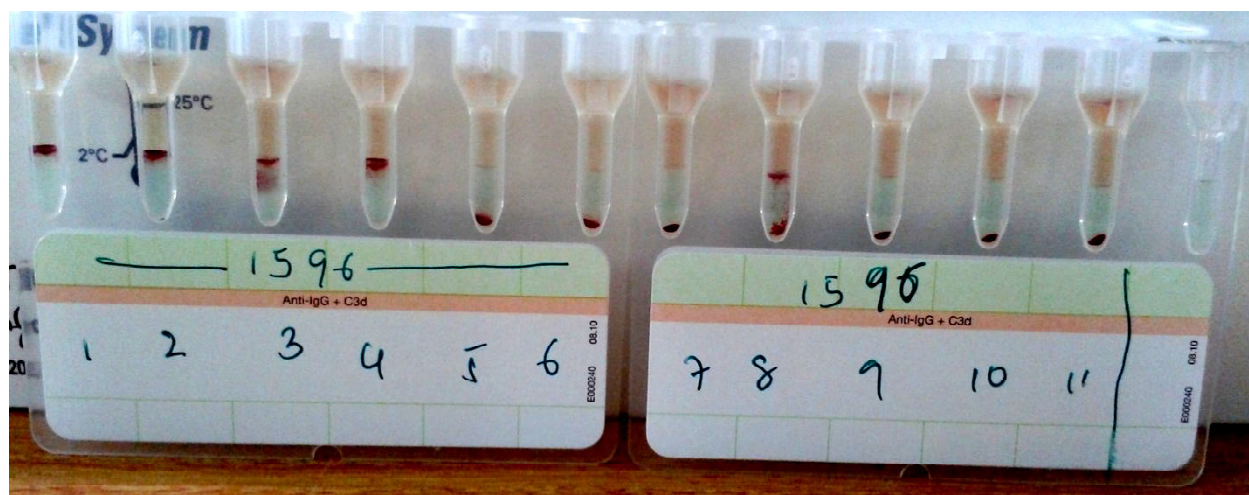
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prophylaxis. Maternal blood was tested for presence of alloantibody in our Department. Her blood group was confirmed to be 'AB' Rh D negative. Antibody screening and identification done using commercially available three and eleven cell antigen panel (ID Diacell; Diamed ID microtyping system, Diamed Switzerland) were positive and both "anti-D" and "anti C" were identified in the maternal serum (Figure 1 and Figure 2). The antibody status in the previous pregnancies was unknown. The antibody titer performed using saline double dilution method with R2R2

## CASE REPORT

A 22-year-old female presented to the Government Maternity hospital at 29 weeks of gestation. She had one previous live child and one abortion (G<sub>3</sub>P<sub>1</sub>L<sub>1</sub>A<sub>1</sub>). As per records her blood group was 'AB' Rh D negative. Her husband's blood group was 'A' Rh D positive and previous child's blood group was also 'A' Rh D positive. There was no history of previous blood transfusion or Rh immunoglobulin (Ig)



**Figure 1:** Antibody identification showing positive reaction in 1,2,3,4, and 8 cell panels of ID Diacell micro typing system

