Recurrent Isolated Neonatal Hemolytic Anemia: Think About Glutathione Synthetase Deficiency

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abstract

Hemolytic anemia (HA) of the newborn should be considered in cases of rapidly developing, severe, or persistent hyperbilirubinemia. Several causes of corpuscular hemolysis have been described, among which red blood cell enzyme defects are of particular concern. We report a rare case of red blood cell enzyme defect in a male infant, who presented during his first months of life with recurrent and isolated neonatal hemolysis. All main causes were ruled out. At 6.5 months of age, the patient presented with gastroenteritis requiring hospitalization; fortuitously, urine organic acid chromatography revealed a large peak of 5-oxoproline. Before the association between HA and 5-oxoprolinuria was noted, glutathione synthetase deficiency was suspected and confirmed by a low glutathione synthetase concentration and a collapse of glutathione synthetase activity in erythrocytes. Moreover, molecular diagnosis revealed 2 mutations in the glutathione synthetase gene: a previously reported missense mutation (c.[656A>G]; p.[Asp219Gly]) and a mutation not yet described in the binding site of the enzyme (c.[902T>C]; p.[Leu301Pro]). However, 15 days later, a control sample revealed no signs of 5-oxoprolinuria and the clinical history discovered administration of acetaminophen in the 48 hours before hospitalization. Thus, in this patient, acetaminophen exposure allowed the diagnosis of a mild form of glutathione synthetase deficiency, characterized by isolated HA. Early diagnosis is important because treatment with bicarbonate, vitamins C and E, and elimination of trigger factors are recommended to improve long-term outcomes. Glutathione synthetase deficiency should be screened for in cases of unexplained newborn HA.

Achieving an accurate diagnosis of hemolytic anemia (HA) in a newborn is essential for their good care. Main causes of HA (Rhesus D alloimmunization excluded) are ABO incompatibility, red blood cell (RBC) membrane disorders, RBC enzyme defects, and hemoglobinopathies.[1] Considering RBC enzyme defects, glucose-6-phosphate dehydrogenase and pyruvate kinase deficiencies are systematically researched due to their high prevalence. However, many other enzymes are involved in RBC metabolic pathways and can cause HA.[2] Among them, glutathione synthetase (GSS) is involved in the synthesis of glutathione (GSH), a strong antioxidant.[3] GSH depletion can be constitutive (genetic defect)[4] or induced by different trigger factors (eg, drugs, severe sepsis, malnutrition).[5,6] Hereditary glutathione synthetase deficiency (GSD) is classified into 3 phenotypes, from a mild form with isolated HA to a severe form with HA, constant 5-oxoprolinuria, metabolic acidosis,

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TABLE 1 Evolution of the Study Patient's Hb and TSB Levels During the First 9 Days of Treatment

Variable	Patient Results								Normal Values	
	Day 1	Day 2	Day 3	Day 4	Day 6	Day 7	Day 8	Day 9		
Hb, g/dL	13.2	12.9	12.3	11.5	7.9	7.3	6.3	14.3	1–7 d 8–90 d	14.5–22.5 10–16
TSB, g/dL	13.1	8.5	11.4	12.4	12.1	14.2	—	2.8	—	0–1

