CASE REPORT

Significant Anti-M in Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Haemolytic Crisis: A Case Report

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ABSTRACT

Anti-M are naturally occurring antibodies and are usually clinically insignificant. However, when anti-M antibodies are found to be reactive at 37°C, they are considered as clinically significant. We report a rare case of anti-M antibody that was found to be reactive at 37°C in a patient with acute haemolysis secondary to G6PD deficiency. In this case, we were able to supply M antigen-negative blood despite the difficulty and time consumed to identify M antigen-negative blood. The patient's condition improved after multiple blood transfusion.

Keywords: Anti-M, Naturally occurring antibodies, Haemolysis

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INTRODUCTION

There are many blood group systems recognised by the International Society of Blood Transfusion (ISBT). Among all, ABO and Rh are the most significant in transfusion (1,2). However, other blood groups such as MNS, Kidd, Duffy and Kell are equally important as it is common to encounter antibodies to these antigens. Transfusion of incompatible blood may result in haemolytic transfusion reaction (2,3,4). Therefore, pre-transfusion testing is performed to ensure safe transfusion practice by selecting compatible donor blood components for transfusion. It includes ABO grouping, Rh typing of the patient's red blood cells and antibody screening with the patient's serum. If the antibody screening detects presence of antibody, antibody identification will be done to identify the specific antibody.

The MNS system was the second blood system that was discovered after the ABO blood group system. The M antigen is found on red blood cell glycoprotein, known as glycophorin A (2). Anti-M antibodies are naturally occurring antibodies and are usually clinically insignificant. However, when reactive at 37°C, they are considered as clinically significant and warrant antigen-negative blood for transfusion (2,3,4). We report a case of atypical anti-M antibody that was found to be reactive at 37°C in a patient with acute haemolysis secondary to G6PD deficiency.

CASE REPORT

A two-year-old boy presented with fever, pale stool, tea-coloured urine and reduced activity two days prior to admission. No abdominal pain or vomiting. There was no history of travelling, ill-contact or traditional medicine consumption. He never had history of hospital admission nor jaundice. G6PD at birth was normal. His mother denied hereditary blood disorder. Upon arrival, he appeared tachypnoeic with respiratory rate of 50 breaths per minute and tachycardic with heart rate of 180 beats per minute. He was resuscitated with intravenous normal saline 20cc/kg bolus and was put on face mask. Subsequently he was admitted to the paediatric intensive care unit for acute haemolytic anaemia possibly due to gastrointestinal bleed. Full blood count on admission showed haemoglobin 3.9g/dL, total white cell 16.2 x 109/L and platelet 279 x 109/L. He was planned for packed red blood cell (pRBC) transfusion 10cc/kg. However, during crossmatching of the blood, we discovered presence of antibodies with cell I 3+, cell II negative and cell III 3+. There was no compatible blood after random trial to crossmatch with the commonly pre-typed blood available in the inventory laboratory, eg. R1R1, R2R2, Jk(a-) and Fy(b-). We proceeded with red cell phenotyping and antibody identification and found that this patient's blood group was A positive, R1R1 with anti-M antibody which was reactive at 37°C and antiglobulin phase. Decision was made to supply M antigen-negative pRBC to the patient

despite the urgency of transfusion. We obtained one to two unit of M antigen-negative pRBC with every ten units pRBC that were randomly crossmatched. The total duration spent was two hours. His clinical condition and haemoglobin improved after multiple transfusion (Table I). While investigating the cause of haemolysis, he was started on antibiotic to cover for mycoplasma infection. Multiple blood investigations were sent simultaneously (Table II). His reticulocyte count was 10%. Full blood picture showed presence of blast cells, bite cells and spherocytes. Direct Coomb's test was negative, but the indirect Coomb's test was 3+. Direct and indirect bilirubin were 9.5μmol/L and 76.9μmol/L respectively. Lactate dehydrogenase was 1203U/L. Infective screening for Human Immunodeficiency Virus, Hepatitis B and Hepatitis C were all non-reactive. Qualitative G6PD test was sent again during this admission and turned out to be normal. The patient's mother then revealed that one of the uncles is G6PD deficient. The patient was treated as G6PD deficiency in haemolytic crisis. G6PD counselling was given to the patient's mother. He was discharged well and planned for G6PD quantitative assay three months after the haemolytic episode. His G6PD enzyme level taken later was 224U/L, which confirmed the diagnosis of G6PD deficiency.

Table I: Serial Full Blood Count

Day of Admission	Day 1	Day 2	Day 3	Day 4
Haemoglobin (g/dL)	3.9	6.9	9.8	12.1
Total White Cell (x 10^9/L)	16.2	15.0	8.2	7.4
Platelet (x 10^9/L)	279	342	286	286

DISCUSSION

The ABO and Rh blood group are the most widely understood and significant in blood banking. The MNS blood group is the second blood group recognised after the ABO blood group. It has 46 antigens, making it equally complex as the Rh blood system (2). A study in Malaysia showed the frequency of M-negative donors among the Malay, Chinese and Indian were 18.5%, 22.6% and 18.3% respectively (1). Shah et al. reported the frequency of anti-M antibody in Mumbai population as 13.98%, whereas M antigen frequency in their donor population was 78% (3).

