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CASE REPORTS

HEMOLYTIC ANEMIA IN FEMALES: AN UNEXPECTED CAUSE

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ABSTRACT
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy and is inherited as an X-linked trait, therefore homozygotic females are rare. We present a five-year-old girl, who had severe hemolytic anemia after fava beans ingestion and an infectious insult. Investigations revealed G6PD deficiency associated with a genetic A- variant, which was also present in both her father and brother.

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy, affecting more than 400 million people worldwide.[1,2] G6PD has an important role in protection against red cells oxidative damage.[2,3,4] The enzyme catalyzes pentose phosphate's pathway first step - oxidation of glucose-6-phosphate to 6-phosphogluconolactone and the reduction of NADP+ to NADPH.[3,4] NADPH maintains a high level of reduced glutathione, protecting hemoglobin against hydrogen peroxide and other oxygen radicals.[3,4] The G6PD gene is located on the long arm of the X chromosome.[5] These gene mutations are related to protein variants with different levels of enzyme activity and an wide range of clinical phenotypes.[1] The most common clinical manifestations are hemolytic anemia and neonatal jaundice, triggered by an oxidative stress exposure (such as drugs, food or infections).[1,3,6] Some variants are related to chronic hemolysis and congenital non-spherocytic hemolytic anemia.[1,3,6] G6PD is inherited as an X-linked trait[3,7], therefore homozygotic females are rarer than hemizygous males 5 and have similar clinical manifestations.

We report the case of a girl with homozygotic G6PD deficiency that presented with severe hemolytic anemia. We discuss G6PD variants, different clinical phenotypes and emphasize the importance of considering this disorder in females.

Case Report

A previously healthy five-year-old female of Brazilian origin presented to the emergency department with fatigue, headache and chest pain evolving over a 7-day period. On the day of admission, she started vomiting and had fever. There was no relevant family history, and she had ingested fava beans on the first day of symptoms. There was no intake of other relevant food or drugs. On examination she was prostrated, with pale skin and conjunctiva, and had tachycardia and a systolic murmur (grade II/VI). Blood work revealed severe hemolytic anemia (Table 1). Auto immune, infectious etiology and hemoglobinopathies were excluded (Table 2). Abdominal ultrasound was unremarkable. Erythrocyte G6PD activity was in the lower limit of the reference range (Table 2). The child was admitted and received red blood cell transfusion on the first day without complications. Throughout the hospitalization, there was remarkable improvement, with no fever and resolution of the complaints. Hemoglobin values progressively increased and hemolysis parameters normalized. She was discharged on day 6 and was kept on regular follow-up. After 3 months, erythrocyte G6PD activity test showed moderate enzymatic deficiency (0.8 U/gHb – 11%). Genetic testing was then pursued which revealed c.202G>A (exon 4) and c.376A>G (exon 5) modifications resulting in p.(Val68Met) and p.(Asn126Asp) amino acids replacement, compatible with homozygotic G6PD A- variant. Family genetic testing revealed G6PD deficiency in her father and brother; her mother had normal enzymatic activity. Her brother has the same variant. Two years after the diagnosis, she avoids food and drugs causing oxidative stress, remains asymptomatic and with no reports of further hemolytic episodes.

Discussion

The World Health Organization classifies G6PD variants according to magnitude of enzyme deficiency and hemolysis severity.[7,8] Class I variants are rare and have severe enzyme deficiency (<10% activity) with chronic hemolysis. Class II variants, such as the Mediterranean variant, are also associated to severe deficiency but present with acute hemolytic anemia. Class III variants (including the A- variant) result in moderate deficiency (10 to 60% activity), causing intermittent hemolysis. Classes IV and V have no clinical significance.[8] The Mediterranean variant causes more severe anemia

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Table 1. Blood investigations of the patient

	Admission	After RBCT	At discharge	3 months later
Erythrocytes ($x10^{12}$ cells/cumm) RR: 3.9-5.3	1.72	2.94	3.25	4.57
Hemoglobin (g/dL) RR: 11.5-13.5	4.7	8.3	9	12
Hematocrit (%) RR: 34-40	14.6	25.8	29	37.4
Mean corpuscular hemoglobin (fL) RR: 25-29	27.5	28.2	27.8	26.3
Mean corpuscular volume (fL) RR: 75-87	85.2	87.7	89.2	81.8
RDW (%) RR: 11.5-14.5	15.7	16	17.5	13.7
Reticulocytes (%/$x10^9$/L) RR: 0.5-2/20-100	7.3/126	-	16.3/530	1.8/80
Platelets ($x10^9$/L) RR: 200-450	357	350	357	258
Peripheral blood smear	Mild anisocytosis and polychromatophilia	-	-	Unremarkable
Haptoglobin (mg/dL) RR: 26-185	<10	-	43	71
Total bilirubin (mg/dL) RR: <1	2.07	-	0.16	0.31
Direct bilirubin (mg/dL)	0.48	-	-	-
Lactate dehydrogenase (U/L) RR: 135-225	1133	-	-	244
Urinalysis	Positive hemoglobin	-	-	-

Note: RR - Reference range, RBCT - Red blood cell transfusion

Table 2. Investigations for autoimmune disorders and infectious agents

Test	Result
Direct and indirect Coomb's test	Negative
Complement C3 (mg/dL) RR: 90-180	148
Complement C4 (mg/dL) RR: 10-40	36.3
Immunoglobulin A (mg/dL) RR: 26-232	86.1
Immunoglobulin G (mg/dL) RR: 560-1307	1150
Immunoglobulin M (mg/dL) RR: 56-242	88.2
Anti-Parvovirus B19 antibodies (IgM)	Negative
Anti-*Mycoplasma pneumoniae* antibodies (IgM)	Negative
Anti-Cytomegalovírus antibodies (IgM)	Negative
Anti-Epstein-Barr antibodies (IgM)	Negative
Erythrocyte G6PD (U/g) RR: 4.6-13.5	4.6
Hemoglobin electrophoresis and quantification of Hb A2 and Hb F	Unremarkable

Note: RR - Reference range

compared to A- (more common in Africa and other regions, such as Brazil)[2], which is usually associated to self-limited and mild anemia.[9] More than 186 variants are identified in addition to the wild-type G6PD gene. The majority are missense mutations causing single amino acid replacement.[5]

G6PD deficient homozygotic females are rare and have clinical manifestations that are similar to those of heterozygotic males. In this case the patient presented with severe hemolysis which is not the most frequent phenotype associated to the identified variant (A-). The severe presentation is likely related to exposure to more than one agent known to trigger oxidative stress; fava beans intake (causing prolonged hemolysis which is corroborated by reticulocytosis) and an infectious insult. G6PD deficiency must be consider in the differential diagnosis of hemolytic anemia with negative Coomb's test in females, even in the absence of suggestive personal or family history. The diverse variants and phenotypes have a broad spectrum of severity. Therefore, there are many undiagnosed individuals with mild symptoms or even asymptomatic.

When diagnosing G6PD deficiency is essential to properly interpretate the normal reference range of G6PD activity. In severe hemolysis there is an increase of enzymatic activity due to the presence of reticulocytes and young red blood cells, which often leads to a false negative result, as described in this case report.[1,9,10] Enzymatic activity should be repeated 2 to 3 months after the acute episode to confirm or exclude the diagnosis of G6PD deficiency.[10] Genetic studies to identify the variant is crucial, since it has prognostic implications and identifies those who require genetic counseling.[4,8]

Compliance with Ethical Standards
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