Advances in the treatment of alpha-thalassemia

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ABSTRACT

Alpha (α)-thalassemia represents a group of recessively inherited hemoglobin disorders marked by deficient or absent synthesis of 1 to all 4 of the α-globin genes. Inactivation of 3 α-globin genes – either by deletional or nondeletional mutations – results in hemoglobin H (Hb H) disease. Patients with Hb H disease produce hemoglobin composed of all beta chains and have moderate to severe hemolytic anemia, a variable degree of ineffective erythropoiesis, and splenomegaly. Transfusion requirements vary depending on the mutation and clinical severity. Treatment for deletional Hb H disease is primarily preventative and transfusions are uncommon. Patients with nondeletional Hb H disease (e.g., Hb H Constant Spring) typically have more severe anemia, and approximately one-third require regular transfusions. These patients often require comprehensive, multidisciplinary care. This chapter focuses on screening, diagnosis, and treatment approaches for patients with Hb H disease.

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1. Introduction

Alpha (α)-thalassemia is the most common genetic disorder of hemoglobin (Hb) synthesis, affecting up to 5% of the world’s population.¹ This family of recessively inherited disorders occurs at a particularly high frequency in populations from sub-Saharan Africa through the Mediterranean region and Middle East, to the Indian sub-continent and East and Southeast Asia, as well as in countries with large immigrant populations from these regions.² α-Thalassemia occurs as a result of deficient or absent synthesis of α-globin chains on chromosome 16, which leads to an excess of gamma (γ)- and beta (β)-globin chains in adult Hb.³ Inactivation of one or both α-globin genes results in advanced α-thalassemia such as Hb H Constant Spring (Hb HCS), which is the most common nondeletional Hb H; these disorders typically have a more severe phenotype compared with the deletional forms.¹ Hb HCS is caused by mutations in the stop codons, resulting in 31 amino acids being added to the α-chain, and the elongated α-chain is unstable.¹ Other clinically important forms of nondeletional α-thalassemia are Hb H-Quong Sze and Hb H-Suan Dok. Although Hb HCS was first described in a Chinese family in Jamaica,⁴ it predominates in people of Southeast Asian and Chinese descent.³ The spectrum of mutations that underlie α-thalassemia vary widely in the different populations affected (see article titled, “Recent advances in the molecular understanding of non-transfusion-dependent thalassemia”). Rare α-thalassemia syndromes associated with multiple developmental abnormalities and distinct physical features are also described in the article on molecular understanding of non-transfusion-dependent thalassemia (NTDT).

There is substantial phenotypic heterogeneity in α-thalassemia resulting from its underlying genetic diversity.¹ ⁵ ⁶ Inactivation of a single α-globin gene results in α-thalassemia silent carrier status, an asymptomatic form of the disease characterized by normal hematology. Inactivation of 2 genes causes α-thalassemia trait; patients are typically asymptomatic with microcytosis, but exhibit no anemia. Inactivation of 3 of the 4 α-globin genes results in significant production of Hb H, a form of Hb composed of all β-chains (β₄). Patients with Hb H have α-thalassemia intermedia, or Hb H disease, which results in moderate to severe hemolytic anemia, a modest degree of ineffective erythropoiesis, and splenomegaly. Inactivation of all 4 α-globin genes results in significant production of Hb Bart, a form of Hb composed of all γ-chains (γ₄). Patients with Hb Bart have α-thalassemia major, which typically results in fatal hydrops fetalis.

Deletional and nondeletional forms of Hb H disease are very different disorders with various clinical phenotypes. A study conducted by the Thalassemia Clinical Research Network (TCRN) compared the clinical features of nondeletional Hb HCS in 46 patients with those of deletional Hb H disease in 106 patients.⁷ More patients with Hb HCS required transfusions (24% vs 2%), had splenomegaly (13% vs 1%), and underwent splenectomy (13% vs 2%) compared with patients with deletional Hb H disease.

In general, α-thalassemia intermedia, or Hb H disease, typically causes mild-to-moderate hemolysis, therefore, as noted above, transfusions are necessary only occasionally depending on the
clinical severity. Most patients with deletional Hb H disease and Hb HCS, are thus considered to have NTDT. This article focuses on screening, diagnosis, and treatment approaches for patients with non-transfusion-dependent Hb H disease.

