Case Report

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Maternal Cold-Reacting Immunoglobulin G Anti-M of MNS Blood Group System Causing Hemolytic Disease of the Fetus

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ABSTRACT

Several cases of the hemolytic disease of the fetus and newborn (HDFN) caused by immunoglobulin G (IgG) anti-M antibodies have been reported, in which almost all the HDFN-associated anti-M were warmly reacting. Here we report two cases of severe HDFN associated with cold-reacting IgG anti-M. In both cases, pregnancy was terminated, in weeks 33 and 23 respectively, due to a diagnosis of fetal growth retardation (FGR). To our knowledge, these are the most severe HDFN cases caused by cold-reacting IgG anti-M.

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INTRODUCTION

Hemolytic disease of the fetus and newborn (HDFN) is caused by maternal alloimmunization against fetal red blood cell (RBC) antigens [1, 2]. During pregnancy, mothers may generate IgG alloantibodies against fetal RBC antigens that enter maternal circulation via transplacental feto-maternal hemorrhage [3]. The IgG alloantibodies then enter the fetus through the placenta and subsequently cause the agglutination and destruction of fetal RBCs [4]. Common symptoms of HDFN include fetal anemia, edema, hepatosplenomegaly, and fetal abortion or stillbirth [5]. Forty-three RBC antigens have been reported to be related to HDFN, most of which belong to ABO and RH blood group systems [6]. Another blood group incompatibility has also been reported to cause

HDFN ,although the cases are rare [7, 8].

Landsteiner and Levine (1927) discovered the MNS blood group as the second blood system. So far, 49 antigens have been discovered, the most important ones being M, N, S, s, and U antigens [9]. The MNS blood group system antigens (M, N, S, s) are fully developed and well detected in the second half of pregnancy [10]. Usually, anti-M is an IgM antibody, however, a component of IgG with IgM occurs occasionally. IgM Anti-M antibodies are cold-reacting and cannot cross the placenta, hence they are considered not to influence the fetus. However, IgG class anti-M antibodies can penetrate the placental barrier and cause agglutination and hemolysis of the fetal RBCs carrying M antigens at 37 °C. In China, the incidence of anti-M antibodies is higher than that of anti-N antibodies [11]. Most anti-M antibodies are naturally occurring IgM ones, showing a detection rate of 10% in pregnant women [12]. Pregnancy and blood transfusion may stimulate the generation of IgG anti-M, besides IgM alloantibodies. Only 0.01% to 0.7% of pregnant women are immunized to fetal M antigen-positive cells for IgG Anti-M production [13]. These antibodies cause hemolysis in fetuses. Various regions like Japan, Turkey, India, Taiwan, etc., have reported the occurrence of HDFN cases caused by anti-M [14-17]. Among those HDFN cases, in the maternal plasma, the titer of IgM anti-M antibody varies from 2 to 512 and IgG anti-M antibody titer shows variation between 8 and 512. Wikman reported a severe case of HDFN induced by anti-M antibodies of titer 1 [13]. More anti-M associated HDFN cases, especially those resulting in serious consequences, have been reported in Asians compared with that in the Caucasian population, indicating there are racial/ethnic disparities in risk factors that cause anti-M related HDFN [7, 15, 18]. Almost all the HDFN-related anti-M IgG have been found warm-reacting, while cold-reacting anti-M IgG has been rarely reported at present.

In this study, two severe HDFN cases that were caused by cold reacting IgG anti-M antibodies are reported. In these two cases, alloimmunization provoked hemolytic anemia and subsequently led to FGR and pregnancy termination.

