

CASE REPORT

Anti-M induced severe haemolytic disease of foetus and newborn in a Malay woman with recurrent pregnancy loss

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Abstract

Haemolytic disease of the foetus and newborn (HDFN) is caused by maternal red blood cells (RBC) alloimmunisation resulted from incompatibility of maternal and foetal RBCs. However, only a few HDFN attributed to anti-M were reported, varying from asymptomatic to severe anaemia with hydrops foetalis and even intrauterine death. A case of severe HDFN due to anti-M alloantibody from an alloimmunized grandmultiparous Malay woman with recurrent pregnancy loss is reported here. The newborn was delivered with severe and prolonged anaemia which required frequent RBC transfusions, intensive phototherapy and intravenous immunoglobulin administration. Although anti-M is rarely known to cause severe HDFN, a careful serological work-up and close assessment of foetal well-being is important, similar to the management of RhD HDFN. Alloimmunisation with anti-M type can lead to severe HDFN and even foetal loss.

Keywords: anti-M, HDFN, Malay woman, recurrent pregnancy loss

INTRODUCTION

Haemolytic disease of foetus and newborn (HDFN) is caused by trans-placental passage of clinically significant maternal red blood cell (RBC) alloantibody which is directed against the RBC antigen on the foetal RBC. The maternal IgG antibodies coat the foetal RBC antigen and cause foetal haemolytic anaemia. Unlike an ABO antigen system, the MN system determinants are fully developed on foetal RBCs and they can be detected as early as 9 weeks of gestation.¹ Although ABO antigens can be detected as early as 5 to 6 weeks of gestation, they are not fully developed until the age of 2 to 4 year old.²

Anti-M is known to cause HDFN, but the incidence is low and the reported cases vary from asymptomatic to severe anaemia with hydrops foetalis³ and even intrauterine death (IUD).⁴ Anti-M antibody of IgM type usually occurs naturally⁵ and is clinically not significant; it causes neither HDFN nor haemolytic transfusion reaction (HTR). However, when the antibody is reactive at 37°C which is of IgG type, it becomes clinically significant and can cause HDFN and HTR.^{5,6} To the best of our knowledge, there is

no report in this region on severe HDFN due to anti-M.

CASE REPORT

A grandmultiparous 39-year-old Malay woman, G₁₀P₂₊₇ at 38 weeks of gestation with a history of recurrent pregnancy loss (RPL), delivered a male infant via emergency lower segment caesarean section due to acute foetal distress. Antenatally, she had regular uneventful follow-ups at the peripheral health centre and serial ultrasound findings prior to delivery showed normal foetal development. Throughout the pregnancy, she was on thromboprophylaxis with subcutaneous enoxaparin as part of the management for thrombophilic risk due to her previous RPL problems. She had also been confirmed to have mild protein S deficiency after her seventh pregnancy. Her RBC alloimmunisation status from the previous pregnancies was unknown. There was no history of blood transfusion in this subject for the reason of RBC alloimmunisation in this case. The chronology of the pregnancy outcomes is outlined in Table 1.

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TABLE 1: Chronology of pregnancies and outcomes

Number of pregnancy	Year	Period of gestation (weeks)	Pregnancy outcome
1	2003	16 weeks	miscarriage
2	2003	20 weeks	miscarriage
3	2004	8 weeks	miscarriage
4	2005	38 weeks	boy, 3.95 kg, macerated intrauterine death, unknown cause
5	2006	12 weeks	miscarriage
6	2006	20 weeks	miscarriage
7	2008	16 weeks	miscarriage
8	2011	32 weeks	boy, 2.7 kg via LSCS due to foetal distress, expired day 1 of life of unknown cause
9	2014	20 weeks	miscarriage
10	2015	38 weeks	boy, 3.4 kg via LSCS due to foetal distress with severe HDFN, the only living child (in this case report)

LSCS=lower segment caesarean section

The baby was born flat (poor Apgar score) with severe pallor and mild jaundice, but no evidence of hydropic or dysmorphic features. The baby was intubated and managed in the neonatal intensive care unit. The baby was suspected clinically of having HDFN and the diagnosis was confirmed after blood investigations. The results showed haemoglobin (Hb) level of 4.0 g/dL, reticulocytes count of 0.81% and total serum bilirubin of 88 μ mol/L. The peripheral blood film showed severe anaemia with spherocytosis and occasional nucleated RBC with normal white cell and platelet counts. There was no significant fetomaternal haemorrhage from the Kleihauer test and the viral screening for intrauterine infections; Toxoplasmosis-Rubella-Cytomegalovirus-Herpes Simplex-HIV screening was negative.