2. Hemoglobin H disease

2.1. Pathophysiology

Hemoglobin H disease is most frequently observed in patients who are compound heterozygotes for 2 different mutations or, less frequently, homozygotes for a moderately severe genetic mutation. These patients usually produce less than 30% of the normal amount of α-globin. The deficit in α-globin expression is most severe in nondeletional Hb H disease. Hemoglobin H tetramers have a high oxygen affinity, lack heme–heme interactions, and have poor oxygen delivery capacity. Therefore, patients with large amounts of Hb H are functionally more anemic than their hemoglobin level indicates. Hemoglobin H is unstable and can be oxidized to form intramolecular inclusion bodies within red blood cells (RBCs). These inclusion bodies present in erythroblasts are thought to cause intramedullary early erythroid cell death and ineffective erythropoiesis. More often, inclusion bodies form in circulating erythrocytes and become attached to the cell membrane, causing local oxidative damage, membrane dysfunction, and shortened red cell survival.

2.2. Clinical characteristics

Table 1 compares the clinical manifestations and laboratory findings in patients with deletional and nondeletional Hb H disease. Complications associated with deletional Hb H disease primarily include anemia, jaundice, and gallstones. Patients with Hb H disease have increased hemolysis and a mild-to-moderate anemia with marked microcytosis and hypochromia due to the instability of Hb H. Hematologic changes vary and are age-dependent. In one study including Hb H deletion patients, the mean hemoglobin level was 8.5 g/dL (range 6.9–10.7) among infants 1–3 months of age; mean corpuscular hemoglobin (MCH) was 16.6 pg (range 14.3–24.7); and mean corpuscular volume (MCV) was 54.0 fl (range 46.0–76.0). Red blood cell indices vary in Hb H disease: mean corpuscular hemoglobin (MCH) was 16.6 pg (range 14.3–24.7); and mean corpuscular volume (MCV) was 54.0 fl (range 46.0–76.0). Red blood cell indices vary in Hb H disease. 

Table 1

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Deletional</th>
<th>Nondeletional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td>8.5</td>
<td>7.2</td>
</tr>
<tr>
<td>(range 6.9–10.7)</td>
<td>(range 3.8–8.7)</td>
<td></td>
</tr>
<tr>
<td>MCV, fl</td>
<td>54.0</td>
<td>65.2</td>
</tr>
<tr>
<td>(range 46.0–76.0)</td>
<td>(range 48.7–80.7)</td>
<td></td>
</tr>
<tr>
<td>MCH, pg</td>
<td>16.6</td>
<td>18.6</td>
</tr>
<tr>
<td>(range 14.3–24.7)</td>
<td>(range 14.8–24.8)</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>Microcytic</td>
<td>Normocytic</td>
</tr>
<tr>
<td>Reticulocytosis</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hypochromia</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Age at first transfusion, yrs</td>
<td>11 ± 5.5</td>
<td>1.5 ± 2.1</td>
</tr>
<tr>
<td>History of blood transfusion, %</td>
<td>3–29</td>
<td>24–80</td>
</tr>
<tr>
<td>Spleenomegaly</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gallstones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth retardation</td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td>Decreased bone density</td>
<td>Rare</td>
<td>Common</td>
</tr>
</tbody>
</table>

Abbreviations: MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

Increased recognition of the clinical morbidity associated with Hb H, particularly the more severe deletional forms, the rising prevalence, and the benefits of early detection has led to development of Hb H newborn screening programs. Genetic testing and counseling for families of newborns with Hb H is important. Because of the risk of having future pregnancies with hydrops fetalis, family testing of all Hb H newborns is necessary. The approach to screening and counseling, however, depends on available regional resources, which are often scarce in geographic regions with a high frequency of α-thalassemia such as sub-Saharan Africa and Southeast Asia. In the United States, California was the first state to implement mandatory screening of newborns for Hb H disease in 1999 following an investigational screening period initiated in 1996; more than 500 cases were diagnosed between 1998 and 2006. However, the US Secretary of Health and Human Services Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) found insufficient data to recommend that all states adopt newborn screening for Hb H disease.