CASE DESCRIPTION

Case 1

Fetal anemia was diagnosed at 31 weeks gestation and the pregnant woman was hospitalized. Complete blood count (CBC) results showed 31 g/L hemoglobin and 10.7% hematocrit HCT, which was much lower than the critical point of 30%. Color Doppler ultrasound showed middle cerebral artery peak systolic velocity (MCA-PSV, 66cm/s (>1.5MOM)). Blood group typing showed that the fetus had phenotype O+, MN, whereas the mother had phenotype O+, NN (Figures 1A & C, Table 1). Anti-M antibodies of both IgM and IgG classes were detected in the mother's serum by Coomb's test (at room temperature). The titer of anti-M IgG was 128 (2-mercaptoethanol treated, 4 °C, Table 2). Direct antiglobulin test (DAT) results showed RBCs of the mother and the fetus were not sensitized by antibodies, however, alloantibodies against RBCs were detected in the mother's serum via Indirect Antiglobulin Test (IAT, Figure 1A, Table 1). After an intrauterine blood transfusion, the CBC showed an increase in hemoglobin level of 58 g/L and an HCT increase of 18%. The patient was discharged after treatment, however, FGR was diagnosed afterward and the pregnancy was terminated at week 33.

The pregnant woman was of Chinese Han ethnicity. She was healthy and had no related diseases or blood transfusion history before. The mother delivered a healthy daughter with O+, and NN blood types several years before the occurrence of the above HDFN case. The mother got pregnant again a few years after the case and smoothly delivered a healthy infant (blood group type O+, NN). Therefore, in the above-described HDFN case, the IgG anti-M antibodies should be naturally occurring but

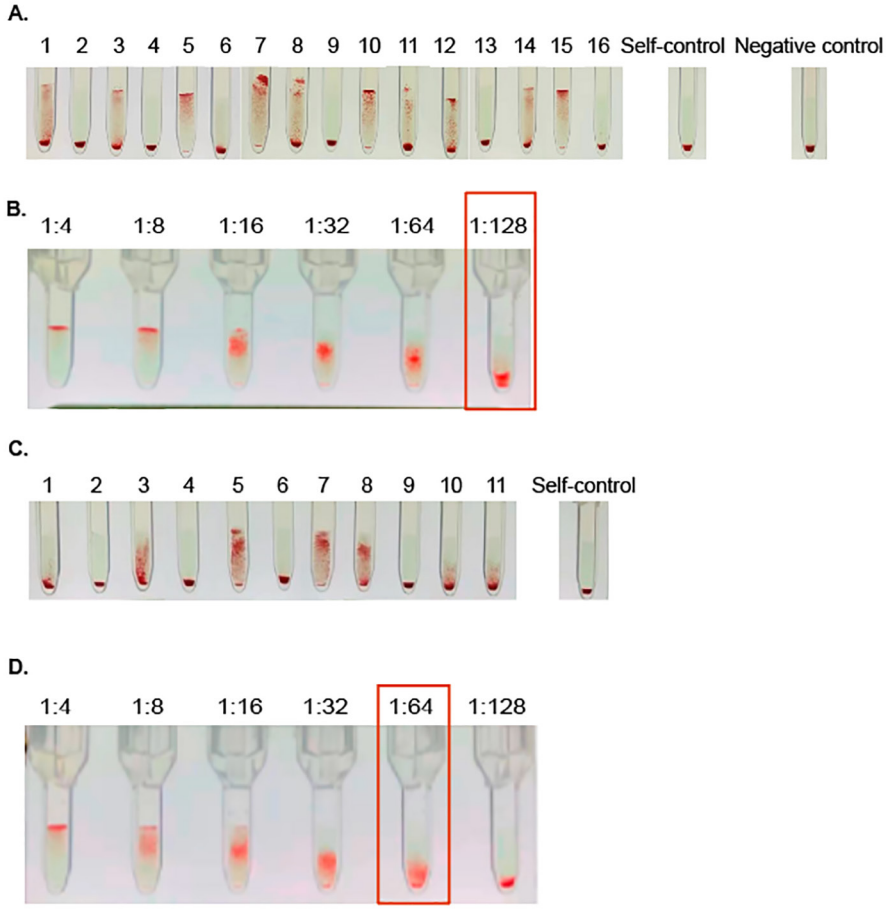

Figure 1. Antibody identification results of two cases. A&C. Mother's antibody identification results at 37 °C of case 1 (A) and case 2 (C). B&D. Titers of IgG Anti-M antibodies at 4 °C in the umbilical cord blood after 2-Me treatment of case 1 (B) and case 2 (D).

not induced by previous immune stimulation.

