Hemoglobinopathies and Thalassemias

Hemoglobin Defects

- Genetically determined abnormalities of the structure or synthesis of hemoglobin molecule.
- Abnormality associated with globin chain
  - Qualitative defects (structural defect) – genetic mutation involving amino acid deletions or substitution
  - Quantitative defect - thalassemia

Nomenclature of Hemoglobin Variants

- First discovered was Hemoglobin S (HbS)
- Originally, given letter designations beginning with Hemoglobin C (except Hemoglobin F = fetal hemoglobin and Hemoglobin M = hemoglobins that tend to form methemoglobin)
- Later given common names according to geographic area in which they were first discovered (e.g. Hb Ft. Worth)
- Disease (homozygous) vs. trait (heterozygous)

Pathophysiology of Hemoglobin Variants

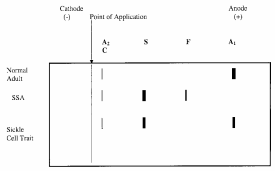
- Altered Solubility – when nonpolar amino acid is substituted for a polar amino acid near the surface of the chain (Hb S and C)
- Altered Function – polar amino acid substitution for nonpolar residue near the hydrophobic crevice may affect oxygen affinity by stabilizing heme iron in Fe^{3+}
- Altered Stability – substitutions in internal residues may prevent folding into proper tertiary structure

Identification of Structural Hemoglobin Variants

- Hemoglobin Electrophoresis
  - Primary diagnostic tool for differentiating types of qualitative hemoglobinopathies
  - Separates hemoglobins based on surface charge and movement in an electrical field
  - Surface charge is affected by the amino acid substiturion
  - Rate of migration depends on support media, pH and ionic strength of buffer, strength of electrical field and time

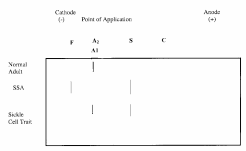
Cellulose Acetate Electrophoresis

- Detection and preliminary identification of normal and abnormal hemoglobins
- Abnormal hemoglobins may require confirmation by citrate agar electrophoresis



Citrate Agar Electrophoresis

- Performed at acid pH (6.0) – vs. pH 8.6 for cellulose acetate
- Used to differentiate Hg S from D and G
- Differentiate C from A_2



Sickle Cell Anemia

- Most common symptomatic hemoglobinopathy – highest in Africa
- Sickle cell disease in 0.3-1.3% of African Americans; trait in 8-10% of African Americans
- HbS in heterozygous state confers advantage against fatal *Plasmodium falciparum* infections

Pathophysiology of SSA

- Mutant hemoglobin (HbS) is produced in which valine (nonpolar) is substituted for glutamine (polar) in 6th position of β chain. ($\alpha_2\beta_2^{6val \rightarrow glu}$)
- Produces a change in net chg. of molecule; solubility in deoxygenated state is markedly reduced and rigid aggregates of hemoglobin form.
- Aggregates polymerize and red cell sickles.

- Rate of polymerization depends of
  - Temperature (temps higher than 37 °C)
  - pH (acidosis)
  - Ionic strength (hypertonicity)
  - Oxygen tension (hypoxia)
- Sickled cells return to normal upon reoxygenation – with repeated sickling the membrane undergoes permanent changes and cells become *irreversibly sickled*

Clinical Findings in SSA

- First clinical signs at about 6 month of age
- Anemia – moderate to severe anemia as result of extravascular hemolysis
  - Changes in attempt to compensate for oxygen deficit lead to cardiac overload (cardiac hypertrophy, cardiac enlargement, and congestive heart failure)
  - Hyperplastic bone marrow (compensation for increased RBC destruction) leads to bone changes

- Aplastic crises during or following viral, bacterial, and mycoplasma infections
- Vaso-Occlusive Crisis – blocking of microvasculature by rigid sickled cells
  - Triggered by infection, decreased oxygen, dehydration, slow blood flow, or without any known cause
  - Pain, low grade fever, organ dysfunction, tissue necrosis

- **Autosplenectomy** – splenic fibrosis and calcification due to infarction
- Dactylitis – painful swelling of hand and feet

- Bacterial infection – reasons for increased susceptibility not fully understood –
- Acute splenic sequestration – splenic pooling of sickled RBCs may cause decrease in RBC mass
- Acute Chest Syndrome – cough, fever, chest pain, dyspnea, chills, wheezing, pulmonary infiltratesl