Several laboratory screening tests can be used to test for α-thalassemia. For example, testing of RBC indices reveals microcytic anemia in patients with deletional Hb H disease and a moderate (between 3% and 6%) amount of reticulocytosis. Peripheral blood smear in patients with deletional Hb H disease typically reveals microcytosis, hypochromia, anisocytosis, poikilocytosis, and very rare nucleated RBCs (i.e., erythroblasts). Red cell inclusions can be found in 5% to 80% of the erythrocytes of individuals with Hb
H disease following supravalve stain analysis (e.g., methylene blue or brilliant cresyl blue). Qualitative and quantitative Hb analysis by electrophoresis and high-performance liquid chromatography (HPLC) may demonstrate high levels of HbH in patients with deletional Hb H disease. Hemoglobin electrophoresis may not detect the Hb H protein; Hb H is very unstable and is best seen on very fresh samples.6 Patients with Hb H disease often have decreased levels of HBA2. Diagnosis of Hb HCS is more difficult because the associated anemia is often normocytic and can be easily missed on blood smears, and the low levels of Hb H are difficult to detect by electrophoresis. Thus, molecular testing may be necessary.11,14

Several molecular genetic testing methods exist that can be used to confirm diagnosis of α-thalassemia and determine which type is present (Table 2).23 Molecular genetic testing of HBA1 and HBA2 detects deletions in approximately 90% and point mutations in approximately 10% of affected individuals. For example, targeted mutation analysis can detect deletions and sequence variants in the α-globin genes. Polymerase chain reaction (PCR)-based methods that use specific primers flanking the deletion breakpoints can detect deletion of a single or both α-globin genes.24,25 Primer panels targeted to the most common mutations found in a given geographic region can be used. Sequence variants often create or destroy restriction enzyme sites within the α-globin genes and may be detected by restriction enzyme digestion of the amplified product.21 When an α-globin deletion is not identified, sequence analysis can be used to detect point mutations, including rare termination codon mutations and hyperunstable α-globin variants, in the coding regions of HBA1 and HBA2.26 It is important to note that mutations in regulatory regions upstream from the α-globin cluster will not be detected by sequence analysis of the α cluster region alone. Deletion/duplication analysis can also be used to detect common, rare, and/or novel deletions and duplications involving HBA1 and HBA2 that are not readily detectable by sequence analysis of genomic DNA.23 A variety of methods including quantitative PCR, long-range PCR, and multiplex ligation-dependent probe amplification (MLPA) may be used. An expanded multiplex gap-PCR has recently been developed for the simultaneous detection of nondeletional Hb HCS and common deletion variants that give rise to α-thalassemia.27 As noted previously, standard electrophoretic techniques often miss unstable structural mutations such as Hb HCS; therefore, it is recommended that DNA analysis be used for detection of these types of variants.14

Patients with α-thalassemia may also co-inherit mutations for other hemoglobinopathies.1 For example, many patients with Hb H disease also have β-thalassemia or sickle cell mutations. Patients with Hb H disease may also have glucose-6-phosphate dehydrogenase deficiency.1 A diagnosis of Hb H disease should therefore not rule out the possibility that other hemoglobinopathies exist.

2.4. Current management strategies

The nature of treatment for Hb H disease is primarily preventative and supportive. Because of the hemolysis and increased erythropoiesis associated with Hb H disease, supplementation with folic acid (2–5 mg/day) is generally recommended, especially in pediatric patients who may not acquire adequate amounts from their daily diet, and in pregnant women during the periconceptional period and beyond.3,10 Patients may develop antioxidant, calcium, and vitamin D deficiency. Supplementation with a non-iron-containing multivitamin is usually indicated.24 The following preventative measures are indicated for patients with Hb H disease: avoidance of oxidative compounds and medications, avoidance of high iron diet, prevention of unnecessary iron therapy unless iron deficiency is documented, prompt treatment of infections, and alertness to the possibility of acute anemic events.1 Management also requires ongoing monitoring of growth, bone health, spleen size, symptoms of cholecytitis, and fatigue level.

Despite having moderate anemia, most patients with deletional Hb H disease are asymptomatic and do not require regular transfusions. However, during a hemolytic crisis, anemia can become severe enough to necessitate transfusion.10 There are several factors that can contribute to hemolytic crisis including infection, pyrexia, oxidative challenge, aregenerative anemia, hypersplenism, or pregnancy.3 Therefore, patients should be monitored closely for severe anemia during acute infections and pregnancy. Patients with nondeletional Hb H disease are more likely to require periodic transfusions, which are usually triggered by an infection, and some patients may require regular transfusions.10,12