Anti-M antibodies are naturally occurring saline agglutinins. They can be found in patients without history of blood transfusion (3,4). Reports regarding anti-M are limited mainly due to the majority of the antibodies are IgM and only react at low temperature (4). However, these antibodies can become clinically significant when they react at 37°C (1,2,3,4). They can cause haemolytic disease of newborn (HDFN) and haemolytic transfusion reaction (2,3). Shah et al. reported thirteen cases of biphasic anti-M antibodies (3). Lin et al. reported a case of severe HDFN secondary to anti-M alloimmunisation requiring serial intrauterine transfusions (2).

Table II: Blood Investigations on Admission

Investigations	Result
Reticulocyte Count	10.1 %
Coagulation Profile	
Prothrombin Time	14.9 sec
INR	1.07 ratio
Activated Partial Thrombin Time (APTT)	29.1 sec
Coomb's test	
Direct	Negative
Indirect	3+
Liver Function Test	
Total Protein	65 G/L
Total Bilirubin	86.4 μmol/L
Direct Bilirubin	9.5 μmol/L
Indirect Bilirubin	76.9 μmol/L
Alkaline Phosphatase	166 U/L
Albumin	43 G/L
Aspartate Transaminase	75 U/L
Alanine Transferase	19 U/L
Globulin	22 G/L
Lactate Dehydrogenase	1203 U/L
Renal Profile	
Urea	6.6 mmol/L
Sodium	137 mmol/L
Potassium	4.8 mmol/L
Chloride	97 mmol/L
Creatinine	37 μmol/L
Urine FEME	
Glucose, Urine	Normal
Urine Ketone Bodies	3+
Nitrite	Negative
Leukocytes	Negative
Blood in Urine	3+
Bilirubin, Urine	Negative
Protein, Urine	2+
Urobilinogen	Negative
Colour	Brown
Clarity	Turbid
pH, Urine	6.0
Urine Specific Gravity	1.020
Infective Screening	
Human Immunodeficiency Virus	Non-reactive
Hepatitis B	Non-reactive
Hepatitis C	Non-reactive
Blood Culture & Sensitivity	No growth
Mycoplasma Serology	Negative
Epstein-Barr Virus Serology	Negative
Parvovirus Serology	Negative

There are various conditions that may lead to haemolytic anaemia. They can be classified into hereditary and acquired causes. G6PD deficiency, being a common X-linked inherited disorder, is one of the hereditary causes of haemolytic anaemia. The triggering factors for acute haemolysis in G6PD deficiency are infection, ingestion of oxidative drugs or fava beans, and stress (5). In this case, the cause of acute haemolysis could not be identified at the early stage as the patient's G6PD at birth was normal. G6PD test sent during the admission was negative as well.

There have been reports of failure to diagnose G6PD deficiency at birth via fluorescent spot test (FST), which is a recommended qualitative test for G6PD screening

due to its cost-effectiveness. However, it is operator dependent, unable to discriminate intermediate fluorescence and requires a cold chain reagent. The high level of G6PD activity due to high level of immature erythrocytes in newborns adds on the challenge to diagnose G6PD deficiency at birth via FST. G6PD test that was sent during an acute haemolytic crisis might not be accurate as G6PD level might appear falsely elevated due to the increased level of compensatory immature erythrocytes (5). All these factors could lead to result discrepancy. On the other hand, quantitative test measures G6PD activity accurately but requires advanced laboratory infrastructure and skilled staffs. Physicians should be aware of the limitations of FST and proceed with quantitative test when clinically suggestive of G6PD deficiency. In our case, the patient was suspected to be G6PD deficient after his mother revealed a positive family history of G6PD deficiency and full blood picture which was suggestive of oxidative haemolysis. The diagnosis was confirmed by the quantitative G6PD assay of 224U/L, which was taken three months after the haemolysis subsided.

There were a few challenges faced while managing this patient during the acute haemolysis. Firstly, was to identify the cause of haemolysis due to the contradicting result of normal G6PD at birth with the full blood picture showing oxidative haemolysis. Secondly, was the immediate supply of the most suitable pRBC for transfusion during haemolytic crisis. Furthermore, there are no reports sharing the experience of managing patients with significant anti-M in G6PD deficiency haemolytic crisis. Blood rescue plays a major role in improving tissue oxygen delivery as well as replacing the blood volume. We decided to supply M antigen-negative crossmatched compatible pRBC to the patient despite knowing the urgency for transfusion. This was to prevent haemolytic transfusion reaction as the anti-M antibodies detected were clinically significant. In our experience, the probability of finding one pint of M antigen-negative crossmatched compatible pRBC was one in five, which is similar to Mumbai (3). Our study highlighted that despite commonly present as cold-reactive antibodies, anti-M could be clinically significant when they react at 37°C.

CONCLUSION

Anti-M antibody is considered clinically significant when it reacts at 37°C and antiglobulin phase. M antigen-negative blood should be supplied whenever blood transfusion is required. A donor registry of complete phenotyped blood should be implemented in all hospitals to ensure a quick supply of antigen-negative blood during emergency situations.

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