Hemoglobinopathies

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Hemoglobin consists of a heme molecule at the center of a quaternary protein structure. Heme consists of an iron atom sitting at the center of protoporphyrin ring and the protein part of hemoglobin consists of 2 alpha globin chains and 2 beta globin chains. The alpha globin gene cluster is encoded by chromosome 16. During the embryonic life the active alpha globin gene is the Zeta (ζ) gene. Activity transitions in the fetal an adult life to 2 copies of alpha 1 and alpha 2 ($\alpha 1, \alpha 2$) genes. The beta globin genes are present on chromosome 11 and the beta chain is encoded by the epsilon (ε) gene in the embryonic life but in contrast to alpha hemoglobin there are separate active genes during fetal and adult lives. During the fetal life, 2 copies of gamma hemoglobin give rise to what is known as fetal hemoglobin ($\alpha 2 \gamma 2$, HbF) then after birth this shifts to beta (β) and delta (δ) chain production. Most of the adult hemoglobin is in the form of alpha 2 betas 2 (HbA) and a minority in the form of alpha2 delta 2 (minor adult hemoglobin, HbA2) and a very small fraction of the adult hemoglobin may normally persist as fetal hemoglobin throughout life. Note that there are normally 4 total copies of adult alpha chain and 2 of beta since there are copies on both of the homologous chromosomes. Knowing the fore mentioned is helpful in understanding the classification of hemoglobin disorders.

Hemoglobin disorders can be either quantitative such as thalassemia the hallmark of which is the imbalance between the production of the 2 main types of globin chains resulting in both anemia and ineffective erythrocytosis, or qualitative resulting in the production of abnormal globin chains as is the case in sickle cell disease. In the normal state, balanced amounts of alpha and beta, or alpha and gamma chains are produced resulting in the presence of HbA during post-natal life and HbF during fetal life. Alpha thalassemia is a situation where there is a decreased production of the alpha chain. This results in less amounts of normal fetal hemoglobin produced during the fetal life and the excess gamma chains form a tetramer (gamma 4) called hemoglobin Bart's. in the post-natal life, this leads to less amount of the normal HbA produced and the excess beta chains form a tetramer (beta 4) called hemoglobin H. On the other hand, in beta thalassemia, we observe less amounts of the normal HbA but no alpha 4 tetramers since they are not stable. The excess alpha and beta chains that don't participate in normal hemoglobin production tend to precipitate in red blood cells resulting in membrane damage and hemolysis adding and element of ineffective erythropoiesis to the anemia. This in turn drives bone marrow space expansion and extramedullary hematopoiesis to compensate coupled with increased intestinal iron absorption.

Thalassemia can be classified either according to the genetic defect (alpha, beta, delta-beta) or according to clinical severity (minor, intermedia and major) where minor thalassemia presents with mild asymptomatic anemia, intermedia thalassemia presents with moderate symptomatic anemia that may require intermittent transfusions and those with thalassemia major are transfusion dependent due to severe anemia.

Following is a summary of the different scenarios:

A) Alpha thalassemia:
   1) 4 genes present: $\alpha\ \alpha / \alpha\ \alpha$: normal state.
   2) 3 genes present: $\alpha\ \alpha / \alpha -$: silent carrier. Normal or low normal hemoglobin values. The clinical significance is risk to offspring when reproducing with a person that is either a carrier of affected by the disease.
   3) 2 genes present: $\alpha\ \alpha / - -$ (cis form, both mutation on the same chromosome) or $\alpha - / \alpha -$ (trans form, one gene is missing from each chromosome). Thalassemia trait. The usual clinical picture is thalassemia minor with mild asymptomatic anemia (Hb 10-11 gm/dl). Characterized by hypochromia and mild microcytosis in addition to minimal amounts of hemoglobin Bart's (2-10%) in neonates. The significant difference between the Cis and Trans forms is that 2 carrier parents with the trans form will have all of their offspring carrier with the trans from too, while 2 carrier parents with the cis form will bring up offspring that is 25% normal, 50% carrier with the cis form and 25% with thalassemia major (--/--) that results in hydrops fetalis and intrauterine demise.

Thalassemia trait can frequently be misdiagnosed as iron deficiency anemia that is refractory to iron replacement therapy.