In this case study all data was collected after the prior informed consent of the participants.

Case 2

A fetus developed severe intrauterine and edema at 21 weeks of gestation. The fetus had blood group phenotype O+, MN whereas the mother had A+, NN (Figures 1B&D, Table 1). Anti-M antibody (IgG+IgM) was detected in the mother's serum by Coomb's test (at room temperature) and the titer of anti-M IgG was 64 (2-mercaptoethanol treated, 4 °C, Table 2). The mother's RBCs were not sensitized by antibodies determined by DAT, while antibodies against RBCs in circulation were detected in IAT (Figure 1, Table 1). Since no cord blood was collected, the DAT of the fetus was not performed. The pregnancy was terminated due to fetal growth retardation at week 23.

The pregnant woman in this case was also of Chinese Han ethnicity. She was healthy and had no related diseases or blood transfusion history before. It was the third time that the woman got pregnant in the reported case. She smoothly delivered a healthy infant of O+, NN blood type in the first pregnancy and ended in miscarriage in the second pregnancy, in which the fetus had a blood grouping phenotype of O+, MN. Therefore, the IgG anti-M antibodies that were detected in the reported HDFN case, should be partially induced by immune stimulation of the second fetus. The spontaneous abortion of the second pregnancy was also associated with the naturally occurring IgG anti-M antibodies.

In this case study all data was obtained after the prior informed consent of the participants.

Table 1. Irregular antibody identification cell profile and IAT result of the two cases

Cellbind ID		1	2	3	4	5	6	7	8	9	10	11
Rh-Hr	C	+	+	0	0	+	0	0	0	0	0	+
	D	+	+	+	+	0	0	0	0	0	0	+
	E	0	0	+	0	0	+	0	0	0	0	+
	c	0	0	+	+	0	+	+	+	+	+	0
	e	+	+	0	+	+	0	+	+	+	+	+
	C^w	+	0	0	0	0	0	0	0	0	0	0
	f	/	/	/	/	/	/	/	/	/	/	/
	V	/	/	/	/	/	/	/	/	/	/	/
Kell	K	0	+	0	0	0	0	+	0	+	0	+
	k	+	+	+	+	+	+	0	+	+	+	+
	Kp^a	0	0	0	0	0	0	0	+	0	0	0
	Kp^b	+	+	+	+	+	+	+	+	+	+	+
	Js^a	/	/	/	0	/	/	/	0	0	/	/
	Js^b	+	+	+	+	+	+	+	+	+	+	+
Duffy	Fy^a	+	0	0	0	+	0	0	0	+	+	+
	Fy^b	0	+	+	0	+	+	+	+	0	0	+
Kidd	Jk^a	+	+	0	+	+	+	+	0	+	+	0
	Jk^b	0	+	+	0	+	0	+	+	0	0	+
Lewis	Le^a	0	0	0	0	0	0	0	+	+	+	0
	Le^b	+	+	+	0	+	+	+	0	0	0	+
P	P1	+	+	+	+	+	+	0	+	+	+	0
MNS	M	+	0	+	0	+	+	+	+	0	+	+
	N	+	+	+	+	0	+	0	+	+	0	+
	S	+	0	+	0	+	0	+	0	0	+	+
	s	0	+	0	+	+	+	0	+	+	+	+
Luther	Lu^a	0	0	0	0	0	0	0	0	0	0	0
	Lu^b	+	+	+	+	+	+	+	+	+	+	+
Xg	Xg^a	+	+	+	+	+	+	0	+	+	+	+
Case1	IS	$2+^s$	0	$2+^s$	0	3+	w+	3+	$2+^s$	0	3+	2+
(Mother)	IAT(37°C)	1+	0	1+	0	$1+^s$	w+	$1+^s$	1+	0	$1+^s$	1+
Case2	IS	$2+^s$	0	$2+^s$	0	3+	w+	3+	$2+^s$	0	3+	2+
(Mother)	IAT(37°C)	1+	0	1+	0	$1+^s$	w+	$1+^s$	1+	0	$1+^s$	1+

A cellbind screen kit (Sanquin Reagents BV, The Netherlands) was used for the identification of antibodies.

