CASE REPORT

Importance of Glucose 6 Phosphate Dehydrogenase (G6PD) Status in the Management of Methaemoglobinaemia

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ABSTRACT

Methylene blue is an antidote for methaemoglobinaemia, but it is not given to individuals with glucose 6 phosphate dehydrogenase (G6PD) deficiency since it could potentially worsen oxidative haemolysis. We report the case of a 36-year-old male with unknown G6PD status who presented with oxidative haemolysis and methaemoglobinaemia. The fluorescence spot test showed a normal G6PD level. Methylene blue was given, but it was stopped as his anaemia worsened with no improvement in methaemoglobin level. This case highlights the challenges in the management of methaemoglobinaemia with concurrent oxidative haemolysis when the G6PD status of the patient is not known. In the acute presentation of oxidative haemolysis, molecular tests to detect G6PD deficiency can be time consuming and fluorescent spot test could give a false negative result. Therefore, it is highly advisable to avoid methylene blue for patients with unknown G6PD status when they have concurrent oxidative haemolysis and methaemoglobinaemia.
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INTRODUCTION

Haemoglobin is a protein component of erythrocytes which plays a vital role in transporting oxygen molecules from the lungs to tissues. An adult haemoglobin (HbA) is made up of two alpha and two beta globin chains with each attached to one haem molecule (1). Each haem molecule consists of a porphyrin ring which has an iron atom in its centre. Normally, this iron atom is in the ferrous state (Fe^{2+}) and can reversibly bind to oxygen molecules (1). Methaemoglobin is formed when the iron atom is in the ferric state (Fe^{3+}) instead of its normal ferrous state. Since methaemoglobin is unable to bind oxygen molecules, the haem component with ferrous iron shows increased affinity for oxygen and results in a left shifted oxygen dissociation curve causing functional anaemia (1). Methemoglobinemia is defined as the presence of an abnormal fraction of methaemoglobin in peripheral blood and its aetiology can be congenital or acquired (1). Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency is a genetic condition which affects the ability of red blood cells to withstand oxidative stress(1). If individuals with G6PD deficiency develop methaemoglobinaemia, a careful treatment approach is required as certain medications used in the management of methaemoglobinaemia can further exacerbate oxidative stress caused by G6PD deficiency (2). In this case, we will discuss challenges in the management of methaemoglobinaemia in a patient of unknown G6PD status.

CASE REPORT

A 36-year-old Burmese male with no underlying medical illness presented with fever, lethargy, and myalgia for four days and dark coloured urine for two days. Otherwise, he has no vomiting, diarrhoea, abdominal pain, history of recent travelling, jungle trekking, or water activities. He also denied taking any illicit drugs or traditional medication. He has been residing in Malaysia for the past two years and works as a waiter in a restaurant. On examination, his Glasgow coma scale (GCS) was E4V5M6, blood pressure was 118/71mmHg, heart rate was 133 beats/minute, respiratory rate was 28 breaths/min and SpO2 was 63% under room air and only improved to 86% with high flow mask. He was then put on a high flow nasal cannula 60/60 but his SpO2

was still 68%-70%. He was subsequently intubated for airway protection. Examination of the cardiovascular system showed dual rhythm and no murmur, his lungs were clear and abdominal examination showed hepatomegaly with liver palpable two finger breadth below the coastal margin. Otherwise, no splenomegaly or lymphadenopathy. Venous blood gas analysis showed pH 7.3, bicarbonate 17.3mmol/L, haemoglobin value of 7g/dL and methaemoglobin level of 24%. Peripheral blood film showed leucoerythroblastic picture with the presence of numerous bite and blister cells suggestive of oxidative haemolysis. A G6PD fluorescence spot test was done since the patient was unsure about his G6PD status, and it was normal. His toxicology investigation panel was negative. Serum bilirubin level was elevated to 148µmol/L and Coombs test was negative. He was then given the first dose of intravenous (IV) methylene blue. After 3-5 minutes post administration, he desaturated and his SpO2 dropped to 30%. Manual bagging was done, and immediate arterial blood gas showed good oxygenation. He was then given two further doses of IV methylene blue. Repeated full blood count on day two of admission showed haemoglobin level dropped to 5.0g/dL and his methaemoglobin level was 23% despite being given three doses of IV methylene blue. No further doses of methylene blue was given because of no significant reduction in methaemoglobin level with the initial three doses given. He was given two units of packed red cell but developed a fever during the transfusion of the second unit. Transfusion reaction workup concluded his febrile episode was not transfusion related. On day three of admission, his methaemoglobin level dropped to 11.4% and his haemoglobin level was 5.4g/dL. His methaemoglobin level was closely monitored and planned for exchange transfusion if his methaemoglobin level was more than 18%. The trend of his haemoglobin and methaemoglobin is summarized in Table I. He was also treated for occult sepsis with IV Tazocin for ten days and developed non oliguric acute kidney injury with metabolic acidosis which resolved by day ten of admission. Repeated peripheral blood film before discharge showed reactive thrombocytosis with no morphological evidence of acute haemolysis. The patient was discharged well after sixteen days of admission.

Table I: Summary to Haemoglobin level and Methaemoglobin level from Day 1 to Day 6 of admission.

Day of Admission	Haemoglobin Level (g/dL)	Methaemoglobin Level (%)		
		Morning	Afternoon	Night
1	7.1	-	24	-
2	5.0	23.1	23.3	-
3	5.4	11.4	6.7	2.5
4	9.5	6.2	1.6	2.7
5	10.2	1.4	1.7	1.1
6	9.5	0.7	-	-

Note: Methaemoglobin level was monitored closely from Day 2 to Day 5 of admission post methylene blue administration. The level was measured twice to three times per day during this period.

