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The Diagnostic Dilemma of Congenital Unstable Hemoglobinopathies

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Abstract

Unstable hemoglobin variants represent a rare etiology of congenital hemolytic anemia. Without a high index of suspicion, plus proper laboratory testing and interpretation, the correct diagnosis can be elusive. We report on 5 children who were initially thought to have other congenital disorders such as hereditary spherocytosis or thalassemia, before β -globin gene sequencing led to the definitive diagnosis. Recognizing the variable clinical presentation and laboratory data reported will aid clinicians in diagnosis of unstable hemoglobins variants in children with atypical forms of hemolytic anemia, particularly those with low pulse oximetry values or whose hemoglobin electrophoresis suggest β -thalassemia trait.

Keywords

Hemolytic anemia; unstable hemoglobin; hemoglobin electrophoresis

INTRODUCTION

The evaluation of children with hemolytic anemia requires a thorough history and physical examination, accurate laboratory testing, and careful interpretation. Patients may present with fatigue, abdominal pain, hepatosplenomegaly, dark urine, or scleral icterus [1]. Laboratory evaluation usually reveals anemia with reticulocytosis and hemolysis; specific testing can reveal defects in the erythrocyte membrane or enzymes and identify acquired disorders of microangiopathic or immune-mediated anemia [2]. Most hemoglobinopathies are identified on newborn hemoglobin screening, although some may require subsequent hemoglobin electrophoresis. There is a small subset of patients whose comprehensive evaluation for hemolytic anemia appears normal or consistent with a mild disorder such as β -thalassemia trait, but have unexplained hemolysis with reticulocytosis.

In patients for whom standard evaluation does not identify the etiology of the hemolytic anemia, the possibility of a congenital unstable hemoglobinopathy should be considered. Among the more than 1000 globin chain mutations described (comprehensive hemoglobin variant database found online at <http://globin.cse.psu.edu>) [3,4], several alpha and beta

globin mutations are highly unstable, causing significant hemolysis. These children often present with hemolytic anemia, brisk reticulocytosis, jaundice, and splenomegaly, but can be difficult to diagnose.

One of the challenges of correctly diagnosing unstable hemoglobins is the potentially misleading results from hemoglobin electrophoresis. Unstable hemoglobin (Hb) variants undergo rapid denaturation, precipitation, and degradation within the erythrocyte. Analysis of the remaining Hbs may reveal a relatively normal hemoglobin electrophoresis pattern, particularly if the analysis is not performed quickly after collection[5]. For some unstable beta globin variants, the electrophoretic pattern may reveal increased Hbs A₂ and F, suggesting the diagnosis of β -thalassemia trait[6]; however, uncharacteristic severe hemolysis in this setting should prompt a more extensive evaluation including β -globin gene sequencing.

METHODS

With local IRB approval, we retrospectively reviewed the medical records of 7 pediatric patients with unstable hemoglobinopathies diagnosed since 2005 at St. Jude Children's Research Hospital. One family had 3 members with the same unstable hemoglobin variant; we report one since the presentations were similar. In all cases the diagnosis was established by DNA sequencing of the β -globin gene by an outside institution. None of the patients had concurrent α -globin gene deletions.

CASE REPORTS

Patient 1

An African-American male was referred as an infant after his newborn hemoglobin screen was consistent with sickle cell anemia. Electrophoresis of the parents revealed sickle cell trait in the father and elevated HbA₂ consistent with β -thalassemia trait in the mother. However, the mother had undergone cholecystectomy and splenectomy at an early age and was known to have anemia with reticulocytosis. Additionally, the patient had two maternal half siblings who had elevated HbA₂, but who were also being followed with uncharacteristically severe hemolytic anemia. He was tentatively diagnosed with HbS/ β^0 -thalassemia and his baseline labs at age 7 years are listed in Table I. During illnesses, he had pulse oximetry of 75–85% with arterial blood gas oxygen saturation of 95%. Review of the peripheral smear revealed target cells and bizarre forms without clear evidence of sickle cell forms, along with extreme reticulocytosis and large number of nucleated red blood cells (Figure 1A). Co-inheritance of an RBC enzyme deficiency such as G6PD deficiency or pyruvate kinase was considered but enzyme levels were normal or elevated. Due to the unusual family history and laboratory findings, β -globin gene sequencing was performed and revealed compound heterozygous HbS with Hb Volga (β Ala27Asp) [7,8]. Sequencing of family members revealed his mother, 2 siblings and one cousin had inherited Hb Volga without HbS.

