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Transient liver injury and severe neonatal cholestasis in infant with glucose-6-phosphate dehydrogenase deficiency due to a new mutation

Short title: Severe Neonatal cholestasis with glucose-6-phosphate dehydrogenase deficiency, new mutation

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Abstract

We report the case of a neonate with a new, previously undescribed glucose-6-phosphate dehydrogenase (G6PD) gene mutation, which was revealed by severe cholestasis, hyperbilirubinemia, and transient liver dysfunction. The severity of the clinical phenotype with ongoing chronic hemolytic anemia suggests that this mutation belongs to class 1 G6PD deficiency. The hemizygous mutation «c.675G>c; p.Trp225Cys» was detected by genomic sequencing. Since severe G6PD deficiency can be revealed by cholestasis, it is important to check G6PD enzyme activity when faced with a case of liver dysfunction in the neonatal period.

Keywords: G6PD deficiency, cholestasis, liver insufficiency, hyperbilirubinemia, new mutation, hemolysis.

1- INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common red cell enzymopathy estimated to affect 400 million people worldwide [1]. The gene that encodes G6PD is located on chromosome X. To date, 400 biochemical G6PD variants have been identified corresponding to 186 G6PD mutations [2]. G6PD deficiency is classified under five categories, based on the level of enzyme activity and clinical manifestations (Table 1).

Typical clinical manifestations of G6PD deficiency are acute hemolytic anemia and/or neonatal jaundice. Most men who are hemizygous for G6PD deficiency remain asymptomatic (as is the case for the A(-) G6PD variant, which is very common), but may be affected by acute hemolytic anemia only after exposure to an oxidative agent or stress.

Here we describe the case of a child presenting with severe cholestasis and liver dysfunction in the neonatal period [3]. Clinical symptoms were related to severe perinatal hemolysis and hyperbilirubinemia: Severe G6PD deficiency was diagnosed, due to a previously undescribed mutation.

2- CLINICAL DATA

A male infant was born at 39 weeks' gestation by cesarean section owing to fetal distress. The mother's origin was Greece (from her father's side) and France (from her mother's side), the father from the Ivory Coast. The infant was eutrophic. Physical examination revealed hepatosplenomegaly, jaundice, and fetal distress requiring initial mechanical ventilation.

Blood tests on the first day of life revealed severe anemia (hemoglobin = 74 g/l) with high reticulocytosis (558 g/l) and macrocytosis (mean corpuscular volume 141.4 fl), requiring blood transfusion. He also had a platelet transfusion because of transient thrombopenia. Owing to hepatocellular insufficiency (prothrombin time 34%, Factor II 20%, Factor V 40%, Factor X 28%), frozen plasma concentrates were administered. Prothrombin time was normalized on the second day of life and coagulation factors were normal at 1 week.

Laboratory investigations at birth also revealed the following: aspartate aminotransferase (AST) 1923 U/l (standard: < 37 U/l), alanine aminotransferase (ALT) 405 U/l (standard: < 32 U/l), total serum bilirubin 359 μ mol/l and conjugated bilirubin 248 μ mol/l, and gamma glutamyltransferase (GGT) 54 U/l, showing severe cytolysis and cholestasis with regenerative hemolytic anemia.

The infant developed several acute hemolytic episodes, which required iterative blood transfusions (three transfusions in 2 months). However, cytolysis gradually improved but cholestasis worsened, with treatment by ursodeoxycholic acid started on the ninth day of life.

The initial suspicion was organ dysfunction secondary to an early bacterial fetal infection, but several medical microbiological test results were negative.