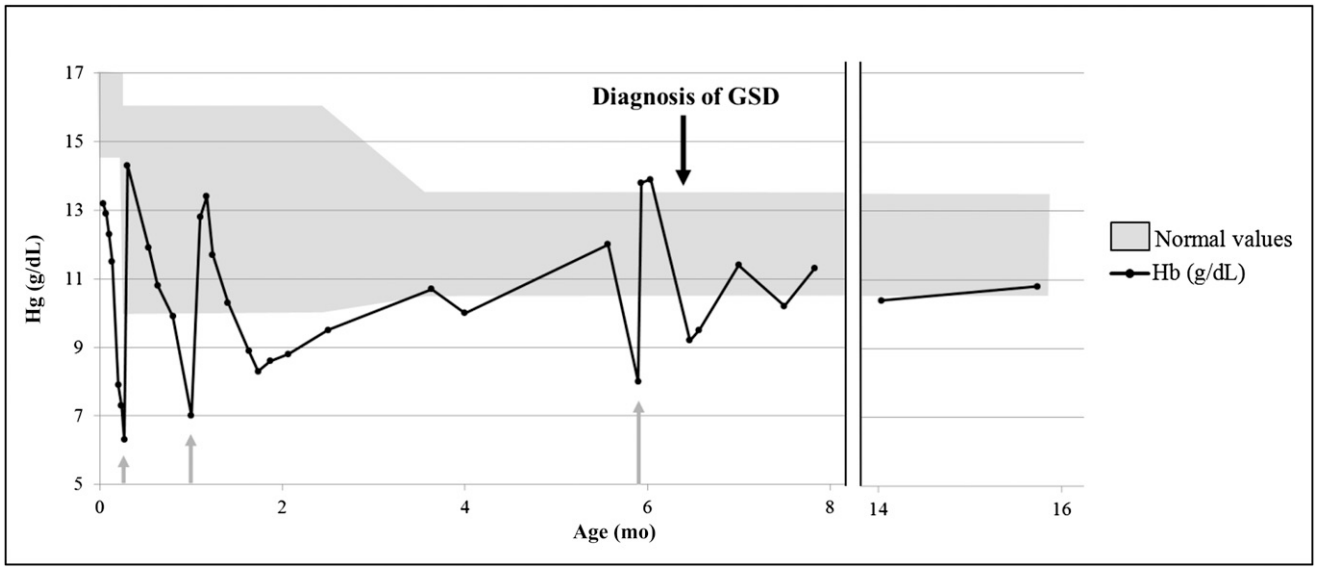

FIGURE 1
Variations in Hb concentrations over time. Before diagnosis, the patient presented 3 severe HA requiring blood transfusions (gray arrows; Hb, <8 g/dL). Since diagnosis, the patient's Hb level, due to appropriate care, has remained within low normal values.

and central nervous system impairment.[7,8]

This case report describes the discovery of GSD in a newborn who had presented with recurrent isolated HA since birth. Diagnosis was made fortuitously, at 6.5 months of age, by identification of 5-oxoproline in urine organic acid chromatography (UOAC) initially conducted to screen for causes of hypoglycemia.

CASE REPORT

The patient was the first child of a nonconsanguineous union. He was born at term after an uneventful pregnancy. His weight was 3.110 kg (50th percentile). At birth, he presented with slight anemia (hemoglobin [Hb], 13.2 g/dL) associated with a high total serum bilirubin (TSB) level (13.1 mg/dL [normal values, 0–1 mg/dL]). At 1 day old, phototherapy was initiated, which led to a decrease in TSB level. During the first week, results of regular blood screening revealed a progressive decline in Hb up to 6 g/dL, associated with an increase in TSB level to 14.1 mg/dL. A blood transfusion at day 8 improved Hb and TSB rates (Table 1).

At this time, no metabolic acidosis was observed, and there was no evidence of growth retardation or neurologic or digestive symptoms. The principal peripheral causes of HA were first ruled out: negative Coombs test result and autoimmune analyses, no Hb abnormality observed (normal Hb profile according to results of Hb electrophoresis), absence of glucose-6-phosphate dehydrogenase or pyruvate kinase deficiency, and normal ektacytometry results. Second, viral infections (Epstein-Barr virus and megalocytovirus) and paroxysmal nocturnal hemoglobinuria were excluded. Finally, a central cause was eliminated by normal findings on the bone marrow aspirate examination. In the follow-up, Hb was initially monitored on a weekly basis. Within 6 months, the patient presented with 3 severe cases of anemia requiring blood transfusions (Fig 1). No trigger factor was found.

At 6.5 months of age, the patient was admitted to pediatric emergency department for acute gastroenteritis for which he had received acetaminophen 48 hours before hospitalization. He presented with fever, episodic diarrhea, and no

signs of neurologic disorder. His Hb level was measured at 9.2 g/dL; a hemolytic cause of this anemia was suggested because of increases in lactate dehydrogenase activity at 520 UI/L and a reticulocyte count at 131 g/L (4.1%), despite normal values of TSB and haptoglobin (0.84 mg/dL and 120 mg/dL, respectively). A detectable haptoglobin concentration could be explained by the infectious syndrome; C-reactive protein level and leukocyte count were increased (data not shown). The presence of hypoglycemia associated with an infectious syndrome led to UOAC being conducted on day 4 of hospitalization to eliminate a fatty acid oxidation defect. The analysis revealed significant excretion of 5-oxoproline (pyroglutamic acid) equal to 8649 μmol/mmol creatinine (normal values, <70 μmol/mmol creatinine). GSD was then suspected because of the association between recurrent HA and 5-oxoprolinuria (pyroglutamic aciduria). However, 15 days later, there was no sign of the 5-oxoprolinuria. Thus, a GSH assay in RBC was performed (1 month after acetaminophen administration), and it revealed a decrease in reduced and oxidized GSH at 0.98 μmol reported to gram of Hb (mean normal value, 5.88 μmol/g Hb) and 0.02 μmol/g Hb (mean normal value, 0.235 μmol/g Hb), respectively.

