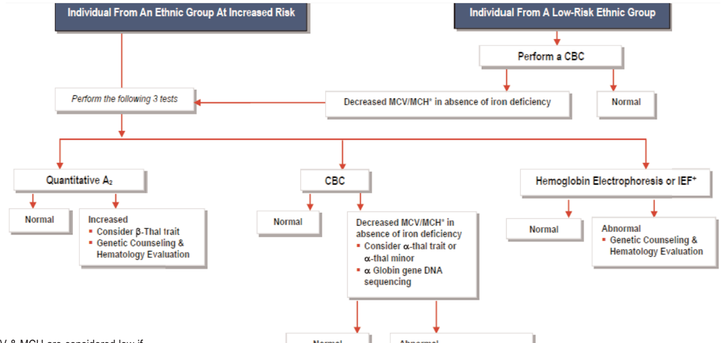
Algorithm for Testing



Key:
* MCV & MCH are considered low if below 80 fL and 27 pg respectively
\+ Isoelectricfocusing

10.3%). In addition a significant decrease in **gestational age** and **birth weight** was observed. While a decreased birth weight is expected to follow a decreased gestational age, another study by Tan et. al.[8] removed pregnancies with additional complications that may affect birth weight and still found an increased risk of low birth weight (<10th percentile) of 14.8% vs. an anticipated 10%. Again in this study a control group was not included and results may be impacted by socioeconomic factors.

Clearly additional research is required to assess the risk for pregnancies in women with sickle cell trait and how they should be managed. These studies do however suggest an increased frequency of complications in pregnancies of women with sickle cell trait.

If you have any questions or if we can be of assistance please contact the IHTC Genetic Counselor at the Indiana Hemophilia and Thrombosis Center: 1-877-256-8837.

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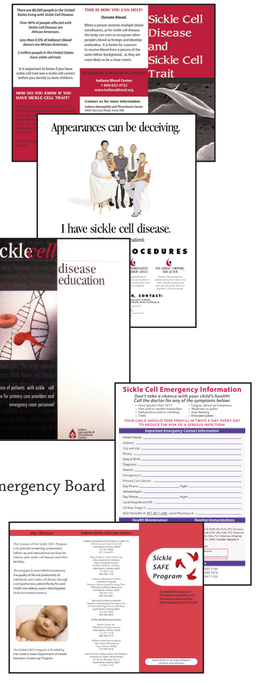
Other resources are available through the IHTC:

- Sickle Cell disease brochure
- Sickle Cell disease poster
- Sickle Cell Handbook
- Sickle Cell Patient Emergency Board
- Newborn Screening (Sickle SAFE) Program brochure



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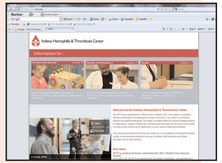
The IHTC launched a new website in 2011, featuring improved navigation and expanded medical and educational content.

There is also a Physician Portal and a Reading Room with previous issues of Blood Type. The website allows sharing features that enable you to e-mail, tweet or post webpages to Facebook. Start exploring at www.ihtc.org!



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Sickle Cell Disease and Hemoglobinopathy Screening in Pregnancy

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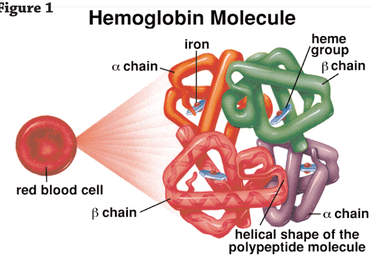
The hemoglobinopathies, including sickle cell disease, alpha and beta thalassemia, are the most common single-gene diseases in the world. Hemoglobinopathies affect hemoglobin production and function and are usually inherited in an autosomal recessive pattern. More than 5% of the world's population is a carrier of a clinically important hemoglobin disorder. Due to eight causative genes and various types of mutations that occur, the hemoglobinopathies are extremely heterogeneous disorders and represent a wide range of clinical phenotypes.

Six different types of globin chains are found in normal human hemoglobins at different stages of development (α, β, γ, δ, ε, ζ). Normal adult hemoglobin A has a tetrameric structure composed of two α chains and two ß chains. This type of hemoglobin comprises approximately 97% of total hemoglobin in normal adults, while 2-3% of total adult hemoglobin is comprised of hemoglobin A2 which contains two α and two δ chains. Hemoglobin F is made up of two α and two γ chains and comprises 50-85% of hemoglobin in newborns but declines after birth with normal adults having <1% Hb F.