The main diagnostic hypothesis was idiopathic neonatal hepatitis, which could be excluded by many differential diagnostic approaches. Metabolic disease and storage disease were excluded (bile acid deficiency, galactosemia, mitochondrial diseases, abnormal beta oxidation, tyrosinemia, urea cycle, CDG syndrome, anti-trypsin deficiency, transaldolase deficiency, abnormal redox point, Gaucher disease, Niemann–Pick disease, hemochromatosis). The bone marrow was normal. A direct Coombs test and Guthrie test yielded negative results. Hemoglobin electrophoresis findings were normal for his age. Several abdominal ultrasound examinations showed hepatomegaly but the gallbladder was still normal (no biliary atresia). Liver biopsy (Figure 1) revealed chronic cholestatic hepatitis with giant cells (intrahepatic cholestasis, portal inflammation, acute cholangitis, ductopenia, and residual cluster of hematopoietic elements), whereas muscle biopsy was normal.

G6PD activity was null on the first day of life but could not be assessed for several months because of repeated red blood cell transfusions. He left hospital at 4 months. However, at nearly 7 months of age, the child was admitted to the emergency department presenting with an acute episode of very severe and life-threatening hemolytic anemia (hemoglobin level at 26 g/l) after he had eaten mashed fava beans. The child's parents had not been correctly informed to avoid oxidative substances, especially fava beans. G6PD deficiency was confirmed 4 months later, after a period of no blood transfusion (enzymatic activity was 5% of normal with a G6PD/pyruvate kinase ratio of 0.05). G6PD gene sequencing revealed the c.675G>C; p.Trp225Cys mutation, present in the hemizygous state, which is a new and previously undescribed mutation. This new mutation has been called « G6PD Grenoble variant, » as the child was born in Grenoble and was initially deemed to carry a sporadic mutation. The G6PD

gene is located on chromosome X. The clinically severe phenotype with neonatal severe cholestasis and liver dysfunction and chronic hemolytic anemia together with the extremely low G6PD activity indicates a class 1 mutation. Genetic analysis of the mother revealed the same mutation, in the heterozygous state. The mother's G6PD activity was normal (84% and 106% of normal), she had no hemolysis and a normal hematological phenotype, due to chromosome X lyonization: This is a classic finding in heterozygous mothers when the mutation is linked to a severe phenotype in the hemizygous state. The father was also diagnosed with hemizygous G6PD deficiency (reduced G6PD activity 7% of normal, G6PD/pyruvate kinase ratio 0.10) and genetic analysis revealed the G6PD hemizygous mutation (c.202G>A, p.Val68Met), which is the most frequent G6PD variant present in tropical Africa and in the world (A-G6PD variant). The A-G6PD variant belongs to class 3 G6PD deficiency, which is a very mild condition, usually asymptomatic but resulting in acute hemolytic anemia (potentially severe) only after exposure to oxidative stress. The father had never experienced hemolysis and had normal hematological parameters.

In the months that followed, we observed the following:

- A persistent chronic hemolytic anemia requiring blood transfusion, folic acid and erythropoietin treatment (Table 2).
- Decreased cholestasis with a treatment by ursodeoxycholic acid (Table 2). The bilirubin level was normal at 6 months.
- Progressive disappearance of hepatosplenomegaly.

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3- DISCUSSION

Severe G6PD deficiency can cause neonatal hemolysis, which in turn leads to an obstruction and dilatation of the biliary ducts as well as progressive cholestasis, hepatosplenomegaly, and

liver dysfunction [4]. Indeed, patients with hemolytic disorders may develop biliary sludge, a suspension of particulate matter precipitated in bile and can cause this obstruction. This suggests that the capacity of hepatocytes to conjugate is exceeded by the excessive amount of bilirubin resulting from hemolysis.

Neonatal indirect hyperbilirubinemia is common for patients with G6PD deficiency. Usually, the diagnosis and treatment are quick and efficient. However, neonatal cholestasis is very uncommon, with only a few rare cases described in the literature [5,6]. In this case, G6PD deficiency was noticed but was not initially linked to the severe cholestasis, hyperbilirubinemia, and transient liver dysfunction. One should consider G6PD deficiency when faced with mixed hyperbilirubinemia in the neonatal period. However, class 1 G6PD deficiency resulting in chronic non-spherocytic hemolytic anemia (CNSHA) is an extremely rare condition with very few cases reported.