4) One gene present: $\alpha-/--$. Hemoglobin H disease (there is a variant in the form of $\alpha\ \alpha^{cs}$/-- called hemoglobin H constant spring that we will not discuss here). Usually presents as thalassemia intermedia with symptomatic anemia requiring occasional transfusions. Hypochromia and microcytosis are more pronounced than in the trait and up to 50% of the neonatal peripheral blood may be Hb Bart's in addition to the presence of HbH later in life. These patients usually have hepatosplenomegaly due to the early destruction of red blood cells and many eventually develop hemochromatosis.
5) All gene deletion: --/--. Thalassemia major usually resulting in hydrops fetalis unless managed by intrauterine transfusion and bone marrow transplant post Nataly.

B) Beta Thalassemia:

1) Both genes present and normal: β/β. Normal state.
2) One normal gene: β/β^0 or β/β^+. Beta thalassemia trait. Usually presenting as thalassemia minor. The β^0 gene carries a mutation that completely abolishes beta chain production while the beta plus gene has a mutation that down regulates beta gene production resulting in a less severe phenotype compared to β/β^0). Patients have normal blood counts in the neonatal period since the predominant hemoglobin then is HbF. As transiting to adult type hemoglobin, they start developing mild microcytic anemia by 4-6 months of age. The delta chain upregulates production to compensate for the decreased beta chain production resulting in increased HbA2 (alpha2 delta 2) on electrophoresis. Beta thalassemia minor is a relatively common disorder that is misdiagnosed and treated as an iron deficiency anemia.
3) Both genes mutated, but at least one copy produces some beta chains: β^+/β^0, β^+/β^+, β^E/β^0 or β^E/β^+. Generally, present as thalassemia intermedia. The beta E chain is the hemoglobin E mutation that results in mild compensated anemia in the homozygous state. These patients also present with normal blood counts at birth and progress to mild to moderate anemia over a few months. The have elevated levels of HbA2 and HbF to compensate for the anemia. They may also develop hepatosplenomegaly and hemochromatosis. Some of them may need occasional transfusions.
4) All genes mutated and don't produce any beta chains β^0/β^0 . Beta thalassemia major or /Cooley's anemia. These patients show normal counts at birth, typical of a beta thalassemia that progresses to severe hypochromic microcytic anemia in a few months. They are transfusion dependent for life with significant hepatosplenomegaly.

The only cure for thalassemia major (and patients with thalassemia intermedia with severe enough symptoms) is hematopoietic stem cell transplantation. This is usually offered if the patient has a suitable sibling donor and must be weight carefully against transplant related toxicity and the patient's baseline performance status. Chronic transfusion is commonly offered and is indicated to alleviate the symptoms of severe anemia, prevent or treat growth failure or delay and suppress extramedullary hematopoiesis this preventing bone deformities (e.g. Frontal bossing) and decreasing organomegaly. Splenectomy may be considered for those with severe hypersplenism especially if it's increasing transfusion requirements. With chronic transfusions comes the issue of iron overload. These patients end up being on chelation therapy to prevent or delay secondary hemochromatosis resulting in cardiomyopathy, arrythmias, diabetes, hypogonadism, hypothyroidism, liver cirrhosis, liver failure, liver carcinomas and other complications. Patients on chronic transfusion need a way to follow their iron liver content. While serum ferritin provides a quick non-invasive way to get such an estimate. It remains a very rough estimate and being an acute phase reactant makes it even less meaningful in inflammatory states. While the gold standard for such an estimate was a liver biopsy until recent years, it's being replaced rapidly by the much less invasive MRI testing.

Now turning to qualitative hemoglobin disorders under which come a very diverse list of disorders that result from a structural abnormality in the hemoglobin molecule. Among these disorders are:

1) Unstable hemoglobins: such as Hb Koln. These tend breakdown shortly after production or under less than usual stress such as oxidative stress. They can range from being asymptomatic to causing severe anemias and jaundice.

2) Decreased solubility: Hb S (sickle) and Hb C is examples.
3) Decreased (Hb Kansas) or increased (Hb Syracuse) affinity to oxygen.
4) Abnormal heme oxidation as methemoglobin.