Set ID - Identifiant P: 4517.1.95.X (Japan: 4517.95.xx)										05361.95.x - 05461.95.x (New: 05361.95.x - 05461.95.x)										V.I.P. Software: P80		ID-DiaPanel-F	
Antigen-Tablet / Antigen-Tablet / Table d'antigènes / Tabella antigenica / Tabla de antígenos / Tabla de antígenos										Antikörper-Identifizierung / Antibody identification / Identification d'anticorps / Identificazione anticorpale / Identificação dos anticorpos													
Spender Donor Donateur Donante Donante Donor	Rh-hr	Kell	Duffy	Kidd	Lowes	P	MNS	Luth.	X0	Spez. Antigene Special types Antigènes spéc. Antigens por tipos Outros Antígenos Tipos especiais	Resultat / Result / Resultat / Resultado / Resultado / Resultado	Urid / Coombs	Enzyme	4°C	Bemerkungen / Remark / Remarques / Observações / Observações / Observações								
	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z	(D) C F E G H I J K L M N O P Q R S T U V W X Y Z														
1	CCCWd.ee R <sub>1</sub> WR <sub>1</sub>	050050	+	+	0	0	+	nt	nt	+	0	+	0	+	+	1	4+						
2	CCD.ee R <sub>1</sub> R <sub>1</sub>	208710	+	+	0	0	+	nt	nt	0	+	0	+	0	+	2	4+						
3	ccD.EE R <sub>2</sub> R <sub>2</sub>	260663	+	0	+	0	0	+	nt	nt	+	0	+	0	+	3	3+						
4	Ccddee r'r	679120	0	+	0	+	0	+	nt	nt	0	+	0	+	0	4	4+						
5	cddEe r'r	212217	0	0	+	0	0	0	nt	nt	0	0	0	+	+	5	0						
6	cdddee rr	054645	0	0	0	+	0	+	nt	nt	+	0	0	+	0	6	0						
7	ccdee rr	699575	0	0	+	0	0	+	nt	nt	0	+	0	+	0	7	0						
8	ccD.ee R-r	018511	+	0	+	0	0	0	+	0	0	+	0	+	0	8	4+						
9	cdddee rr	984971	0	0	0	+	0	0	+	nt	nt	0	0	+	0	9	0						
10	cdddee rr	502600	0	0	0	+	0	0	+	nt	nt	0	0	+	0	10	0						
11	cdddee rr	036765	0	0	0	+	0	0	+	nt	nt	0	0	+	0	11	0						
Patient / Patient / Patient / Patient / Patient / Patient										Eigenkontrolle / Autocontrol / Autocontrol / Autocontrol / Autocontrol / Autocontrol													
Anmerkungen siehe rückseitig / Remarks see overleaf / Voir les remarques au verso / Per le note consultate il retro / Ver observaciones en el reverso / Ver observações no verso																							
Name / Name / Nom / Nome / Nombre / Nome		Blutgruppe + Antigene / Blood group + antigens / Groupe sanguin + antigènes / Gruppo sanguigno + antigeni / Grupo sanguíneo + antígenos / Grupo sanguíneo + antígenos				Interpretation / Interpretation / Interpretation / Interpretation / Interpretation / Interpretation				Datum / Date / Date / Data / Fecha / Data													
						Most probable antibodies: "anti-D, anti-C"																	

**Figure 2:** Antigram showing most probable antibodies anti-D and anti-C

(D+C-) cells was found to be 1 in 32 dilutions and with r'r (D-C+) cells, it was 1 in 4 dilutions and with R1R1 (D+C+) cells, the antibody had a titre of 1 in 32. Then the patient was lost to follow-up and she came back only at 32 weeks with premature rupture of membranes. Emergency caesarean section was conducted. The baby showed features of HDFN with serum bilirubin levels of 32 mg/dL. The baby was treated postnatally in neonatal intensive care unit with phototherapy and double volume exchange transfusion with compatible blood, and was discharged in stable condition.

### DISCUSSION

Antenatal services in India are variably available and there is a limited amount of published data on alloimmunization rates among pregnant women in India. Although guidelines for antibody screening in the sera of both Rh D positive and Rh D negative women during the first antenatal visit have been laid down by the Director General of Health Services, India<sup>1</sup>, it is being done primarily for Rh D negative women or those presenting with an adverse obstetric history.

