Neonatal hemolytic disease due to anti-A1 accompany fetomaternal transfusion: A case report

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Case Report

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Abstract

Background: A neonatal with severe anemia HB:48g/L was detected before blood transfusion. A major crossmatch-incompatible result was found with a AB donor and the further some of random 8 donors. To diagnosis the reason of anemia and the mismatched, a series serological tests were detected.

Case presentation: The AB positive neonatal was born by B positive mother and AB dad. The neonatal DAT was negative, but the elution was positive with A\textsubscript{1} cell and negative with A\textsubscript{2} cell. In his mother serum, titer 1024 anti-A were checked out in saline tube, after treated by DTT, titer 64 anti-A\textsubscript{1} and 2 anti-A were checked out. The neonatal was diagnosis with Hemolytic disease of the newborn (HDFN). His mother red cell showed a weak agglutinated with anti-A under microscopy. Then was diagnosis as fetomaternal hemorrhage (FMH) through the Kleihauer-Betke test and flow cytometry. A2B red blood were given combine with phototherapy, the neonatal HB was 108g/L on the day 11 before discharged.

Conclusions: This is a rare case concurrent FMH and HDFN. The massive red cell of fetus entered maternal blood because of FMH, and then AB fetus red blood tiggered the high titer anti-A\textsubscript{1} IgG and caused HDFN.

Introduction

Hemolytic disease of the fetus and newborn (HDFN) is an alloimmune hemolytic disease of newborns due to red blood type incompatibility between the mother and fetus. The IgG antibody transferred from maternal serum is reactive against fetal red blood cell (RBC) antigens inherited from the father, destroy fetal cells via the phagocytosis system of the fetus and newborn causing hemolytic disease in the fetus and newborn. ABO incompatibility between mother and newborn appears in 20% of all pregnancies, while 1–4% develops HDFN. The prevalence is higher among non-Caucasians (3–5%) than among Caucasians.\textsuperscript{1,2} Most cases of HDFN are ABO incompatibility from O mothers. The IgG antibody (anti-A/B) in O maternal blood transfer to the newborn blood and combine with A/B/AB antigen on the red blood cells, then cause hemolysis. HDFN secondary to ABO incompatibility due to anti-A\textsubscript{1} antibodies is very rare in non-O blood types.\textsuperscript{1,2}

Fetomaternal transfusion (FMH) is defined as the various amount fetal blood loss by entry into the maternal circulation, resulting in severe fetal blood loss and/or fetal hydrops, sometimes lead maternal hemolytic responses.\textsuperscript{3–5} Both FMH and HDFN are major causes of anemia in newborns. However, to date, there are seldom reports of HDFN following FMH. Herein, we report a case of HDFN second to FMH by the anti-A\textsubscript{1} antibodies, which provide new insights into the etiology of HDFN, Although the infant suffered from a long severe anemia due to FMH, the anti-A\textsubscript{1} antibody lead the further AB red blood cell destroyed.

Case Presentation

Infant’s medical history
A male infant with a birth weight of 3200 g at 6 hours after birth was admitted to our hospital on account of a 6-hour history of pallor. His mother had experienced gravida 2 para 1, and without blood transfusion history. This baby was delivered via cesarean section at a gestational age of 37 weeks + 4 caused by reduced of fetal movement. The Apgar scores of 8 at 1 minute and 8 at 5 minutes (with a 2-point reduction for skin color). He had a pale skin, shortness of breath, no sputum, spat up immediately after birth. Physical examination: T: 36.2 °C, P: 136 / min, R: 53 / min, SpO2 85%, pale skin, shortness of breath, without body edema. The laboratory results showed he was AB positive, red blood cell count of $1.2 \times 10^{12}$ /L, hemoglobin concentration of 43 g/L, hematocrit of 14.78%, platelet count of $157 \times 10^9$ /L, reticulocyte percentage of 18.8%, total bilirubin level of 26.5 µmol/L, conjugated bilirubin level of 12.3 µmol/L, unconjugated bilirubin level of 14.2 µmol/L, and lactate dehydrogenase level of 921 U/L. The baby was applied for transfused with red blood cells. But a major crossmatch-incompatible result was found in serologic cross-match test with AB positive donor. A series serological tests were then detected.

No personal information of enrolled patients was revealed in this case report. The need for ethical approval was waived by the hospital ethics committee.