Hemoglobin is the oxygen carrying molecule in red blood cells. The α chains are encoded by a gene cluster on chromosome 16. The β chain genes are clustered on chromosome 11. Like most genes, globin has "sensitive areas" in which mutations cause disease and "insensitive areas" in which genetic variation often has no effect on health. Mutations in the globin genes can cause:

- Qualitative changes in the structure of globin, such as sickle cell anemia.
- Quantitative changes causing decreased synthesis of globin chains. This type of globin abnormality is classified as thalassemia.

Figure 1



Sylvia S. Mader, Inquiry Into Life, 8th edition. Copyright (c) 1997. The McGraw-Hill Companies, Inc. Used with permission.

Sickle Cell Diseases

Sickle cell disease refers to a collection of diseases including sickle cell anemia (Hb SS), Hb SC, and sickle cell/β-thalassemia. Sickle cell disease is an autosomal recessive

Glossary

Definitions	
Hemoglobin	The molecule within red blood cells by which oxygen is transported to the tissues. Each hemoglobin molecule is composed of four subunits: two α chains and two β chains while each subunit is composed of a plolypeptide chain, globin and an iron containing heme group.
MCV	Mean corpuscular volume or mean cell volume indicates microcytosis
MCH	Mean corpuscular hemoglobin or mean cell hemoglobin indicates reduced content of Hb per cell.
cis	The presence of two mutations on the same chromosome (ex. aa/--).
trans	The presence of two mutations on separate chromosomes (ex. a-/a-).
Hemoglobin electrophoresis	The method by which hemoglobin variants are separated by placing on a filter paper or gel and exposing them to a charge gradient.
Isoelectric focusing (IEF)	The method by which hemoglobin variants are separated on a gel by exposing them to a pH gradient.

condition. Approximately 1/12 African Americans carry the sickle cell trait (Hb S), while 1/300 African American newborns has some form of sickle cell disease and 1/600 has sickle cell anemia (Hb SS).

Hb SS is classic sickle cell anemia while Hb SC is a condition with a milder phenotype caused by the presence of one gene with a sickle cell anemia mutation and a second gene with a mutation causing Hb C. Sickle cell/beta thalassemia has a still milder phenotype and occurs in the presence of one gene containing the Hb S mutation and a second gene carrying β-thalassemia.

Table 1. Genotypes for Alpha thalassemia

Genotype	Description
aa/aa	Four normal α globin alleles (no disease)
aa/a-	Silent carrier of α-thalassemia trait
	No clinical symptoms
	Suspected when an individual has microcytosis not explained by iron deficiency or ß-thalassemia.
	Hb electrophoresis is typically normal except for possible reduction in Hb A_2.
	Definitive diagnosis requires DNA analysis
a-/a-	Two α globin gene mutations in trans-orientation (α^+ thalassemia, α-thalassemia minor)
	Mild anemia
	Slight abnormalities in CBC (decreased MCV and/or MCH) in the absence or iron deficiency.
	Definitive diagnosis requires DNA analysis.
aa/--	Two α globin gene mutations in cis-orientation (α^0 thalassemia, α-thalassemia minor)
	Mild anemia
	Slight abnormalities in CBC (decreased MCV and/or MCH) in the absence of iron deficiency.
	Definitive diagnosis requires DNA analysis.
a-/--	Two α globin gene mutations in cis-orientation (α^0 thalassemia, α-thalassemia minor)
	Hb H disease
	Due to lack of alpha globin chains, beta chains group together to make hemoglobin H which is a poor oxygen transporter.
	Hb H is also insoluble and causes the membrane of the red blood cell to break open. This results in a hemolytic anemia.
--/--	Alpha thalassemia major or Hb Bart's
	No alpha protein is produced.
	This results in hydrops fetalis and usually fetal loss.

Sickle cell anemia is caused by a specific genetic mutation in the β globin gene which causes polymerization of hemoglobin under deoxygenated conditions. The polymers distort the red cell's shape into a crescent or sickle shape. The shape prevents normal blood flow through capillaries and increased adhesion to endothelial cells. Decreased blood flow leads to tissue infarction and a wide range of clinical symptoms, the hallmark of which include splenic infarction and painful episodes termed vasooclusive crises.

While in the United States, sickle cell trait most commonly occurs in African American individuals, sickle cell trait is also observed at a higher frequency in other populations including Mediterranean, Middle Eastern, Hispanic Caribbean, and Asian Indians. Table 3 provides additional gene frequency information for the other types of abnormal hemoglobin.