HDFN may be classified as either immune or non-immune. Immune HDFN results from maternal antibodies that are capable of crossing the placenta to react with foetal antigen causing a reaction that manifests as foetal red cell hemolysis. HDFN due to anti-D tends to be more severe than that due to anti-c. Anti-K is next in importance after anti-c. Anti-C, Anti-E and Anti-e do not often cause HDFN; when they do, it is usually mild.<sup>5</sup>

In the largest retrospective review<sup>6</sup> on the aetiology of hydrops involving 598 patients, the cause of hydrops was established unclearly in 26.3% of cases, isoimmunization in 4.5% of cases, and non-immune aetiology in 69.2% of cases. When isoimmunization was identified as the cause, Rhesus antigens (c, C, D, e, E) were responsible for 4.2% of cases and the remaining 0.3% were due to Kell and Duffy

antibodies.<sup>6</sup> An overall alloimmunisation rate of 1.25% has been reported in pregnant women.<sup>7</sup> Among this anti-D was about 78.43%, anti-c about 1.96% and the remaining all are antibodies against C, Kell and MNS.<sup>7</sup>

In a prospective study which was carried out on 624 antenatal cases, red cell antibody screening was positive in 9 (1.4%) out of 624 cases. These were identified as anti-D antibody (6 cases, 66%), anti-D with anti-C antibody (2 cases, 22%), and anti-M antibody (1 case, 11%).<sup>8</sup> In our case, we identified a combination of anti-D and anti-C antibodies which caused a severe HDFN mainly because of significant anti-D. Whenever anti-D with anti-C combination is present, anti-G should be suspected as anti-G apparently will have anti-D+C specificity. It is generally accepted that anti-G, and not anti-C+D, is present in pregnancy whenever the titer against C+D- (r'r) red cells is higher than the titer against C-D+ (R2R2) red cells.<sup>9</sup> But in our patient the titre was higher with R2R2 cells (1 in 32) than with r'r cells (1 in 4). In order to identify the true specificity of the sera, one aliquot of the patient serum was adsorbed with D-C+ cells (aliquot 1) and another aliquot was adsorbed with D+C- cells (aliquot 2). The adsorbed serum from aliquot 1 gave reaction with R2R2 cells and not with r'r cells whereas the adsorbed serum from aliquot 2 gave reaction with r'r cells and not with R2R2 cells. If only anti-G would have been present, then both the adsorbed serum should have given negative reaction with both R2R2 and r'r cells. Thus presence of both anti-D and anti-C was identified (Table 1). Thus, the antibody specificity in our case was due to both anti-D and anti-C and not due to anti-G alone. However, presence of anti-G in addition to anti-D and anti-C in our case could not be ruled out because of lack of elution method.

When the antibody titer is less than 1 in 8 dilutions, whether directed to D or another paternal antigen capable of causing severe

**Table 1: Specificity of antibody**

<b>Aliquot 1</b> (Adsorbed serum with D-C+ cells) <b>Reaction with</b>		<b>Aliquot 2</b> (Adsorbed serum with D+C- cells) <b>Reaction with</b>		<b>Antibody identified*</b>
R2R2	r'r	R2R2	r'r	anti-D and anti-C
+	0	0	+	

\*If anti-G alone was present, then the adsorbed serum from both aliquots would give negative reaction with R2R2 and r'r cells

erythroblastosis, no intervention is necessary; when the titer is greater than 1 in 16 dilutions in albumin or 1 in 32 dilutions by indirect antiglobulin test, doppler study of middle cerebral arterial peak systolic velocity can be used for monitoring. It correlates with the severity of the anemia and to be diagnostically equivalent to amniocentesis, without the risks.<sup>11</sup> However, critical titre needs to be standardized with respect to individual laboratories. In our patient, a titre of 1 in 32 dilutions was associated with HDFN.

The management of anti-C isoimmunization or iohimmunization with any other irregular red blood cell antibody is similar to the management of anti-D isoimmunized pregnancy, with a specification that blood used for foetal and/or neonatal transfusion should be negative for the specific antigen. There was a significant improvement in foetal and neonatal anemia following transfusion with compatible (D and C antigen negative) blood, attributing it as the sole cause of fetal red cell haemolysis.

Despite prophylactic use of RhIg, anti-D is still a common antibody identified as the major cause of alloimmunisation. In this case, a moderately severe type of HDFN was detected in the neonate, which was caused by a combination of anti-D and anti-C antibody. We recommend prenatal antibody screening at first visit and a close follow-up throughout pregnancy if unexpected red cell antibodies are present.

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