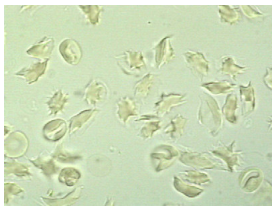
Laboratory Findings in SSA

- Peripheral Blood
  - Severe anemia (5-9 g/dL) – N/N
  - Poikilocytosis –sickle cells, target cells
  - Anisocytosis – Increased RDW
  - Nucleated RBCs, polychromasia
  - Leukocytosis (WBC = 12,000-16,000) – absolute neutrophilia with shift to left
  - Thrombocytosis common – thrombocytopenia during aplastic crises

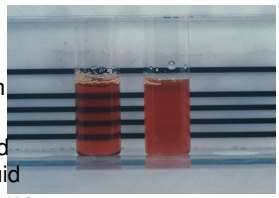
- Electrophoresis on cellulose acetate at pH of 8.4 85 – 100% HbS and <15% HbF
- Chemistry tests
  - Increased bilirubin
  - Increased LDH
  - Decreased haptoglobin

Diagnostic Tests for Hgb S

- Sickle cell prep
  - Sodium metabisulfite added to blood
  - Reduces oxygen tension → sickling
  - Viewed microscopically
  - Rare hemoglobin variants may also sickle



- Dithionite Tube Test
  - Red cells lysed by saponin
  - Sodium dithionite removes oxygen from test environment.
  - Hb S polymerizes and forms tactoids, or liquid crystals…..solution turns turbid



Therapy

- Preventative – eliminate conditions that precipitate vaso-occulsion
- Transfusion during aplastic crises or splenic sequestration
- Hydroxyurea to reduce intracellular sickling – reactivated fetal genes and elevated HbF

Sickle Cell Trait

- Heterozygous for sickle cell gene
- Usually asymptomatic
- May have crisis if oxygen tension is sufficiently lowered
- Hemoglobin electrophoresis shows 50-65% HbA_1, 35-4% HbS, normal HbF and normal to slightly increased HbA_2

Hemoglobin C

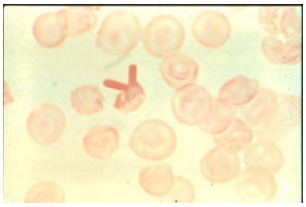
- Lysine substituted for glutamate on the sixth position of beta chain – $\alpha_2\beta_2^{6\ lys}$
  - Same type of substitution as HbS – decreased hemoglobin solubility
- Exclusively in black population – greatest incidence in West Africa – 25% are carriers
- 3% of American blacks are carriers – 0.02% have the disease

Clinical Features

- Trait is usually asymptomatic
- Disease is mild – sometimes asymptomatic
  - Abdominal pain from splenomegaly
  - Gallstones, mild jaundice
  - Mild hematuria
- Treatment not required – excellent prognosis

Laboratory Features

- Peripheral blood
  - Hgb, Hct, RBC are normal to sl. decreased
  - Indices are N/N
  - Target cells, polychromasia
  - Hemoglobin C crystals -



- Hgb electrophoresis-
  - Hgb C Disease – 93 - 100 % Hgb C; remainder Hgb F
  - Hgb C Trait – 25-40% Hgb C; remainder Hgb A_1
  - Hgb C migrates with Hgb A_2 on cellulose acetate

Hemoglobin SC Disease

- Doubly heterozygous
- Approximately 1/3 as common as SSA
- Symptoms appear later in life than in SSA
- Clinical symptoms similar to SSA, but milder, complications are fewer
- Splenomegaly common
- Blood viscosity is higher than in SSA – so retinal problems are seen and more severe

Laboratory findings

- Anemia may or may not be present – mild N/N
- Target cells and "pocketbook" cells present.
- Hemoglobin Electrophoresis
  - Hgb F from normal to 7%
  - No Hgb A_1
  - Hgb C = Hgb S
- Sickle cell prep and tube solubility tests will be positive

Thalassemias

- Variety of genetic defects in globin chain synthesis – decreased or absent synthesis
- Classified according to globin chain that is affected – e.g. β–thalassemia vs. α-thalassemia
- Heterozygous: minor
- Homozygous: major

Pathophysiology

- If α chain is affected, excess of β chains produced. If β chain is affected, excess of α chains produced
- Imbalance in chain synthesis causes
  - Decrease in total hemoglobin production
  - Ineffective erythropoiesis
  - Chronic hemolysis
- Excess α chains are unstable – precipitate within cell – precipitates bind to cell membrane, causing membrane damage