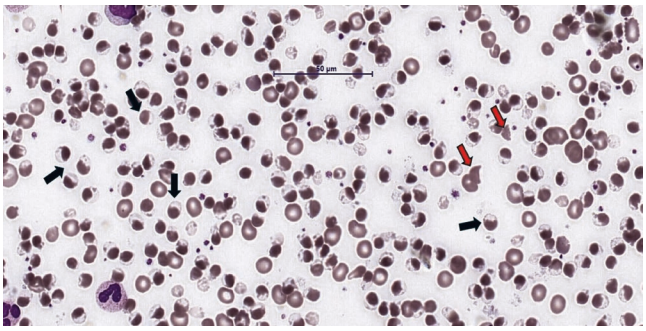

Figure 1 (H&E,40X) : Peripheral blood film showed blister cells (black arrow) and bite cells (red arrow).

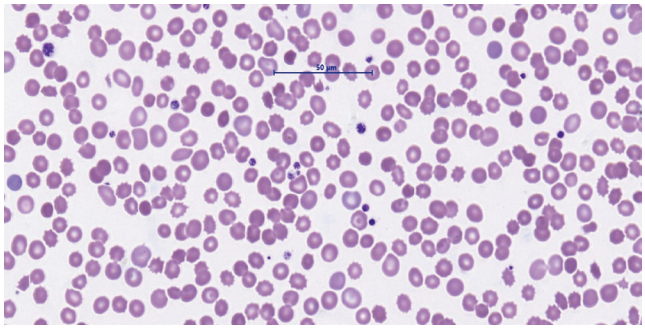

Figure 2 (H&E,40X): Peripheral blood film showed resolution of oxidative haemolysis prior to discharge of patient.

DISCUSSION

G6PD deficiency is considered the most common red blood cell metabolic disorder with more than 400 million people affected globally (1). A higher incidence of this disorder is observed in Africa, Asia, the Mediterranean, the Middle East and Southeast Asia (1). It is an X-linked disorder and the gene involved in encoding G6PD enzyme is located at Xq28 (1). This enzyme plays a crucial role in the Pentose phosphate pathway by catalysing the conversion of nicotinamide adenine dinucleotide phosphate (NADP) into its reduced form, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) using glucose 6 phosphate (G6P) as the substrate (1). NADPH is vital to protect the cells from damage caused by oxygen-free radicles. When the erythrocytes are damaged by oxidative stress in G6PD deficiency, aggregation of denatured haemoglobin is formed (Heinz bodies) and removed prematurely by the spleen resulting in haemolytic anaemia (1). G6PD deficiency also can indirectly cause methaemoglobinaemia by inhibiting NADPH-flavine reductase, which prevents the reduction of methaemoglobin (3). The concurrent occurrence of oxidative haemolysis and methaemoglobinaemia can be observed in individuals with G6PD deficiency although it is a rare phenomenon (4).

The most challenging diagnostic difficulty in this case was identifying the patient's G6PD status during an acute oxidative haemolytic crisis. Although his fluorescent spot test result was normal, it is not reliable since it could

be a false negative result. This is due to haemolysis of the older erythrocytes with remaining young red blood cells having higher levels of G6PD enzyme. Therefore, this test should be performed 2-3 months post haemolytic episode (5). The molecular study can be performed during a haemolytic crisis, but the disadvantage is that the activity level of the G6PD enzyme will not be known (5). In this case, the fluorescent spot test proceeded despite the acute oxidative haemolytic crisis of the patient since the result could be obtained faster as compared to the molecular study. It is of utmost importance to know the G6PD status of a patient with methaemoglobinaemia as it plays a crucial role in the management of the patient. IV methylene blue is an antidote for methaemoglobinaemia. Once administered, methylene blue is converted to leucomethylene blue by NADPH methaemoglobin reductase which in turn reduces methaemoglobin to haemoglobin (2). In G6PD deficiency, there is an insufficient level of NADPH which makes NADPH methaemoglobin reductase ineffective (2). Methylene blue also can cause the production of free radicals which worsens oxidative haemolysis in G6PD deficiency (2). Therefore, methylene blue is not advised to be administered in cases of methaemoglobinaemia with underlying G6PD deficiency and alternative treatments such as ascorbic acid or exchange transfusion are recommended (2).

Although the fluorescent spot test of this patient was done during acute haemolysis, the result was interpreted with caution and methylene blue was administered under close observation. When there was a drop in haemoglobin level, the antidote was immediately stopped. After excluding possible acquired causes, the most likely cause of methaemoglobinaemia in this case could be an underlying G6PD deficiency. Exchange transfusion was not performed as his methaemoglobin level did not exceed 18%. Improvement of his haemoglobin and reduction of methaemoglobin levels was observed from day 4 onwards, potentially due to the resolution of oxidative stress. His G6PD assessment needs to be repeated after three months. In the absence of G6PD deficiency, other rare yet possible congenital causes of methaemoglobinaemia such as deficiency of cytochrome b5 reductase or haemoglobin M need to be investigated (1).

CONCLUSION

This case highlights the importance of individuals knowing their G6PD status as it plays a vital role in the management of haematological emergencies such as methaemoglobinaemia. A careful treatment approach is required if G6PD status is unknown, and it is advisable not to give methylene blue to individuals with unknown G6PD status when there is a concurrent occurrence of oxidative haemolysis and methaemoglobinaemia.

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