The diagnosis of sickle cell trait (Hb S) is made by performing hemoglobin electrophoresis or isoelectric focusing (IEF) with the presence of Hb S and Hb A, with Hb A representing a greater percentage than Hb S. The MCV and MCH are normal except when there is coexisting α or β- thalassemia trait. Hemoglobin C is similarly diagnosed by performing hemoglobin electrophoresis or IEF indicating the presence of Hb C and Hb A, with Hb C representing a lower percentage. Information regarding detection of the carrier state of β-thalassemia follows.

The Thalassemias

Alpha thalassemia, β-thalassemia and other rare forms are due to abnormalities in the globin genes. Deleterious effects are caused by globin chain subunits that are produced at a decreased rate skewing the balance between α and β globin chain production; α and β globin chains are required to be produced in equal amounts to form normal adult hemoglobin. The clinical features of α-thalassemia and β-thalassemia vary widely and research continues on identification of additional genetic factors that modify the phenotypes of these conditions.

Alpha thalassemia

Alpha thalassemia is due to impaired production of the α globin (protein) chains leading to an excess of β globin chains. The α globin genes are located on chromosome 16. Each individual has two α globin genes with a total of four alleles. The severity of alpha thalassemia depends on the combination of the number of affected genes inherited.

Table 2. Comparison of MCH & MCV values for β^+ and β^0

Average ±	β^+	β^0
MCV	66.24-77.70	60.94-68.86
MCH	20.76-24.52	18.63-21.17

Adapted from Millard et. al., Br. J. Haematol., (1977); 36:161-170.[5]

Beta thalassemia

Beta thalassemia is due to impaired production of β globin chains resulting in excess of α globin chains which results in damage of red cells and precursor red cells causing profound anemia. There is one gene on chromosome 11 coding for β chains. Like α-thalassemia the severity of the disease depends on the combination of both number and type of genes inherited. There are more than 200 different genetic mutations causing impaired β protein production, therefore this disease is highly heterogeneous.

1. **Beta thalassemia minor or β-thalassemia trait**
   - Heterozygosity for a β globin gene which codes for decreased (β^+) or absent (β^0) β protein product
   - Usually clinically asymptomatic
   - CBC often shows elevated RBC number with decreased MCV and/or MCH, in the absence of iron deficiency
   - Mean values are significantly different for those with β^+ trait versus β^0 trait, however there is overlap
   - Diagnosis is based on detection of increased Hb A_2, some individuals also have increased Hb F
   - Genetic testing is available for confirmation or to detect "silent" β-thalassemia trait
2. **Beta thalassemia intermedia**
   - Homozygosity (β^+/β^+) or compound heterozygosity (β^+/β^0)
   - These individuals have clinical symptoms that range between hose seen with β-thalassemia minor and β-thalassemia major
   - Laboratory findings may be similar to those in β-thalassemia trait but are generally more severe
3. **Beta thalassemia major or Cooley's anemia**
   - Homozygosity for absent protein product (β^0/β^0) resulting in the inability to make β globin genes that results in absent normal adult hemoglobin
   - These patients are profoundly anemic and transfusion dependent
   - Bone marrow transplantation is frequently used to treat β-thalassemia major

Genetic Testing

Genetic testing of the α and β globin genes is available and may be useful for identification of heterozygosity, prediction of the clinical phenotype, presymptomatic diagnosis or prenatal diagnosis.

Who Should be Screened?

Due to migration and the mixing of ethnic groups in the United States everyone should be screened for hemoglobinopathies. Determining risk based on ethnicity is not always accurate as individuals may be from mixed ethnic backgrounds. Healthcare providers should be familiar with the clinical features, inheritance and prevention of these disorders as they are associated with life-altering or life-threatening medical sequelae and/or chronic illness.

When Should Individuals be Screened?

It is ideal that both parents undergo screening prior to conception as it can be difficult to perform antenatal screening of both parents within the first trimester. In the absence of pre-conception screening, testing should be completed for the mother as early in pregnancy as feasible. Testing of the father should always be pursued if the mother is found to carry a hemoglobin abnormality. Fathers may want to be screened concomitantly as some individuals who carry a hemoglobin abnormality will be missed despite performing the recommended screening tests. If the mother or father is identified as a carrier of a hemoglobin abnormality the couple may wish to pursue DNA testing for the other member of the couple to provide definitive information about the couple's chance to have an affected child.

Individuals that have screening performed should be informed of their results, whether an abnormality is identified or not.

