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Original Research Paper

Immunohaematology

ANTI-M ANTIBODY - NOT ALWAYS AN INNOCENT BYSTANDER. A REPORT OF TWO CASES

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ABSTRACT Anti-M antibody is a common naturally occurring antibody that is seldom clinically significant. We hereby present two cases of anti-M antibody. An unbooked, severely anemic, pregnant lady presenting with crossmatch incompatibility. And one year old child referred to our Regional Blood Transfusion Centre (RBTC) for immunohematology work-up in view of blood group discrepancy.

KEYWORDS : Anti-M, immunizing, naturally occurring, antenatal, Anti-M alloimmunization

Introduction

The MNS system was described by Landsteiner and Levine in 1927.[1] Incidence of 'M' antigen is fairly common worldwide, about 75%.[2] Anti-M is the most common antibody of MNS system and was first described by Wolff and Jonsson in 1933.[3] It is a naturally occurring antibody reacting optimally at 4°C and is usually clinically insignificant. Though majority of anti-M are IgM type and are non-complement activating; IgG component may exist causing hemolysis by complement activation.[4] Thus anti-M becomes clinically significant when active at 37°C either due to IgG component or wide thermal amplitude of IgM component. It is detected incidentally as cross-match incompatibility or blood group discrepancy. In addition, anti-M can cause immediate or delayed hemolytic transfusion reaction, intrauterine death (IUD), hemolytic disease of foetus & newborn (HDN), and neonatal red cell aplasia.[5,6] Therefore, it is important to identify anti-M, determine its thermal amplitude and type especially in pregnant females.

Case 1

A 26 years old, G5P2L1A3 female presented to casualty in acute labor at 32 weeks of gestation. Patient had bad obstetric history and her only live child was from first pregnancy, healthy full-term delivery with no history of neonatal anemia or jaundice. There was no past history of blood transfusion. Medical & family history was insignificant. On physical examination, severe pallor was noted with absence of icterus. Complete hemogram with peripheral smear examination revealed severe macrocytic anemia. Coagulation profile, hemoglobin High Performance Liquid Chromatography and biochemical parameters were within normal limits.

An urgent requisition for packed RBCs was received in our RBTC. Patient's blood group was O Rh (D) positive by tube method at room temperature. Three cross-match compatible packed RBCs ("c,E,K" negative) were transfused. Patient's thyroid function tests were within normal limits. Tests for Lupus anticoagulant, anti-nuclear and anti-phospholipid antibodies were negative, contradicting an autoimmune etiology. The patient delivered a preterm still-born baby with severe pallor. Cord blood was unavailable for analysis.

On postpartum day2, patient needed another transfusion. However, multiple blood units put-up for cross-match were found 3+ incompatible. Extended blood group revealed discrepancy in forward and reverse grouping at 4°C & 37°C. However, blood group was O Rh+ at 22°C. Autocontrols were negative at 3 temperatures ruling out autoantibody. [Table 1] Also, polyspecific direct antiglobulin test (DAT) was negative. The indirect antiglobulin test (IAT) using 3-cell panel was positive. [Figure 1 & 2] An extended 11-cell IAT at 37°C and 4°C suggested possibilities of anti-E/ anti-M and/or anti-Kpa antibody. [Figure 3 & 4]

Rh/Kell/extended antigen profile of patient & her husband was done. [Table 2] Presence of Kpa antigen on self-RBCs ruled out anti-Kpa alloantibody. Husband's RBCs were positive for M antigen whereas the patient was negative suggesting anti-M antibody. Both the spouses were negative for E antigen.

Critical analysis of immunohematology results revealed that the 3rd and 5th cells of 11-cell panel were both E+M+, so anti E could not be confirmed. Therefore, IAT was repeated after 2 weeks with a different commercial 11-cell panel, having E+M- 5th cells. Negative reaction with 5th cells ruled out Anti-E antibody. Also, reactions were stronger with M+ reagent cells indicating anamnestic response.

The Anti-M titres were done at 37°C (1:32) and 4°C (1:16), suggesting either a combination of IgG+IgM or IgM alone with wide thermal amplitude. However, immunisation from pregnancy, bad obstetric history, severe fetal anemia, antibody activity at 37°C and anamnestic response; favoured IgG anti-M.

Case 2

A 1year old female child with orthopedic abnormalities was planned for surgical intervention and packed RBCs were required. The patient had past history of one unit cross-matched, A Rh positive, packed RBCs transfusion at the age of 3 months. There was no history of jaundice, recent fever or drug intake.