Immunohaematological tests showed that both mother and her newborn were blood group O, Rhesus D positive. Antibody screening and identification (Diamed-ID Gel micro typing system) detected the presence of alloantibody with anti-M specificity in both mother's and baby's plasma which reacted at 37°C and later was destroyed by enzyme test. The auto control result was negative. The titration of the anti-M in the mother's plasma at room temperature and 37°C (indirect antiglobulin test) using known M positive red cells revealed a titre of 1:256 and 1:128 respectively. However, the immunoglobulin class and IgG subclass was unable to be determined due to limited

resources. The cross-matching test using mother's and newborn's plasma together with a few other M antigen positive (M⁺) donor red cells were incompatible at antihuman globulin (AHG) phase, thus confirming the presence of clinically significant nature of this anti-M (IgG type) in the mother's plasma. However, the direct Coombs test (DCT) of the newborn was negative. The mother and newborn phenotypes were homozygous N⁺ (NN) and heterozygous M⁺N⁺ (MN) respectively.

The baby was treated with intensive phototherapy and received packed red cell (PC) transfusions with compatible O RhD positive and M antigen negative (M⁻) PC with a total transfusion of 30 ml/kg during the few days of life. The intravenous immunoglobulin (IVIg) infusion was also given on day 5 of life after noticing that the trend of Hb level was dropping. He was discharged well on day 7 of life at Hb level of 12.3 g/dL. However, the baby still required a few episodes of PC transfusion because of prolonged anaemia until 2 months of age.

DISCUSSION

The incidence of HDFN related to anti-M was reported to be low and only a few cases with severe disease are reported.⁴ The previous study on RBC alloimmunisation in Malay pregnant women reported that only 0.04% (2 out of 5163 women) has anti-M⁷ and none of the newborn developed HDFN.⁸ Very few cases of severe

HDFN associated with anti-M have been reported and most of the reported cases were associated with severe foetal anaemia and RPL between 10 to 35 weeks of gestation.⁴ Thus, a history of previous RPL between 8 to 38 weeks of gestation in this case initially was thought to be due to thrombophilia (mild Protein S deficiency), though it could also be due to anti-M HDFN. However, it is difficult to confirm as the status of anti-M alloimmunisation in the previous pregnancies was unknown.

Anti-M induced HDFN was considered in this case, as the clinically significant anti-M was detected in both mother's and newborn's plasma which reacted at 37°C and AHG phase. The blood investigations carried out on the baby supported the ongoing haemolytic process. In addition, other causes of neonatal anaemia such as blood loss, fetomaternal haemorrhage, and infection,⁹ had been excluded. The baby up until this case report was presented, had shown no recurring anaemia and has normal milestone development. The finding of severe anaemia and negative DCT was consistent with a few other reported cases of HDFN due to anti-M.^{1,4,10} A negative DCT and poor reticulocyte response in anti-M mediated HDFN could be explained by 2 hypotheses: a) due to destruction of erythroid progenitors in the bone marrow rather than the mature RBC in the peripheral blood since MNS antigen is fully expressed on immature erythroid precursors.⁵ This explanation is similar to cases affected by anti-K,^{10,11} which may lead to poor reticulocyte response to anaemia; and b) due to rapid intravascular haemolysis, thus all the anti-M coated RBC were lysed and gave a negative DCT upon investigation.¹⁰ Inappropriate low reticulocyte count despite severe anaemia and prolonged anaemia for over a month have been reported previously in anti-M induced HDFN.^{10,12} This could be explained as above by the destruction of erythroid precursors and delayed erythroid regeneration from the anti-M effect in the baby's bone marrow.

It was reported that the severity of HDFN is not directly correlated with anti-M titre since the detection of antibody titre varies depending on the technique, the incubation temperature and suspension media against M antigen expression on RBC used.^{3,10} In this case report, the anti-M titre was showed to be high in the maternal plasma. It is important to determine the immunoglobulin class or subclass of anti-M (either IgM or IgG), as the clinically significant anti-M is of IgG type.^{5,6} However,

the immunoglobulin class (IgM or IgG) and IgG subclass of anti-M in this case report was unable to be determined due to limited resources in our laboratory.