- Excess β chains combine to form Hb H (four β chains)
  - High oxygen affinity – poor oxygen transporter
  - unstable

Clinical Findings

- Anemia/hypoxia
  - Decreased hemoglobin production
  - Ineffective erythropoiesis
  - Presence of high-affinity hemoglobins
  - Increased extravascular hemolysis
- Splenomegaly
  - Splenic removal of abnormal erythrocytes
  - Extramedullary hematopoiesis

- Gallstones – due to increased intravascular and extravascular hemolysis
- Skeletal abnormalities – expansion of bone marrrow
- Pathological fractures – thinning of calcified bone
- Iron toxicity – multiple transfusions

Laboratory Findings

- Decreased RBC, hemoglobin, hematocrit
- Microcytic/hypochromic (↓MCV, ↓MCH, ↓MCHC)
- Increased reticulocyte count
- Anisocytosis and Poikilocytosis
- Target cells, basophilic stippling, nucleated RBCs
- Increased bilirubin, decreased haptoglobin
- Increased serum iron and decreased TIBC

α-Thalassemia

- There are two α genes on each of two chromosome 16 structures (four α genes in the diploid state)
- Mutations can affect one or more of the α genes resulting in four levels of severity
- When all four genes deleted – no α chains, hydrops fetalis or α–thalassemia major
- 3 of the four deleted, hemoglobin H disease

- 2 of the 4 deleted, α-thalassemia minor
- 1 deletion, silent carrier
- Primarily affects people of Mediterranean, Asian and African ancestry

Hydrops fetalis

- Deletion of all four α genes
- No adult hemoglobin can be formed - incompatible with life – infants are stillborn or die within a few hours
- Hemoglobin is made using γ, δ and β chains

Hemoglobin H Disease

- Usually result when two heterozygous parents (--/αα and the other –α/αα) bear children
- Excess of β chains leads to formation of Hb H
- At birth, excess of γ chains leads to Hb Bart's (γ_4)
- Hb H is unstable – triggering chronic hemolytic anemia
- High oxygen affinity

Clinical Findings

- Wide variation in degree of anemia
- Splenomegaly and hepatomegaly present
- Less than ½ of patients exhibit skeletal changes

Laboratory findings

- Microcytic/hypochromic anemia (hemoglobin levels 8 to 10 g/dL)
- 5-10% reticulocytes
- Nucleated red blood cells
- 25% Hb Bart's with ↓ levels of Hb A_1, Hb A_2, and Hb F in neonates
- 2-40% Hb H, ↓ levels of Hb A_2, normal Hb F,remainder Hb A_2 in adults

α-Thalassemia minor

- Two α genes either on same or opposite chromosomes are missing
- Unaffected globin genes are able to compensate for the affected genes
- Mild anemia – signficant microcytosis
- Normal lifespan

Silent carrier

- Affects greater than 25% of African Americans
- 3 remaining genes direct synthesis of adequate number of α chains
- Totally benign – MCV is borderline (78 – 80 fl)

β-thalassemia

- Only 2 β globin genes, one on each chromosome 11
- Defect is not deletional
  - β^+ gene mutation causes partial block in β chain synthesis
  - β^0 gene mutation results in complete absence of β chain production
- Over 180 mutations resulting in partial to complete absence of β gene expression

β thalassemia Major – Cooley's Anemia

- Homozygous (β^+/β^+ or β^0/β^0) or double heterozygous (β^+/β^0) inheritance
- Pathophysiology: dramatic reduction or complete absence of β chain synthesis –
- Symptoms begin to manifest at age 6 months
- Increase in non β containing hemoglobins
- Excess α chains precipitate in cells - hemolysis

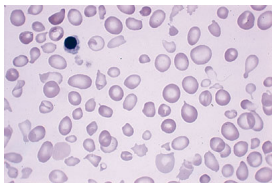
Clinical Symptoms

- First observed in infants – irritability, pallor, failure to thrive
- Enlarged abdomen
- Sever anemia – burdens cardiovascular system- cardiac failure in first decade of life
- Growth is retarded; brown pigmentation of skin

- Bone changes – facial deformities
- Splenomegaly – extramedullary hematopoiesis