Recommendations For Screening in Pregnancy

1. Individuals of African, Asian, Mediterranean, Carribean, Middle Eastern and Central American descent should have a CBC, hemoglobin electrophoresis (or IEF) and quantitative A_2 performed.
2. Individuals of other ethnic backgrounds should have a CBC performed. If the MCV is low this should be followed by hemoglobin electrophoresis (or IEF) and quantitative A_2.
3. A CBC and hemoglobin electrophoresis or isoelectric focusing are the appropriate lab tests to screen for hemoglobinopathies. **Solubility tests, also known as Sickle Dex, alone are inadequate.**
4. Iron studies should be performed as iron deficiency can decrease the MCV/MCH falsely, suggesting the presence of a hemoglobin abnormality.
5. Couples at risk for having a child with sickle cell disease or other hemoglobinopathy should be offered genetic counseling to review prenatal testing and reproductive options.

Table 3. Incidence of Carrier

Ethnicity	Sickle Cell (Hb S)	Hb C trait	Alpha-thal	Beta-thal
African American	1/12	1/50	1/30 trans	1/75
Asian	rare	rare	1/20 cis	1/50
Asian, Southeast	rare	rare	>1/20 cis	1/30
Asian Subcontinent (India, Pakistan)	1/50-100	rare	variable	1/30-50
Hispanic Caribbean	1/30	rare	variable	1/75
Hispanic Mexican, Central American	1/30-200	rare	variable	1/30-50
Mediterranean	1/30-5	rare	1/30-50 trans	1/20-30
Middle Eastern	1/50-100	rare	variable	1/50
Non-Hispanic Caribbean, West Indian	1/12	1/30	1/30 trans	1/50-75
West African	1/6	1/20-30	1/30 trans	1/50

**adapted from the March of Dimes "Genetic Screening Pocket Facts"*[3]
***additional risk estimates are available by country, for specific information please contact the IHTC's genetic counselor*

Why Should Screening be Performed?

Early diagnosis: Newborn screening does not detect all hemoglobinopathies. Knowing that an infant's parents carry a gene for a hemoglobinopathy allows for earlier diagnosis in the presymptomatic period.

Make options available: Identify parents at risk to have a child with a hemoglobinopathy and make reproductive and prenatal options available as well as provide genetic counseling.

Complications in Pregnancy for Carriers of Sickle Cell Disease

Research on pregnancy in carriers of sickle cell trait has produced mixed results. A variety of complications has been demonstrated to occur at an increased rate in women who are sickle cell trait carriers (AS) in several studies; however, there are a few studies that have not found an increased rate of pregnancy related complications.

A 1983 study by Tuck et. al.[10] looked at 334 pregnancies of women with sickle cell trait compared to 717 patients of the same racial and social background. The authors determined that the only "serious" difference observed between the two groups was an increased frequency of recurrent **urinary tract infections** (6% vs. 3%) and **microscopic hematuria** (16% vs. 6%). No significant differences were found between the groups in regards to gestational age, low birth weight, neonatal morbidity or hypertension. Incidentally, there was also a greater incidence of **fetal distress in labor** leading to emergency caesarean section. A 1990 paper by Baill and Winter[1] found a significantly increased rate of **bacteriuria and pyelonephritis** in pregnant women with sickle cell trait. Birth weight of these infants was evaluated as well with the absence any significant difference from the control group. More recent studies since these two papers have been conducted investigating additional aspects of pregnancy in women with sickle cell trait with differing results. Placental findings in pregnancies of women with sickle cell trait were evaluated retrospectively by completing a pathologic evaluation of 131 pregnancies ≥16 weeks gestation as well as the analysis of obstetric/early neonatal information.[9] This study found a significantly increased rate of **IUGR** (10.6%) and **intrauterine fetal demise** (8.13%). Placental pathology indicated acute **amniotic fluid infection** in 50% of specimens and **meconium histocytosis** in 92%. All of the placentas had **sickling in the intervillous space** and there was also **sickling of the decidual vessels**. There were several limitations to the study including skewed socioeconomic status and the lack of a control group from this institution. It does however raise the question of what effect such placental findings have on pregnancy and pregnancy outcome. Alternatively a case report by another author suggested that natural sickling of the red blood cells occurs after placental separation from the uterine wall during delivery.[7]

A prospective study was performed with a control group evaluating **preeclamsia**, gestation age at delivery, and birth weight.[4] They found a significantly increased rate of preeclampsia among women with sickle cell trait (24.7% vs