Extended blood grouping at 4°C, 22°C and 37°C was suggestive of A Rh positive by forward grouping and pan-agglutination with pooled A, B and O cells; making the results invalid. The patient's RBCs agglutinated with commercial anti-A1 lectin ruling out weaker subgroups of A antigen. Blood group could not be determined definitively. [Table 1]

Autocontrols at 3 temperatures & polyspecific DAT was negative. Three-cell panel & 11-cell panel were suggestive of anti-M alloantibody at 37°C and 4°C. The reactions showed dosage effect with homozygous M+N- cells showing strong reaction relative to heterozygous M+N+ cells. [Figure 5] Rh/Kell/extended antigen profile of patient & both parents was done. [Table 2]

Table 1: Extended Blood grouping results of the patients.

Temp-erature	Forward Grouping						Reverse Grouping				
	Anti A	Anti B	Anti D 1	Anti D 2	NS*	BG†	A cells	B cells	O cells	AC‡	BG
Case 1											
4°C	Neg§	Neg	3+	3+	Neg	O+	4+	4+	1+	Neg	Invalid
22°C	Neg	Neg	4+	3+	Neg	O+	4+	4+	Neg	Neg	O +
37°C	Neg	Neg	4+	4+	Neg	O+	4+	4+	2+	Neg	Invalid
Case 2											
4°C	4+	Neg	2+	2+	Neg	A+	3+	3+	3+	Neg	Invalid
22°C	4+	Neg	2+	3+	Neg	A+	1+	2+	2+	Neg	Invalid
37°C	4+	Neg	4+	4+	Neg	A+	3+	3+	2+	Neg	Invalid

§ Negative, * Normal Saline, † Blood Group, ‡ Auto-control

Table 2: Rh/Kell/extended antigen profile of both cases.

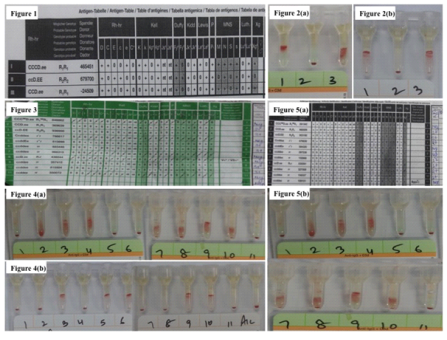
BG*	ABO	C	c	E	e	K	Control	M
Case 1 Husband of case1	O+	4+	mf†	Neg‡	4+	Neg	Neg	Neg
	AB+	4+	3+	Neg	3+	Neg	Neg	4+
Case 2 Mother of case2	Invalid	4+	Neg	Neg	4+	Neg	Neg	Neg
	A+	4+	4+	4+	4+	Neg	Neg	Neg
Father of case2	B+	4+	4+	Neg	4+	Neg	Neg	4+

BG- blood group, † mf- mixed field reaction, ‡ Neg- negative

Since child & mother were negative and father was positive for M antigen, maternal serum was screened by IAT for anti-M alloantibody, which was negative. Thus the possibility of M antigen exposure during previous transfusion was suspected.

The irregular alloantibody in child was active at 4°C, 22°C and 37°C. Anti-M titres were 1:2 and 1:8 at 37°C and 4°C respectively. Patient's sample was compatible with A Rh positive, "c, E, K" negative, M negative packed RBCs which retrospectively confirmed the blood group to be A positive. The patient was successfully transfused.

Figure 1: Phenotype of commercial reagent cells of three-cell panel. Figure 2: Antibody screening panel in case1 (a) at 37°C and (b) at 4°C. Figure 3: Phenotype of commercial reagent cells of 11-cell panel. Figure 4: 11-cell panel showing anti-M alloantibody in case1 (a) at 37°C and (b) at 4°C. Figure 5: 11-cell panel of case2 at 37°C (a) antigram and (b) gel microtyping cards demonstrating dosage effect of anti-M with stronger 2+ reaction with homozygous M+N- cells in 3rd, 4th & 9th columns.