Molecular analysis of the GSS gene according to DNA sequence analysis confirmed the diagnosis, revealing 2 heterozygous mutations (c.[656A>G;902T>C]; p.[Asp219Gly; Leu301Pro]). Each parent was heterozygous for 1 of these mutations and had never shown any clinical signs of GSH deficiency. These mutations were associated with very low GSS activity in erythrocytes in the patient, equal to 2% compared with the control sample (0.1 vs 4.6 pkat/mg Hb in patient versus control, respectively [analysis conducted at the Karolinska Institute, Stockholm, Sweden]). Once the diagnosis was evoked, treatment with vitamin E (10 mg/kg/d) and vitamin C (100 mg/kg/d) was initiated. The parents were instructed to avoid drugs and food known to precipitate HA in GSH deficiency.

At 18 months of age, the patient's neurologic development was normal, and he had been free of hemolytic crises requiring transfusion or hospitalization for 1 year (Fig 1). Considering his healthy lifestyle, vitamin supplementation was stopped. The patient is now 4 years old and has moderate HA that is well tolerated.

DISCUSSION

If HA is frequently observed in neonatology, its association with 5-oxoprolinuria is uncommon. This scenario should evoke GSD, a rare metabolic disease that is characterized by depletion of GSH. This case report draws attention to this inherited metabolic disease associated with HA in newborns.

Clinically, HA is characterized by the appearance of jaundice within the first 48 hours of life. Hemolytic disease of newborns should be considered before prolonged hyperbilirubinemia. History combined with chemical and hematologic laboratory testing are essential tools for making a specific etiologic diagnosis.[1] In the present case, results of the various laboratory tests remained normal, and only a symptomatic treatment by transfusion was initiated. A few months later, the diagnosis of GSD was made fortuitously by identifying 5-oxoprolinuria.

GSD is a rare autosomal metabolic disease (OMIM 266130). GSD can be divided into 3 clinical forms: a mild form, presenting with HA and variable 5-oxoprolinuria; a moderate form, associated with HA, constant 5-oxoprolinuria, and metabolic acidosis; and the severe form, associated with HA, constant 5-oxoprolinuria, metabolic acidosis, and neurologic defects.[4,7,8] Pathophysiology is explained by a decreased concentration of GSH due to the mutated GSS enzyme, which is unable to fulfill its role in the γ-glutamyl cycle.[4] GSH exerts negative feedback on γ-glutamyl cysteine synthetase, thus regulating its own formation (Fig 2A).[9]

When GSS is deficient, as in GSD, negative feedback is lost, leading to an increase in γ-glutamyl cysteine and thus an increase in 5-oxoproline via the γ-glutamyl cyclotransferase pathway.[9] Subsequently, 5-oxoproline accumulates in body fluids and can be removed in urine when the capacity of 5-oxoprolinase is exceeded (Fig 2B); 5-oxoprolinuria is most often secondary in various situations likely to result in GSH depletion. Inherited metabolic diseases, underlying infection, malnutrition, and drugs in particular are the main causes.[5,6] Among these, acetaminophen, via its hepatic metabolite (the N-acetyl-p-benzoquinone imine), is a well-known cause of depleted GSH stores.[10]

Observations of acetaminophen-acquired 5-oxoprolinuria are regularly reported in literature, attesting to its deleterious effect.[11] Reviewing the clinical history, we observed that the patient received acetaminophen at therapeutic doses across a 48-hour period before his hospitalization. As noted earlier, several factors may affect GSH stores, causing local fluctuations in levels. In mild forms of GSD, such as in the present case, the intracellular GSH level is usually sufficient to avoid 5-oxoprolinuria.[12] Acetaminophen administration, which briefly decreases GSH rate, might explain the large and transient urinary excretion of 5-oxoproline 4 days later. GSH, a ubiquitous molecule found particularly in RBC, is essential for cell membrane integrity, protecting