Table 3 summarizes the guidelines for management of Hb H disease with hemolytic crisis.10 Iron overload may occur in older patients regardless of transfusion history, but occurs much earlier in those who have been transfused.3 Because serum ferritin monitoring may underestimate the degree of iron overload, quantitative measurement of liver iron is indicated for adults or children placed on regular transfusions (see article titled, “Iron overload in non-transfusion-dependent thalassemia: a clinical perspective”). Chelation therapy is indicated in all patients with hemosiderosis. Indicators for regular transfusion are based on quality of life. Some patients develop severe fatigue with older age; other indicators include persistently severe anemia (<6.5 g/dL), poor growth and weight gain, or bony changes. Although growth retardation and delayed puberty are not typical features of deletional Hb H disease, bone density and growth should be monitored. Monitoring of bone density and growth is particularly important in patients with nondeletional Hb H disease. For patients with Hb H disease and marked splenomegaly and hypersplenism, splenectomy can result in highly significant hematologic (≥2 g/dL increase in Hb) and clinical improvements.1,10 Patients with nondeletional Hb H disease who have a more severe clinical phenotype may be considered for splenectomy. In some populations, splenectomy is commonly performed in patients with nondeletional Hb H disease.3,12,14 There are, however, risks associated with splenectomy, including septicaemia, deep vein thrombosis, and pulmonary embolism.3,30,31 For patients undergoing splenectomy with post-splenectomy thrombocytosis, aspirin prophylaxis is recommended.10 Cholecyctectomy may also be indicated for patients with persistent gallstones that do not respond to intravenous antibiotics or surgical removal of the stones.3,32

<table>
<thead>
<tr>
<th>Test method</th>
<th>Mutations detected</th>
<th>Percent of alleles</th>
<th>Mutation detection frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted mutation analysis</td>
<td>Deletions</td>
<td>∼90%</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>HBA2 sequence variants</td>
<td></td>
<td>Variable</td>
</tr>
<tr>
<td>Sequence analysis</td>
<td>HBA1 and HBA2 sequence variants</td>
<td>∼9% to 10%</td>
<td>Theoretically 100%</td>
</tr>
<tr>
<td>Deletion/duplication analysis</td>
<td>Deletions and duplications</td>
<td>Unknown</td>
<td>Variable</td>
</tr>
</tbody>
</table>

Table 3 Guidelines for management of Hb H disease with hemolytic crisis.13

1. Restore patient's hemoglobin to 8–9 g/dL by red cell transfusion
   - Provide filtered RBCs or leucocyte depleted blood 5–12 mL/kg/dose depending on patient's clinical severity and levels of anemia
   - A close monitoring on total body fluid and cardiovascular status is highly recommended
   - Serial Hb and Hct evaluation should be done at least daily since hemolysis could be continued as the cause has not been removed or properly treated

2. Give adequate hydration
   - Intravenous fluid therapy should be provided to maintain circulation and withheld during transfusion support
   - The amount and rate should be carefully calculated to avoid possible heart failure from volume overload

3. Check blood electrolytes and provide appropriate correction
   - Metabolic acidosis is usually observed but mostly resolved by transfusion support and fluid therapy; only rare cases require alkali therapy

4. Try to control body temperature by various means
   - Frequent tepid sponge
   - Acetaminophen 10–12 mg/kg every 4–6 hours
   - The usage of NSAIDs in hemolytic crisis of Hb H has limited data

5. Identify the cause of infection/inflammation and provide appropriate treatment
   - Blood and urine culture should be done
   - Empirical antibiotic with the coverage of gram-negative bacteria and/or encapsulated bacteria (depending on splenic condition) such as Streptococcus and Salmonella species, as well as meningococci, should be promptly provided

Hb, hemoglobin; Hct, hematocrit; NSAIDs, nonsteroidal anti-inflammatory drugs; RBC, red blood cell.
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3. Conclusions

There is a wide spectrum of clinical presentations of Hb H disease resulting from genotypic diversity. Despite having moderate anemia, most patients with deletional Hb H disease have good quality of life and do not require regular transfusions. However, during a hemolytic crisis, which can be caused by a variety of factors, anemia can become severe enough to require acute or chronic transfusion therapy. As patients with deletional Hb H disease age, progressive fatigue and clinically significant hemosiderosis occur, requiring closer monitoring of adult patients. Patients with nondeletional Hb H disease typically have more severe anemia, are at greater risk for iron overload and other complications, and should be monitored closely. Because of its clinical morbidity and increasing prevalence, population screening for Hb H disease is increasing.

Conflict of interest statement

Dr. Vichinsky has disclosed that he has received consulting fees from HemaQuest and Novartis and contracted research support from Emmaus, HemaQuest, and Novartis.

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