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Case Report

Hemolytic disease of newborn due to anti-A_1 allo-antibodies: a case report

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ABSTRACT

Hemolytic disease of newborn (HDN) is an alloimmune hemolytic disease which occurs due to red blood type incompatibility between mother and fetus. An AB blood type neonate was admitted to Shengjing hospital with severe anemia. Major crossmatch incompatibility was found with some random donors. Serological tests were administered to the neonate and his parents. The mother was B blood type, while the father was AB blood type. The neonate's direct antiglobulin test (DAT) was negative, but the elution test was positive with A_1 cell and negative with A_2 cell. Titers 64 anti-A_1 and 2 anti-A in the mother's serum were detected after treated by dithiothreitol (DTT). The mother's red cells showed a weak agglutination with anti-A under microscopy. The neonate was diagnosed with HDN. After phototherapy and A_2B red blood cell (RBC) transfusion, the neonate was discharged with a recovery of his hemoglobin and physiological index. This study describes a rare case of HDN caused by anti-A1 allo-antibodies.

Keywords: hemolytic disease of newborn, anti-A_1 allo-antibody, transfusion

INTRODUCTION

Hemolytic disease of newborn (HDN) is an alloimmune hemolytic disease which occurs due to red blood type incompatibility between mother and fetus. When the IgG antibody is transferred from the mother's serum, it is reactive against fetal red blood cell (RBC) antigens inherited from the father, destroying fetal cells. ABO incompatibility between mother and fetus appears in 20% of all pregnancies, while 1%–4% of newborns develop HDN. Most cases of HDN stem from ABO incompatibility in type O mothers. HDN caused by ABO incompatibility due to anti-A_1 antibody is very rare in non-O blood type[1]. Herein, we report a case of HDN caused by the anti-A_1 allo-antibody.

CASE REPORT

A male premature infant was admitted to Shengjing Hospital due to severe anemia. The physical examination for the infant showed pale skin, shortness of breath, and no sputum, and the infant spat up immediately after birth. The infant's oxygen saturation (SpO_2) was 85%, with no signs of edema. Laboratory results showed AB positive, an RBC count of 1.2×10^{12}/L, hemoglobin at 43 g/L, hematocrit at 14.78%, a platelet count of 157×10^{9}/L, reticulocyte at 18.8%, total bilirubin at 26.5 μmol/L, conjugated bilirubin at 12.3 μmol/L, unconjugated bilirubin at 14.2 μmol/L, and lactate dehydrogenase at 921 U/L. Transfusion testing for the neonate showed a major crossmatch-incompatible result with AB and A RBCs,

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but a negative result with B and O RBCs, as well as some A and AB blood cells.

ABO blood typing was administered to the neonate and his parents by using the ABO gel method (Ortho Clinical Diagnostics, State of New Jersey, USA, Lot No. ABR228A). Reverse typing of ABO blood type was carried out by using A_1, A_2, B, and O cells (Hemo-Pharmaceutical & Biological Company, Shanghai, China). Antigens A, B, and A_1 were assessed using anti-A, anti-B and anti-A_1 (Hemo-Pharmaceutical & Biological Company, Lot No. 20170719) in saline tubes. Neonatal antibody screening, identification, cross-matching, direct anti-globulin test (DAT), and elution test were performed using Coomb's gel card (Bio-Rad Laboratories, CA, USA, Lot No. 50531.16. 09). All procedures were performed according to the manufacturers' instructions. The results are shown in ***Table 1***.

The neonate was A_1B positive, while his father was A_1 positive and his mother was B blood type. However, the mother's sample before delivery showed a mixed reaction with anti-A in gel cards (***Fig. 1A***) and under microscopy in saline tube tests, indicating the presence of an additional A antigen. The forward blood typing is shown in ***Fig. 1B***.

The neonate tested negative for A_1B antibody and DAT. The elution test showed a positive reaction with A_1 cells and a negative reaction with A_2 cells, confirming HDN caused by anti-A_1 from his mother. After dithiothreitol (DTT) treatment, the maternal serum showed a titer of 64 with A_1 cells and 2 with A_2 cells. Then, a Kleihauer-Betke test was performed on the mother's peripheral blood for fetomaternal hemorrhage (FMH) diagnosis, which showed that the KBt was 12%.

After admission, the neonate was given low-flow oxygen. Matched A_2B RBCs were then prepared for transfusion and 0.25 units of RBCs were administered on both the first and second days of hospitalization. The hemoglobin level increased to 108 g/L after blood transfusion. Phototherapy started on the day of hospitalization and was continued for 4 days. The highest serum bilirubin level was recorded as 109.7 μmol/L on the third day of therapy. Following 11 days of hospitalized therapy, the neonate was discharged and the treatment was considered satisfactory. The laboratory results during hospitalization are shown in ***Table 2***.