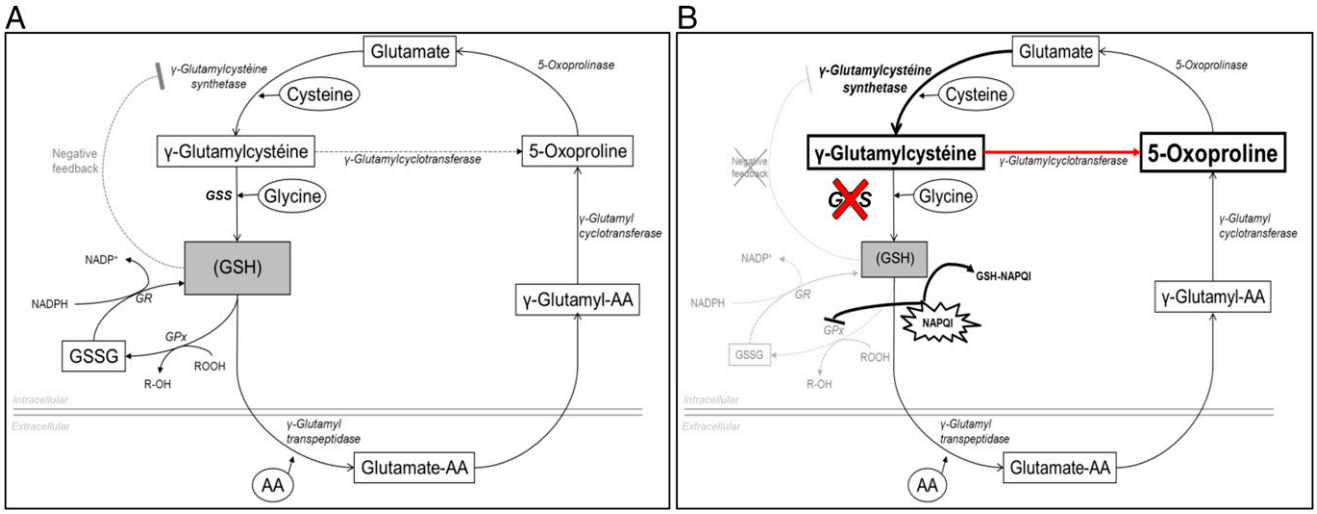

FIGURE 2

γ-Glutamyl cycle. (A) Normal cycle (GPx, GSH peroxidase; GR, GSSG reductase; GSSG, oxidized glutathione; NADPH / NADP+, reduced and oxidized forms of nicotinamide dinucleotide phosphate). (B) Patient's cycle: acetaminophen amplifies the decrease in GSH and eventually forces the γ-glutamyl cycle out of balance. Capacity of 5-oxoprolinase was exceeded, and transient 5-oxoprolinuria was observed (N-acetyl-p-benzoquinone imine [NAPQI]).

against reactive oxygen species and repairing oxidative damage.[13] Its deficiency decreases the reductive power of RBC and, hence, increases its susceptibility to oxidative stress. This scenario explains cellular and membrane fragility and the resulting nonspherocytic HA observed in GSH depletion due to GSD.[14–17]

Currently, there are ~70 recognized patients worldwide who have mild to severe forms of GSD. Biochemical diagnosis is based on the organic aciduria profile, cellular glutathione quantification, and enzymatic assay in RBC and nucleated cells.[4] In our case, the patient presented with a mild form of GSD, revealed by isolated HA. In this form, only some of the affected patients present with concomitant 5-oxoprolinuria, and involvement of a trigger factor in the anemic episode is rarely described.[6,8,16,17] Anemia was observed in a 56-year-old man after fava bean ingestion (a common trigger factor for anemia in glucose-6-phosphate dehydrogenase deficiency) and in a woman, 1 of her anemic episodes was observed during pregnancy which induces a glycine consumption.[17] No trigger factor has been identified in the other cases. Acetaminophen as a trigger factor has only been mentioned in moderate forms of GSD.[18]

The diagnosis of GSD in our patient was confirmed by a decrease in GSH concentration and the collapse of GSS activity in erythrocytes. A very low activity has already been reported in the mild form,[7] suggesting a complex relationship between genotype and phenotype.[7,8] Furthermore, GSD was confirmed by GSS gene sequencing. This gene is located on chromosome 20q11.22, contains 13 exons, and encodes a 474 amino acid enzyme.[19] Our patient presented with 2 heterozygous missense mutations, which both affect conserved amino acids in the catalytic domain. The mutation c.656A>G; pAsp219Gly has been previously reported only in mild form[7,8] and showed decreased kinetics. To our knowledge, the other mutation (c.902T>C; p.Leu301Pro) has not yet been described in the literature. This variation is located at the binding site between substrate and enzyme in a highly conserved amino acid and was predicted to be "deleterious" by SIFT (J. Craig Venter Institute, La Jolla, CA) and MutationTaster (Charité, Berlin, Germany), 2 pathogenicity prediction programs.

GSD is a rare cause of hereditary HA; 5-oxoprolinuria is an interesting biological marker but inconstant and transient in mild forms of GSD. In our case, without the acetaminophen exposure, diagnosis could have been delayed. Because early treatment with vitamins C and E and removal of trigger factors are the most predictive for long-term outcome, we propose to systematically search for GSD in cases of unexplained and isolated HA in childhood by using GSH assay in RBC before molecular analysis, even if results of the UOAC are normal.

ABBREVIATIONS

GSD: glutathione synthetase deficiency
GSH: glutathione
GSS: glutathione synthetase
HA: hemolytic anemia
Hb: hemoglobin
RBC: red blood cell count
TSB: total serum bilirubin
UOAC: urine organic acid chromatography

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