Materials and methods

Blood sample was collected in EDTA vial under strict warm conditions. The RBCs and serum were separated immediately by centrifugation at 2000rpm for 5minutes. The cells were washed with warm normal saline. Extended forward and reverse blood grouping along with autocontrols was done at 4°C, 22°C and 37°C by tube method. A polyspecific DAT was performed using a 0.8% patient's RBC suspension and low ionic strength saline (LISS)/Coombs gel card (anti IgG and C3d ID-cards, Diamed, Switzerland). A commercial three-cell panel (ID-DiaCell I-II-III Asia, Diamed) was used for antibody screening by IAT. The patient's serum was reacted with reagent RBCs using LISS/Coombs ID-cards (Diamed) at 37°C in AHG (anti human globulin) phase and ID-Cards (NaCl, Enzyme Test and Cold Agglutinins, Diamed) at 4°C in saline phase. The cards were incubated for 15 minutes and centrifuged in ID-centrifuge for 10 minutes. An extended 11-cell panel (ID-DiaPanel, DiaMed) was used for antibody identification. Rh/Kell/extended antigen profile (C, c, E, e, K, M/other antigen) was done by using DiaClon gel cards, Diamed. The Anti-M titres were done with O+M+ RBCs by tube method. Blood group cross-match was done by column agglutination technology using LISS/Coombs (AHG) ID-cards. Dithiothreitol (DTT) treatment to confirm the IgG component could not be done due to non-availability of reagents.

Discussion

Anti-M seems to be more common in infants.[7,8] It is detected in just 10% of pregnant women with a positive IAT.[9] The frequency of homozygous M+N– and the heterozygous M+N+ phenotype is approximately 38-42% and 36-43% respectively whereas M–N+ phenotype is only 14-24% in north Indian blood donors. Therefore, immunising type of Anti-M is rarely reported.[10] About 0.01-0.7% women develop anti-M IgG that can lead to HDN.[11] Anti-M is the second most common non-Rh antibody, after anti–Kell, to cause HDN.[8]

Tondon R et al reported two cases of anti-M, one 'immunizing' type reactive at 37°C with IgG component and showing dosage effect. While the other 'naturally occurring' type was reactive well below 37°C.[7]

Since M antigen is expressed only on RBCs and is fully developed on fetal RBCs, anti-M can severely affect even early gestation.[12] The clinical spectrum of anti-M may vary from IUD to fetal hydrops, HDN or neonatal red cell aplasia due to destruction of erythroid progenitors.[8,12-14]

When IgG anti-M is identified in the maternal serum, paternal antigen profile must be done. If the father is M+, fetus may be at risk of HDN. Although, there is no documented evidence correlating anti-M titres & severity of HDN.[6,12,15] Anti-M IgG titre of 1:16 to 1:32 have been reported to cause neonatal red cell aplasia and IUD.[6,9] Therefore, Scientific Section Coordinating Committee of AABB recommends if IgG anti-M titer is >16, amniotic fluid bilirubin should be analysed.[16] The anti-M titres may vary depending on the technique, incubation temperature, suspension media and phenotype of reagent RBCs.[17] Reactions can be falsely interpreted as positive in AHG phase if a high titre IgM-type anti-M reacts at room temperature and agglutination is carried forward to AHG phase. Therefore, strict warm conditions should be maintained and results should be read immediately.[5] Incidence of anti-M in donor sera is 1:2500 when tested with homozygous M+N- cells while incidence is reduced to 1:5000 when screened with heterozygous M+N+ cells, indicating that weak anti-M may be missed with heterozygous cells. MN antibodies are often pH dependent (optimum pH of 6.5) and are mostly inactive at pH 7.5. [2] Also, anti-M don't react with ficin or papain-pretreated cells.[7]

DAT may be deceptively negative even when neonatal RBCs are M+

and hemolysis is occurring. It is hypothesised that it might be either due to very rapid intravascular hemolysis, or it could be similar to Kell and Gerbich antigens that are present on erythroid progenitors rather than mature RBCs. Hence, infant's MN antigen status should be determined.[6,15]

Combined plasmapheresis, IVIG therapy and intrauterine transfusion (IUT) with M negative cross-matched RBCs, are mainstay of treatment.[6] Furukawa K *et al* reported a woman who had multiple IUDs due to anti-M and delivered a live child when intensive plasmapheresis was started from first trimester.[12]

CONCLUSION

Anti-M is not always naturally occurring insignificant antibody. We recommend that a detailed immunohematology work-up for thermal amplitude & IgG component of anti-M should always be done to prevent and manage feto-maternal complications. Also, Case 2 demonstrates that apparently compatible blood unit may and do result in sensitization with uncommon antigens with significant long-term consequences. Therefore, transfusion should always be done after careful assessment of the risk-to-benefit ratio.

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