Here, we will mainly discuss sickle cell disease as its one of the most common and significant hemoglobinopathies. It's one of the recognized abnormal structural beta hemoglobin chain variants that results from the replacement of glutamic acid at position 26 in the beta chain with valine resulting in loss of flexibility and deformability of the hemoglobin molecules. This mutation is common Africa, India and the Mediterranean region. In the heterozygous state (HbSA), the individual will be a silent or asymptomatic carrier. But patients with homozygous HbSS, suffer from sickle cells anemia, a form of sickle cell disease. There are many other structural variants of the beta hemoglobin chain, among the more common ones are HbE (homozygous individuals have a mild anemia), HbC (homozygous individuals have a mild anemia) and $HbO^{Arab.}$ Patients get sickle cells disease by either being homozygous for HbS that is, HbSS phenotype or by being for heterozygous for HbS along with certain other beta globin qualitative mutations such as HbO^{Arab}, HbC, $Hb\beta^0$, or $Hb\beta^+$. we note these variants of sickle cell disease as $HbSO^{Arab}$, HbC, $Hb\beta^0$ and $Hb\beta^+$. These variants differ from each other in severity and certain presentation aspects. Note that only HbSS is referred to as sickle cell anemia. Also, note that not all beta chain mutations heterozygous with sickle cell trait result in sickle cell disease. For example, HbSE patients have sickle cell trait and not disease.

Since sickle cell disease doesn't present at birth due to the fact that the predominant hemoglobin type during that period is fetal hemoglobin, newborn testing is utilized in some countries with very high incidence of sickle cell disease given the debilitating nature and significant impact of this disease on individuals. This is usually done through a variant of hemoglobin electrophoresis that detects small amounts of HbS present in the neonatal period. If the testing is suspicious for sickle cell disease, a full hemoglobin electrophoresis is performed for confirmation. Red blood cells with HbSS or other sickle hemoglobin content tend to behave normally while oxygenated. They take the sickle cell shape and become less flexible in the deoxygenated state due to polymerization of sickle hemoglobin molecules. This process is initially reversible but with repeated cycles becomes irreversible leading to decreased red blood cell life span as the irreversibly sickled cells will breakdown as they pass through capillaries and through the spleen explaining the hemolytic nature of this disease. Among factors that promote sickling are cold weather, heat, stress and dehydration.

Sickle cell disease is a hematologic disorder with multi system manifestations. The hematologic presentation is a chronic hemolytic anemia resulting in jaundice, pallor, fatigue and gall stone formation. Acute worsening of anemia may occur and is usually either due to splenic sequestration crisis or parvovirus induced aplastic crisis. Differentiating these 2 entities from each other can sometimes be difficult but the presence of splenomegaly, reticulocytosis and thrombocytopenia usually points to the first condition while the reverse picture along with decreased jaundice and absence of nucleated red blood cells points to the second. With recurrent splenic sequestration episodes, splenectomy may be indicated. Acute and chronic manifestations are many and can vary in severity and incidence based on the specific mutation. The most commonly encountered include the following:

1) Fever: patients with sickle cell disease are at much higher risk for bacterial sepsis particularly encapsulated bacteria due to either functional or anatomic asplenia. Splenic involution (auto infarction) starts at around 3 months of age in HbSS patients and is complete by teenage. Add to this sickle cell patients with history of splenectomy. The risk of sepsis is life long but is at its highest the first few years post splenectomy. For this reason, these patients are placed on Penicillin VK prophylaxis for the first years of life and for several years post splenectomy. Thus, fever in patients with sickle cell disease is considered a medical emergency and timely evaluation and management is vital. Features of higher risk in sickle cell patients include high grade fever, age under 1 year, non-compliance with Penicillin V prophylaxis, white blood count above 30,000 or history of pneumococcal sepsis. Higher risk patients are typically managed in the inpatient setting. All sickle cell disease patients should be up to date on pneumococcal vaccination.
2) Vaso-occlusive pain crisis: the initial presentation of this usually occurs between 4-6 months of age in patients with more severe phenotypes and typically takes the form of dactylitis. That being said, pain can be located anywhere and can range from vague abdominal pain to severe bony pain. The trigger to the episode is usually not

identified but can be fever, infection, cold weather, or dehydration and other stressors. These patients are managed with hydration to help decrease sickling in addition to pain management. Pain control is typically achieved through coadministration of NSAIDs and narcotics. The importance of this is not only for symptomatic management but also helps decrease sickling. Attention should be paid not to overtreat pain with narcotics at the same time resulting in hypopnea, deoxygenation and worsened sickling. Transfusion may help patients who present initially with lower hemoglobin values as is dilutes HbS percentage in the blood steam. If this is done, care should be taken to avoid over transfusion (Hb above 10 gm/dl) as this increases blood viscosity and can lead to overt stroke.