Patient 2

A two year old African-American female was referred for evaluation of hemolytic anemia (Hb 9.0 gm/dL, Absolute Reticulocyte Count $700 \times 10^9/L$) and splenomegaly. Review of her peripheral blood film revealed spherocytes with pronounced polychromasia, but also marked anisocytosis and moderate poikilocytosis with small cells resembling schistocytes, helmet cells, echinocytes and other bizarre forms, along with basophilic stippling (Figure 1C). Hemoglobin electrophoresis (HPLC) revealed elevated HbF 11%, and slightly elevated HbA₂ 3.6%. Osmotic fragility testing, RBC enzyme, and serum lead levels were normal. She underwent β -globin gene sequencing at the same time as her half brother (Patient 1), and another half sibling with milder hemolytic anemia which revealed heterozygous Hb Volga (β Ala27Asp) [7]. She continues to have brisk hemolysis, splenomegaly, and has undergone cholecystectomy. At age 8 years, her baseline labs reveal a partially compensated hemolytic anemia (Table I).

Patient 3

A Caucasian female was referred at 1 year of age for evaluation of palpable splenomegaly and a month history of jaundice. She was found to have significant hemolytic anemia and was initially diagnosed with congenital spherocytosis, although no other family members were affected. Her baseline labs at age 13 years (Table I) and peripheral blood smear (Fig 1B) were not fully consistent with spherocytosis, since in addition to spherocytes she had massive reticulocytosis and bizarre RBC morphology, including basophilic stippling. She underwent splenectomy at age 7 years and had improvement in her anemia, but increased reticulocytosis. Also noted were low pulse oximetry readings (88–92%) on room air, despite a documented PaO₂ of 99 mm Hg. Comprehensive hemolytic anemia work-up revealed normal osmotic fragility and RBC enzyme activity. Hemoglobin electrophoresis revealed elevation of HbF of 12% with normal amounts of Hbs A and A₂ (83% and 3%, respectively). β -globin gene sequencing revealed heterozygous Hb Mizuho (β Leu68Pro) [9].

Patient 4

A one year old Caucasian male presented to an outside hospital after a breath holding spell where diagnostic work-up noted anemia and reticulocytosis. During the hospitalization he had persistent low pulse oximetry in the upper 80% to mid 90% range, but arterial blood gas revealed PaO₂ of 75mm Hg (low normal). Hemoglobin electrophoresis revealed a small abnormal peak that was later identified by β -globin sequencing as Hb Santa Ana (β Leu88Pro) [10]. Peripheral blood smear revealed moderate anisocytosis and poikilocytosis, marked polychromasia and irregularly shaped small erythrocytes with no well-defined abnormal morphology (Figure 1D). At age 21 months, his baseline labs show ongoing intravascular hemolysis (Table I).

Patient 5

A 7 year old Caucasian female presented for evaluation of anemia noted during routine school physical. Family history revealed Italian ancestry in her father, who was diagnosed with β -thalassemia minor as a child, but due to the presence of prominent basophilic stippling on his peripheral blood smear was later thought to have 5' nucleotidase deficiency.