Table 1 **Results of blood groups for the neonate and his parents**

Samples	Anti-A	Anti-A_1	Anti-B	Ac	Bc	A_1 cell		A_2 cell		Antibody screen
						Saline	Gel	Saline	Gel	
Neonate	4+	4+	4+	0	0	0	0.5+	0	0	0
Mother	0.5+	/	4+	3+	0	3+	3+	3+	3+	0
Father	4+	4+	0	0	4+	/	/	/	/	0

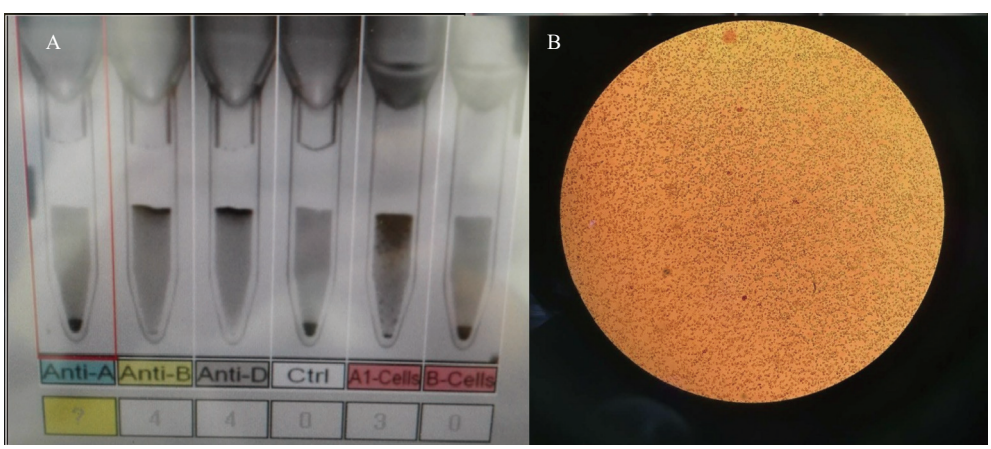

Fig. 1 **Blood type of the mother.** A: The mother's blood type could not be identified by gel card because of anti-A weak positive. B: Forward blood typing of the mother's blood with anti-A in the tube test with saline under microscope showed weak agglutination with anti-A.

DISCUSSION

This study describes a case of HDN caused by anti-A_1 antibodies in B maternal serum. Cases of ABO-HDN in neonates born to non-O mothers are rarely reported. Jeonh *et al*. reported a case of HDN caused by IgG anti-B allo-antibodies from an A_2 mother[2]. Wang M *et al*. reported another HDN case caused by

Table 2 **Results of laboratory examinations performed after admission**

Age (days)	Hemoglobin (g/L)	Red blood cells ($\times10^{12}$/L)	Hematocrit (%)	Percentage of reticulocytes (%)	Total bilirubin (μmol/L)	Indirect bilirubin (μmol/L)	Direct bilirubin (μmol/L)
1	43	1.2	14.78	18.8	26.5	14.2	12.3
2	108	3.4	33.09	14.2	/	/	/
3	/	/	/	/	120.5	109.7	10.8
7	112	3.8	35.22	4.09	70.1	51.1	19.0

an A_2 mother with 1024 titer IgG anti-B allo-antibodies[3].

In individuals with B and O blood types, two natural antibodies, anti-A_1 (mainly IgM) and anti-A, can be detected[4], which occasionally cause similar symptoms such as the hemolytic transfusion reaction[1–4]. The vast majority of spontaneous FMH are low-volume bleedings with no hemodynamic significance, but can cause alloimmunization[5–7].

Not all pregnancies routinely undergo FMH screening before delivery, but Rhesus (Rh) negative women receive the FMH test after delivery. If FMH is ≥6 mL, additional RhIg should be given, even though RhIg is received regularly at 28-week gestation and 36-week gestation[7], indicating that FMH is a higher risk factor leading to an increase in antibodies.

In this case, during pregnancy, A_1 fetal RBCs continuously entered the mother's blood, stimulating the mother to produce high titer IgG anti-A_1 and anti-A. Although the ABO antigen is a tissue antigen that can absorb anti-A and anti-A_1 from the mother, the higher titer anti-A_1 remains. Furthermore, in this case, because FMH continued to occur and the placental barrier was open, a large amount of IgG antibodies was able to transfer to the fetus.

FMH was not diagnosed prenatally in this case. After birth, the neonate's hemoglobin was 48 g/L with severe anemia, requiring an urgent transfusion. Major crossmatch incompatibility was detected before the transfusion. In the mother's B blood sample, a small amount of RBC agglutination was confirmed with anti-A serum. However, reverse typing and results from a previous blood test suggested that the mother's blood group was B, indicating that the A cells in the mother's blood sample came from the fetus. A study conducted by Wei GA showed the difficulty of identifying RhD blood type in cases of FMH[8]. Li Y *et al*. also reported a case of severe FMH, in which B fetal RBCs interfered with blood type identification of a blood type A mother[9]. In such cases, the Kleihauer-Betke test helps the diagnosis of FMH.

In contrast to FMH, the symptoms of this HDN were not always obvious. In this case, the highest level of serum bilirubin was 120.5 μmol/L on the third day after birth, and jaundice was not severe. Anti-A_1 antibody was detected in time on the day of admission, indicating the presence of ABO-HDN. This resulted in early phototherapy, and A_2B cells were chosen for transfusion.

Although many reports on FMH have been published, few report on FMH combined with HDN. In this case, after FMH, the fetal blood stimulated the production of anti-A_1 in the mother which in turn triggered HDN. When FMH occurs, ABO incompatibility acts as a trigger of secondary HDN. Future research focuses on improving the sensitivity of the test to promote the timely identification of the presence of fetal blood in maternal circulation so as to predict FMH and HDN.

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