**Laboratory tests**

**Diagnosis of FMH**

The baby was A₁B positive, his father was A₁ positive and mother was B positive before this pregnant, but the mother sample before delivery showed ABO discrepancy and there was a mixed appearance with anti-A in gel card and under microscope, indicated the additional A antigen in mother sample, the reverse typing is B. (Figure). The Flow cytometry was used to check the percent additional red blood cell, the result showed the 12.8% red blood cell with A antigen in her B red blood cells.

Then the Kleihauer-Betke test for mother peripheral blood for diagnosis of FMH. This case KBt was 12%, combined with follow history: The fetal appeared movement and heart rate falling from 37 weeks + 4 of gestation. After birth, the infant is pale, severe anemia appearance, hemoglobin was 43g /L. so the infant diagnosis as FMH.

**Identified HDFN**

The infant antibody screen negative, and DAT negative. But when cross-match for the him, his serum showed mismatched with a random AB, the further cross-match tests were performed with 8 randomly donors. In the gel card, his serum matched with all B and O, but partly matched with A and AB donors, indicating that probably IgG anti-A₁ may existed in his serum. Then all the A and AB donors red blood cells were tested the expression of A₁ antigen. The donors which red cell mismatched with patient serum were A₁ positive. And the matched donors were A₁ negative. Ensured the IgG anti-A₁ exsited in this A₁B infant serum.
The elution tested showed positive reacted with A1 cell and negative reacted with A2 cell. Anti-A₁ antibody was detected in infant’s elution fluid, which confirmed HDNF caused by anti-A₁ from his mother.

Then maternal anti-A titer was investigated on the first day postpartum. After DTT treatment, the serum showed titers 64 with A1 cell and 2 with A2 cell, indicating the titer of IgG anti-A is 64. The anti-A₁ titer before DTT treatment was 1024.

**Treatment**

The laboratory results during hospitalization are shown in Table. After admission, the baby was given low-flow oxygen. And matched A₂B red blood for transfusion, 0.25 units of RBCs were given on the first and second days of admission, respectively. The hemoglobin level increased from 43 g/L to 108 g/L after blood transfusion. Phototherapy was started on the day of admission and continued for 4 days. The highest serum bilirubin level was recorded as 109.7 µmol/L on the age third day. The baby recovered stable spontaneous breathing on the eighth day, and oxygen therapy was stopped. On the 11th day after admission, the baby breathed naturally. The percutaneous oxygen saturation without oxygen uptake was maintained above 90%. The bilirubin level determined percutaneously was 69 µmol/L, the blood glucose level was 5.8 mmol/L, and the body weight was 3240 g. The baby was then discharged.

**Discussion**

This is a rare HDFN case caused by anti-A₁ antibody from B maternal serum, accompany with severe FMH.

The cases of ABO-HDFN in neonates born by non-O mothers are rarely reported. IgM ABO antibodies are naturally occurring and largely do not cross the placenta in the A or B individuals. But IgG anti-A or B antibody can also be found in serum of B or A mothers. Jeonh et al⁶ reported a A₂ mother with titer 256 IgG anti-B in serum leaded HDFN in B newborns. Wang M et al⁵ reported a A mother with titer 1024 IgG anti-B leaded HDFN in her B newborns.

In B and O individuals, two natural antibodies anti-A₁ and anti-A can be detected⁷,⁸. Anti-A₁ mainly are IgM.⁹ Sometimes caused the positive and reverse stereotype inconsistent and rarely develop HDFN. Some reports anti-A₁ can caused acute or delayed hemolytic transfusion reaction¹⁰⁻¹⁶. Only few reports HDFN caused by anti -A₁¹⁷,¹⁸. In 1955, Hubinont¹⁸ first reported a A₁ female HDFN infant caused by anti-A₁ from her O mother. Our case is the first report of HDFN in A₁B infant caused by anti-A₁ from his B mother. In this case, mother was B, we found both anti-A and anti-A₁ in her serum, and the titer were 1024 in saline medium. After DTT treated, also titer 64 anti-A₁ was check out, and anti-A was titer 2. The higher titer of anti-A₁ results higher antibody transfer percent.