3) Acute chest syndrome: is usually defined by new pulmonary infiltrate on chest X ray, hypoxia/ pulmonary symptoms and fever. This triad may not be evident at the time of diagnosis and the complete picture tends to evolve over time. Acute chest syndrome can be infectious in etiology or simply secondary to vaso-occlusive crisis of pulmonary vessels or bronchospasm. Management relies on the empiric use of antibiotics along with pain management and careful hydration in addition to supportive measures such as correction of hypoxemia.
4) Stroke: can be either acute or silent repetitive minor cerebral infarctions. This is promoted by occlusive cerebral vasculopathy (Moya Moya syndrome). Patients presenting with acute infarction should undergo emergent red blood cell exchange transfusion to lower HbS percentage to the lowest level possible (less than 10%). Silent infarctions eventually lead to cognitive impairment and other complications. these patients are also at risk for reversible posterior leukoencephalopathy. The optimal way to deal with stroke is to prevent it in the first place. Sickle cell patients should undergo yearly transcranial doppler ultrasounds starting at the age of 2 to measure cerebral blood flow speed. Patients with abnormally elevated values should be started on chronic transfusion. Once stroke occurs in a patient, secondary prevention should also be initiated with chronic transfusions, too. Stem cell transplant can be considered as both primary and secondary prophylaxis for patients with a suitable sibling donor.
5) Priapism: relatively common. Mainstay of management relies on pain control and hydration and pharmacologic agents such as psudoephedrine. Transfusion has not shown to be of any help for this complication. Urologic consultation may be considered in prolonged refractory cases.
6) Renal complications: these can range for reversible inability of the kidney to concentrate urine (reversible hyposthenia) in the early years of life that becomes later irreversible. This progresses later in life to proteinuria then RTA and hematuria. Most patients develop end stage renal disease by the age of 30-40 years.
7) Other complications: include retinopathy, Avascular necrosis, chronic lung disease and pulmonary hypertension, cardiomyopathy and sudden death.

In addition to the previous management strategies for sickle cell disease complications, hydroxyurea therapy has shown to improve sickle cell disease symptoms by increasing the percentage of fetal hemoglobin in the circulation which has an inhibitory effect on sickling. Patients initiated on this drug need to be monitored frequently for toxicity, most importantly neutropenia. The only cure to sickle cell disease currently is stem cell transplantation but gene therapy approaches are under the way.

The Iron atom in the heme molecule is normal in the reduced ferrous (+2) state. This form can combine reversibly with oxygen to form oxyhemoglobin. Under certain oxidative stresses such as certain drugs such as lidocaine or Pyridium, Aniline dyes, toxins such as bluing agents, nitrates or nitrites it will change to the ferric (+3) state forming methemoglobin. This form has a very high affinity to oxygen and binds irreversibly shifting the oxygen hemoglobin dissociation curve to the left. This results in poor tissue oxygen delivery although both O2 partial pressures are normal and saturation is at 100%. O2 sat measured by probes will be low due to the fact that methemoglobin has different physical characteristics than normal hemoglobin. Normal methemoglobin values are under 1%. Cyanosis occurs at around 10% and cardiopulmonary compromise and neurologic manifestations can occur above 40%. Blood samples drawn from these patients have a characteristic chocolate brown color. This condition can also be congenital if the patient have an M hemoglobin variant, an autosomal dominant condition (a hemoglobinopathy), or NADH MetHb Reductase Deficiency (autosomal recessive) these conditions result in a hemoglobin that is more prone to oxidative stress than normal hemoglobin. Management is based on removing the inciting agent, oxygen therapy and Methylene blue infusion which is a reducing agent.