Laboratory findings

- Hemoglobin as low as 2-3 g/dL
- Markedly microcytic/hypochromic
- Marked anisocytosis and poikilocytosis
- Basophilic stippling and polychromasia
- Hemoglobin electrophoresis – 90% Hb F and increased Hb A_2

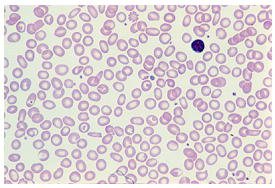


Thalassemia minor syndromes

- More common than once thought –
- Most common in Mediterranean areas and Asia
- Mild compensatory increase in production of chain not affected – e.g. in β-thalassemia minor increase in gamma and delta chains

Thalassemia minor syndromes Laboratory findings

- Mild to non-existent anemia
- Microcytosis – (hypochromia not striking)
- Target cells, basophilic stippling
- RDW is normal
- Normal iron, ferritin, TIBC



Hemoglobin Electrophoresis

- 2-6 % Hgb F (N = < 1% after age 1 year)
- 3-7 % Hgb A_2 (N = 2-3.5%)
- 87-95% Hgb A_1 (N=95.5-100%)

Mentzer Index

- Calculation that **may** (or may not) be useful in differentiating thal minor from Fe deficiency
- Mentzer Index = MCV/RBC Count
- <13 – Thalassemia minor
- >13 – Iron Deficiency

	Incidence	Population	Mechanism
Fe deficiency	Common	All	Lack of Iron
Thalassemia beta alpha	 Common Common	 Greeks, Italian Asians, Blacks	 Def. beta chain synhesis Def.alpha chain synthesis
Sideroblastic Herediatary Primary Acquired Secondary	 Rare Rare More common	 Males All All	Defective heme synthesis
ACD	Common	All	Defect in iron utilization

	RDW	Serum Iron	TIBC	Ferritin	Hgb A_2
Iron Deficiency	Inc	Dec	Inc	Dec	Norm
Thalassemia **alpha** **beta**	Norm	Norm	Norm	Norm	Inc
Anemia of Chronic Disease	Norm	Dec	Dec	Inc	Norm
Sideroblastic Anemias	Inc	Inc	Norm	Inc	Norm
Lead Poisoning	Norm	Norm	Norm	Norm	Norm

Sickle Cell – β Thalassemia

- Doubly heterozygous
- Severity varies from as severe as SSA to asymptomatic
  - β^0 Thalassemia – no β chain production – more severe
  - β^+ Thalassemia – reduced β chain production

Hemoglobin S - β^0 Thalassemia

- Many of same findings and crises as in SSA
- Hgb from 5-10 g/dL with retic count from 10-20%
- Microcytic/hypochromic with marked anisocytosis
- Target cells and sickle cells

Hemoglobin S -β^+ Thalassemia

- Hgb will range between 7-10 g/dL to normal.
- Few red cell abnormalities
- Decrease in MCV or MCH may be only clues to abnormality

Hereditary Persistence of Fetal Hemoglobin (HPFH)

- Group of disorders in which Hgb F production continues throughout life – absence of any significant clinical abnormalities
- Heterozygous HPFH – asymptomatic and Hgb F only slightly increased
- Homozygous HPFH – microcytosis and mild hypochromasia – no anemia – 100% Hgb F
- Important to differentiate thalassemias with high levels of Hgb F from HPHF

Summary of qualitiative hemoglobinopathies

Hemoglobin Disorder	Anemia/ Severity	Clinical Features	Blood Smear	Electrophoresis
AS	None	None	Normal	Hgb A 50% Hgb S 50%
SS	Severe	Pain Crisis Infection Bone Infarcts	Anisocytosis Poikilocytosis Target cells Sickle cells	Hgb S 80% Hgb F 10%
SC	Mild to moderate	Pain crisis Retinopathy Splenomegaly Bone infarcts	Anisocytosis Poikilocytosis Target Cells Sickle Cells	Hgb C 50% Hgb S 50% Hgb F slight

Hemoglobin Disorder	Anemia/ Severity	Clinical Features	Blood Smear	Electrophoresis
S/Thal	Variable Moderate/Severe	Pain Crisis Bone Infarcts	Aniso and Poik Target Cells Microcytic/Hypo Sickle Cells	Hgb S 60-90% Hgb A1 0-30% Hgb F 1-2% Hgb A2 slight
CC	Mild/Moderate	Usually None	Target Cells Hgb C Crystals	Hgb C 100 % Hgb F slight
AC	None	None	Normal	Hbg A 50% Hgb C 50%