We speculate that severe FMH is the main reason to stimulate the mother to produce high titer anti A₁ antibody. Massive peripartum FMH has been reported in approximately 1 in 9000 births¹⁹. Massive acute
FMH can cause fetal death. Hydrops, abnormal fetal heart rate. Reduced or absent fetal movements are the most common symptom of massive FMH, as our case. Usually, the mother is almost asymptomatic, occasionally symptoms appear transfusion reaction (fever, chills, nausea)\textsuperscript{20}. But this mother did not complain similar symptoms like transfusion reaction. The vast majority of spontaneous FMHs are low-volume bleedings with no hemodynamic significance but can cause alloimmunization.\textsuperscript{21}

FMH is not a routine prenatal test for all pregnant women, but Rhesus (Rh) negative women were routinely tested for FMH after birth, and if the fetal to maternal hemorrhage (FMH) $\geq 6$ ml (the whole red blood $\geq 12$ ml, the additional RhIg should be given even though regular received RhIG at 28 weeks gestation, 36 weeks gestation.\textsuperscript{22} This indicated that FMH is a higher risk arouse the antibody increasing.

In this case, during pregnancy, A\textsubscript{1} fetal red blood cells continuously entered the maternal blood, stimulating the mother to produce high titer IgG anti-A\textsubscript{1} and anti-A. Although the ABO antigen is a tissue antigen, which can absorb anti-A and anti-A\textsubscript{1} from mother, but the higher anti-A\textsubscript{1} left. And in this case, because FMH continue occurred, the placental barrier was open, the more IgG antibody can easily transfer to the fetus.

This FMH was not diagnosed prenatal, after birth, the child's hemoglobin is 48g / L, with severe anemia. Urgent transfusion is needed. Crossmatch incompatibility before transfusion detection. In his B mother blood sample, we checked small amount of red blood cell agglutination with anti-A serum, but reverse typing and previous blood type test supported B, confirmed that the A cells in the mother's blood sample came from the fetus. A study conducted by Wei\textsuperscript{23} showed the difficulty in the identification of RhD blood type in cases of FMH. Li et al.\textsuperscript{24} also reported a case of severe FMH in which B fetal red blood cells interfered with the identification of the blood type of a A mother. Then the flow cytometry and Kleihauer-Betke test help the diagnosis the FMH.

On the contrast to FMH, the symptoms of this HDFN were not obvious, the highest level of serum bilirubin was 120.5umol/l on the third day of birth, and jaundice was not serious. It may be that we detected anti-A\textsubscript{1} antibody in time on the day of admission, suggesting that after the clinical presence of ABO-HDFN, early phototherapy was carried out, and the A2B cell were chose for transfusion.

**Conclusion**

Although there are many reports on FMH, little is reported on FMH combined with HDFN. In this case, after FMH, the fetal blood stimulated the production of anti-A\textsubscript{1} in the mother to trigger HDFN. It is suggested that when FMH occurs, ABO incompatibility acts as a trigger of secondary HDFN. Future research is focused on improving the sensitivity of the test to the timely identification of the presence of fetal blood in the maternal circulation to predict FMH and HDFN.

**Declarations**
a. Ethics approval and consent to participate: This study was approved by the Ethics Committee of Shengjing Hospital, China Medical University.
b. Consent for publication: All the authors agree to publication
c. Availability of data and materials: in the supplementary file
d. Competing interests: There are no conflicts of interest to declare.
e. Funding: This work was supported by the Liaoning Science and Technology Program, grant No. 2021JH2/10300045.
f. Authors’ contributions (This statement must exactly match on Editorial submission system and in the manuscript):

Yanjing He: The author design all the serum tests for the infant and his parent

Qiushi Wang: The author is responsible for the design of this paper and write the case report.
g. ACKNOWLEDGMENTS We would like to thank Editage (www.editage.cn) for English language editing.
h. Conflicts of Interest: The authors have no conflict of interest to declare.

References

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Table

**Table 1** Results of laboratory examinations performed after admission
<table>
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<tr>
<th>Age day</th>
<th>Hemoglobin (g/L)</th>
<th>Red blood cells (10^{12}/L)</th>
<th>Hematocrit (%)</th>
<th>Percentage of reticulocytes (%)</th>
<th>Total bilirubin (µmol/L)</th>
<th>Indirect bilirubin (µmol/L)</th>
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**Figures**

![Figure 1](image)

**Figure 1**

Blood type of the mother

(A) Maternal blood type cannot be identified in gel card because of anti-A unclear (Ortho Clinical Diagnostics).

(B) Microscopic examination of the maternal blood revealed forward-blood typing with anti-A in the tube test with saline, showed weak agglutination with anti-A