Acute hemolytic anemia in G6PD-deficient patients is largely preventable by avoiding exposure to fava beans, drugs, and chemicals that can cause oxidative stress. The identification and discontinuation of the precipitating agent are critical in managing hemolysis in patients with G6PD deficiency [7]. Management of the disease is essentially supportive and no specific treatment is available [8,9].

Neonatal cholestasis may be caused by other hemolytic diseases of the newborn such as severe pyruvate kinase deficiency, and other inherited hemolytic anemias in their severe forms (Rh hemolytic disease, stomatocytosis).

The discovery of a new mutation leads to the question of its classification among G6PD deficiencies. These are divided in five groups. The usual classification of G6PD variants is based on the G6PD activity rate and on its clinical and hematological characteristics (WHO classification, Table 1). Therefore, this case illustrates a probable rare class 1 G6PD

deficiency with very low enzyme activity. Usually, severe mutations leading to class 1 G6PD deficiency affect exons 6, 10, and 13. These mutations are often sporadic: In this case the mutation was not sporadic but following the classic X-linked transmission by the mother. Class 1 mutations result in the more severe phenotype of G6PD deficiency: chronic non-spherocytic hemolytic anemia [10].

The severe clinical phenotype of this child has led to discussions of an antenatal diagnosis for a subsequent pregnancy. Antenatal diagnosis for G6PD deficiency should be offered to couples when they are at risk of giving birth to a severely affected child, only for class 1 mutations leading to CNSHA.

4- CONCLUSION

We present the case of a severe G6PD deficiency resulting in chronic non-spherocytic hemolytic anemia and severe liver injury due to a new mutation. This case is a reminder that we must consider G6PD deficiency when faced with liver cholestasis and severe hemolytic anemia. It is equally important to follow recommendations concerning preventive measures when dealing with G6PD deficiency, as severe episodes of acute hemolytic anemia in G6PD deficiency can be life-threatening.

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Figures:

Figure 1: Liver biopsy

- a : HES ×100 numerous hematopoietic cells inside sinusoids (arrows)
- b: HES ×200 : numerous hematopoietic cells inside sinusoids (arrows) along with hepatocytes injuries with giant cells (*)
- c : Perls staining, ×200: a few macrophages with hemosiderin deposits (blue)
- d : Masson's trichrome staining, ×40: portal tract fibrosis with bridges (arrows)

Figure 1.