Table 2. Serological tests of the two cases

Serological tests	Case1	Case2
Blood group of mothers	O, D+, NN	A, D+, NN
Blood group of fathers	O, D+, MN	A, D+, MN
Blood group of fetuses	O, D+, MN	O, D+, MN
DAT of mother	Negative	Negative
Isoantibody test of mother (Coombs test)	Anti-M (IgM+IgG)	Anti-M (IgM+IgG)
Anti-M antibody titer of mother (4 °C, IgG)	128	64
DAT of fetus	Negative	No detection

DAT: Direct antiglobulin test

DISCUSSION

Although Anti-M antibodies are mostly of naturally occurring IgM class whose optimal reaction temperature is 4 °C [19], IgG class was detected in about 78% of subjects in which anti-M alloimmunization was confirmed [20]. Extant studies show little clinical significance

of the non-reactive anti-M antibody at 37 °C, however, the consensus remains elusive currently on the clinical significance of anti-M antibodies [21]. In general, fetal anemia caused by anti-M is considered mild and not fatal[5]. However, in recent years, cases of severe fetal anemia caused by anti-M antibodies have been increasingly reported, especially in Asian populations [7, 15, 18, 22]. Two HDFN cases have been reported to be related to IgG anti-M that reacted optimally at cold temperatures. In both cases, the fetuses were delivered smoothly and recovered after the treatment [23, 24].

The two cases reported in this work were quite similar: fetuses were MN blood type and mothers were NN blood type. It was the first time for both mothers to have M antigen-positive fetuses and encountered severe fetal hemolysis and edema that led to the termination of pregnancy. To the best of our knowledge, this is the first report to show fatal intrauterine hemolytic anemia cases that were caused by cold-reacting IgG anti-M antibodies.

It was reported that HDFN caused by anti-M antibodies might not show typical laboratory changes, such as DAT negative [13, 15], however, in both cases of our work negative DAT results were obtained. Therefore, the correct judgment of prenatal hemolytic disease caused by the anti-M antibody is very important for diagnosis. When anti-M reactivity is detected in maternal serum during pregnancy an additional test of anti-M reactivity using 2-Me-treated samples at cold temperatures is needed to determine the presence of a clinically relevant low-temperature IgG component.

Although in both cases of this report, the patients received appropriate treatments, the pregnancies were still terminated due to the occurrence of FGR, indicating the fatality risks of anti-M alloimmunization. The severity of the consequences caused by anti-M may range from asymptomatic to severely hydropic with intrauterine or neonatal demise [7, 25]. Meanwhile, it seems that anti-M-related HDFN occurs more frequently in Asian populations than in the Caucasian ones [7, 15]. In addition, it was reported that a pregnant woman of NN blood type successfully gave birth to a living child of MN blood type after experiencing several failed pregnancies including miscarriage and intrauterine fetal death. All the pieces of evidence indicate that some other factors may also contribute to the severity of anti-M-related HDFN. Therefore, it is important to examine more changes other than anti-M alloantibodies, e.g. dysregulation of the complement activation [26], to better evaluate the conditions of the fetus and mother for appropriate treatment.

CONCLUSION

The incompatibility of the MNS blood group between the mother and fetus may result in very severe consequences, such as FGR and termination of pregnancy in the Chinese Han ethnicity. Anti-M antibodies can play significant roles in causing HDFN even at low titer, possibly due to other changes in the patient's immune system during a particular period, such as dysregulation of the complement system. More attention is needed for early detection of anti-M antibodies in NN blood type pregnant mother even though negative standard DAT testing results are demonstrated.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the data were obtained with the informed consent of participants.

Conflict of Interest: None declared.

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