Anti-M generally is predominantly of IgM type, but about 50% to 80% of anti-M antibodies also contain a component of IgG forms.¹ Anti-M which was detected in untreated plasma might be misinterpreted as only IgM since IgG anti-M also often agglutinate M+ red cells directly in saline because M antigen epitopes are located at the terminal end of glycoprotein A (GPA) and furthermore GPA is abundantly expressed on red cells.^{1,2} Dithiothreitol (DTT)-treated plasma is able to determine IgG type of anti-M as IgM will be inactivated by DTT.^{1,3} Thus, the persistence of the reaction after DTT treatment suggesting the presence of an IgG component of anti-M. Meanwhile, the IgG subclass can be determined by using gel cards (DAT IgG1/IgG3) after adsorption of antibody on red cells homozygous for M antigen.³

Our case showed that the newborn required intensive treatment including frequent PC transfusion, intensive phototherapy and IVIg administration. Previous studies reported a few cases of severe HDFN due to anti-M which were successfully treated with more invasive treatment including intrauterine transfusion with M antigen negative PC,³ antenatal plasma exchange and direct injection of pooled IVIg into foetal abdominal cavity.¹³ In conclusion, clinically significant anti-M could cause severe HDFN and IUD. A thorough history of previous pregnancy morbidities and careful serological work-up accompanied by serial assessment of foetal well-being in anti-M alloimmunised pregnant woman is important, similar to the management for anti-D alloimmunisation. Obstetricians and pediatricians should be aware for the possibilities to treat anti-M alloimmunisation cases with intrauterine or exchange blood transfusion, immediately post delivery.

Conflict of interest: Authors declare no conflict of interest.

REFERENCES

1. Yasuda H, Ohto H, Nollet KE, *et al.* Hemolytic disease of the fetus and newborn with late-onset anemia due to anti-M: a case report and review of the Japanese literature. *Transfus Med Rev.* 2014; 28: 1-6.
2. Cooling L. ABO, H, and Lewis blood groups and structurally related antigens. In: Roback JD, Combs MR, Grossman BJ, Hillyer CD, editors. *Technical*

- manual. 16th ed. Bethesda (MD): AABB Press; 2008. p. 361-85.
3. Bajpayee A, Dubey A, Sonker A, Chaudary RK. A case of severe foetal anaemia due to anti-M isoimmunization salvaged by intrauterine transfusions. *Blood Transfus.* 2014; 12: s302-4.
 4. Wikman A, Edner A, Gryfelt G, Jonsson B, Henter JI. Fetal hemolytic anemia and intrauterine death caused by anti-M immunization. *Transfusion.* 2007; 47: 911-7.
 5. Klein HG, Anstee DJ, editors. *Mollison's blood transfusion in clinical medicine.* 11th ed. Oxford: Blackwell Publishing; c2005. 865 p.
 6. Tondon R, Kataria R, Chaudhry R. Anti-M: Report of two cases and review of literature. *Asian J Transfus Sci.* 2008; 2: 81-3.
 7. Hassan MN, Mohd Noor NH, Noor SRJ, Sukri SA, Mustafa R. Red blood cell alloimmunisation among Malay pregnant women: a tertiary hospital experience. *Int Med J.* 2015; 22: 154-8.
 8. Hassan MN, Mohd Noor NH, Johan Noor SR, Sukri SA, Mustafa R, Luc Aster HVR. Hemolytic disease of fetus and newborn due to maternal red cell alloantibodies in the Malay population. *Asian Transfus Sci.* 2014; 8: 113-7.
 9. Widness JA. Pathophysiology of anemia during neonatal period, including anemia of prematurity. *Neoreviews.* 2008; 9: e520.
 10. Arora S, Doda V, Maria A, Kotwal U, Goyal S. Maternal anti-M induced hemolytic disease of newborn followed by prolonged anemia in newborn twins. *Asian J Transfus Sci.* 2015; 9: 98-101.
 11. Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA. Inhibition of erythroid progenitor cells by anti-Kell antibodies in fetal alloimmune anemia. *N Engl J Med.* 1998; 338: 798-803.
 12. Ishida A, Ohto H, Yasuda H, *et al.* Anti-M antibody induced prolonged anemia following hemolytic disease of the newborn due to erythropoietic suppression in 2 siblings. *J Pediatr Hematol Oncol.* 2015; 37: e375-7.
 13. Matsuda H, Yoshida M, Wakamatsu H, Furuya K. Fetal intraperitoneal injection of immunoglobulin diminishes alloimmune hemolysis. *J Perinatol.* 2011; 31: 289-92.