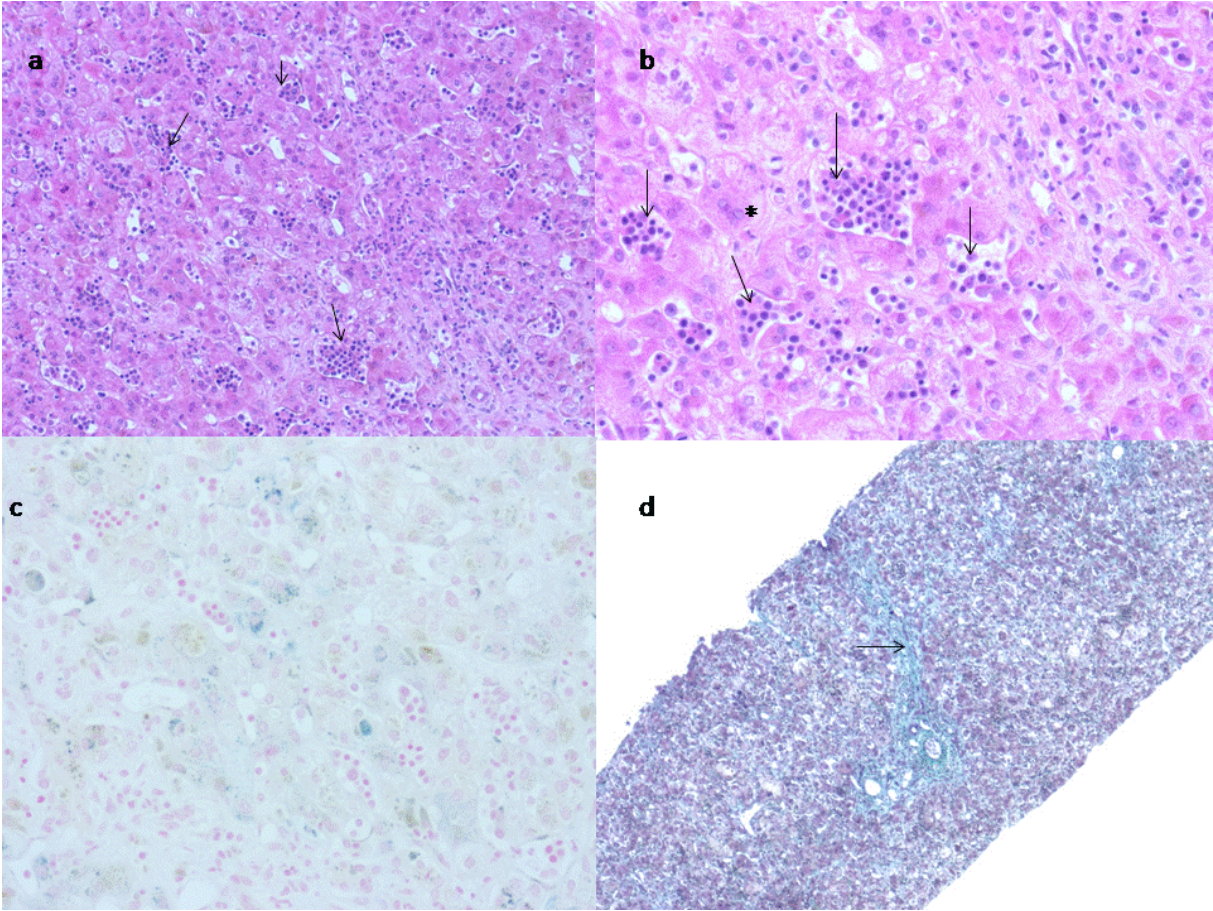


TABLE 1: WHO classification of G6PD variants [11]

G6PD: glucose-6-phosphate dehydrogenase

CLASS	LEVEL OF DEFICIENCY	ENZYME ACTIVITY	PREVALENCE
I	Severe	Chronic non-spherocytic hemolytic anemia	Uncommon , occurs across populations , mainly sporadic mutations
II	Severe	< 10% of normal, episodic hemolytic anemia	Varies ; more common in Asian and Mediterranean populations
III	Moderate	10–60% of normal, intermittent hemolysis with stressors	10% of black males in the United States
IV	Mild to none	60–150% of normal, no hemolysis	normal state
V	None	> 150% of normal, no hemolysis	normal state

TABLE 2. Biological parameters

	Day of Birth	Day 7 *	5 months **	7months ***	1 year	3 years
Total bilirubin ($\mu\text{mol/l}$)	351	570	13	70	81	44
Conjugated bilirubin ($\mu\text{mol/l}$)	244	453		3	6	7
AST (U/l)	1923	724		350	42	38
ALT (U/l)	405	450	70	83	36	24
GGT (U/l)	54	131	19	22	15	18
Alkaline phosphatase (U/l)	228		412	347	283	
LDH (U/l)			2180	1985	310	
Hemoglobin (g/l)	74	114	96	38	79	98
Reticulocytes (G/l)	558		544	360		599
Platelets (G/l)	72	168	273	334	342	319
Leukocytes (G/l)	18.6	29.3	3	28.8	7.2	6
Prothrombin time (%)	34	79	98	60	94	
Activated partial thromboplastin time (ratio)	1.34	1.19	1.08	1.08	1.09	1.27
Factor II (%)	20	63		73	80	90
Factor V (%)	40	114	174	68	124	98
Factor X (%)	28	76		91	98	79

* : after transfusion

** : after treatment by ursodeoxycholic acid, folic acid, and erythropoietin

*** after fava bean ingestion

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyltransferase; LDH : lactate dehydrogenase