He required cholecystectomy and had persistent but stable splenomegaly and jaundice. Her labs revealed a partially compensated hemolytic anemia (Table I) and peripheral blood smear had notable reticulocytosis and basophilic stippling. No variant hemoglobin was identified on electrophoresis, but her HbA₂ and HbF were elevated at 4.1% and 6.1% respectively. β -globin gene sequencing revealed Hb Wien (β Tyr130Asp) [11].

DISCUSSION

We describe 5 cases of unstable hemoglobinopathy in pediatric patients which presented as a hemolytic anemia. These unstable hemoglobins are the result of a single amino acid substitution in the β -globin protein. The amino acid changes in our patients primarily affect β -globin contact with the heme pocket, resulting in degradation of hemoglobin within the erythrocyte. Most of these unstable hemoglobins were described as single case reports and named for the location they were first identified. A comprehensive index of unstable hemoglobins is located at <http://globin.cse.psu.edu>, which describes the clinical and laboratory features of the each globin variant and lists pertinent case reports.

Hemoglobin electrophoresis may be helpful in identifying hemoglobin variants; however, unstable hemoglobins that undergo rapid denaturation or are represented in the HbA fraction may not be detected by this method. A normal hemoglobin electrophoresis should not rule out the diagnosis of unstable hemoglobin in patients with otherwise unexplained hemolytic anemia. Alternatively, hemoglobin electrophoresis may reveal elevation of Hb A₂ and F, suggesting inheritance of β -thalassemia trait. However, persons with β -thalassemia trait do not have evidence of brisk hemolysis; reticulocytosis in this setting should prompt more comprehensive evaluation of the hemolytic anemia. One caveat is that denatured hemoglobin precipitates (Heinz bodies) or micronuclei (Howell-Jolly bodies) may artificially elevate the apparent reticulocyte count for some instruments. In patients with unstable hemoglobinopathies, the blood smear characteristically contains Heinz bodies and polychromasia, and basophilic stippling can be prominent. Conversely, peripheral blood smears from patients with β -thalassemia trait typically reveal only microcytosis and hypochromia.

Variant unstable hemoglobins often have a different oxygenation-deoxygenation curve than HbA. Changes within the hemoglobin that result in decreased affinity for oxygen appear as low pulse oximetry readings as seen in 2 of our patients with steady state pulse oximetry readings of 85–90%. Normal PO₂ by arterial blood gas can confirm adequate tissue oxygenation despite apparent hypoxemia by digital pulse oximetry. The diagnosis of unstable hemoglobin should be considered in patients with low steady state pulse oximetry without symptoms of hypoxemia and with normal PaO₂.

In summary, unstable variant hemoglobins are a rare etiology of childhood hemolytic anemia, and should be suspected when the degree of hemolysis or the red cell morphology are not consistent with the proposed diagnosis. Establishment of the correct diagnosis can be challenging without high clinical suspicion and appropriate laboratory evaluation. Accurate diagnosis allows the clinician to appropriately counsel the family on the expected course of

the disease including potential clinical complications, and provide crucial information for genetic counseling.

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Figure 1.

Table I
Steady-state laboratory values and diagnosis for 5 cases of unstable hemoglobinopathy

Patient	Hemoglobin (g/dL)	ARC (X 10 ⁹ /L)*	LDH (units/L)**	Total Bilirubin (mg/dL)	AST (units/L)	O ₂ saturation (room air)	Hemoglobin Variant	Mutation
1	11.2	2450	592	3.6	66	90%	Volga _S	βAla27Asp, βGlu6Val
2	10.9	2619	280	3.9	24	96%	Volga	βAla27Asp
3	11.0	1291	535	3.3	46	92%	Mizuho	βLeu68Pro
4	9.5	637	1177	2.7	86	88%	Santa Ana	βLeu88Pro
5	10.0	247	540	1.2	40	98%	Wien	βTyr130Asp

* Absolute Reticulocyte count. Normal 50–75 X 10⁹/L;

** Lactate dehydrogenase. Normal 165–310 units/L