Hemoglobinopathies

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NORMAL HEMOGLOBINS

- Consist of 2 alpha chains and 2 non alpha chains
- Hb A = α2β2
- Hb F = α 2γ2
- Hb A2 = α2δ2
Hemoglobin Variants

Altered the conformational dynamic of globin: globin interaction

Altered oxygen affinity

Altered the rheology and physiology of the red cell

Principle of Hemoglobin Electrophoresis

- Hemolysate is prepared
- Globin is run on a gel at acid and alkaline pH
- Mobility base on charge
Abnormal Hb variant

- Hemolysate run on HPLC column (cation)
- Molecule is split into heme and globin
- Globins are dissociated but not denatured
- Globins eluted based on mobility (charge difference)
- As Hb elutes it pass thru a photometer generate a chromatogram
New Born Screening

- New York State one of 43 states that test for hemoglobin variant as part of newborn screening
- NYS Newborn screening for sickle hemoglobin began in 1976
Molecular Genetics

• SSD is due to the substitution of thymine for adenine in the glutamic acid DNA codon (GAG $\uparrow$ GTG) resulting in valine substitution at the $\beta_6$ position
• HbS = $\alpha_2\beta_2$ ($^{6}$glu$\uparrow$val)

Genetics of Sickle Cell Disease

• Autosomal recessive inheritance
• 8-10% African American (AA) carry the Hb S gene
• Gene arose as evolutionary selection for resistance to falciparum malaria
• Approximately 1 in 600 AA are affected with SCD
• The offspring of two carriers has 1:4 chance being affected
Hb S

- Hb S tends to aggregates (valine)
- Deoxygenated Hb S in solution forms gel causes elongation of the cells and resulting in a distorted red cell
- Sickling is also influenced by amount and type of Hb in the cell
- 2,3 DPG
Clinical Laboratory SCD

- Chronic hemolytic anemia
- Steady state Hb 5-11gm/dl
- Normochromic anemia
- Reticulocytosis
- Normal MCHC
- Decrease amount of erythropoietin relative to degree of anemia

Table 1. Sickle Hemoglobinopathies: Neonatal Screening and Diagnostic Test Results.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Neonatal screening results</th>
<th>Hb F distribution</th>
<th>Hematologic studies by 2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MCV²</td>
</tr>
<tr>
<td>SS</td>
<td>F8</td>
<td>N or I</td>
<td>&lt;3.6</td>
</tr>
<tr>
<td>SC</td>
<td>FSC</td>
<td>N or D</td>
<td>NA</td>
</tr>
<tr>
<td>S beta-thal</td>
<td>FSA or FS²</td>
<td>N or D</td>
<td>&gt;3.6</td>
</tr>
<tr>
<td>S beta-thal</td>
<td>FS</td>
<td>D</td>
<td>&gt;3.6</td>
</tr>
<tr>
<td>S delta/beta-thal</td>
<td>FS</td>
<td>D</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>S-HPPH</td>
<td>FS</td>
<td>N or D</td>
<td>&lt;2.5</td>
</tr>
</tbody>
</table>

Hb = hemoglobin, thal = thalassemia, N = normal, I = increased, D= decreased
New Model of Vaso-occlusion

• Unlike normal red cells, sickle cells adhere to the endothelium cells in vitro
• Membrane damage induces oxygen radical generation
• Increased expression of adhesion molecules

Role of Leukocytes

• Suggestion by clinical observation
  - Severity of disease
  - Increased leukocytes associated with increased mortality
  - Increased silent infarcts
• Neutrophils binds sickle cell in vitro
Natural History of SCD

- **FIRST DECADE**
  - Infection/Sepsis
  - Cerebral infarct
  - ACS
  - Arthropathy
  - Cholelithiasis
  - Priapism
  - Poor growth/Development

- **SECOND DECADE**
  - Chronic organ damage
  - Renal disease
  - Leg ulcers
  - Neurologic disease
  - Sudden death

**Functional asplenia**

- The red pulp of the spleen is hypoxic and acidotic and favors sickling.
- Sickle cells lack the deformability necessary for passage through the splenic sinus gaps.
- Sluggish flow, increased viscosity and low O2 and pH lead to chronic congestion of the red pulp and results in multiple micro-infarcts and hemorrhages.
Splenic Sequestration

- Occurs when there is rapid enlargement of the spleen accompanied by an acute fall in hemoglobin.
- The mechanism for the triggering of these episodes are not clear but be accompanied by viral illnesses.
- 10-20% of children with sickle cell disease between the ages of 6 mons to 3 years will have at least one episode.

Stroke in Sickle Cell Disease:

- Affects as many as 15-25% of patients with SS disease (rare in Hgb SC)
- Hemorrhage or thrombosis
- Peak age for cerebral thrombosis and infarction is 7-10 years
- Subarachnoid hemorrhage more common in the second decade.
Patho-physiology of Stroke in Sickle Cell Disease:

- Intimal and medial hyperplasia and proliferation secondary to damaged endothelial from sickle cells.
- Narrowing or complete occlusion of the cerebral vessels; anterior and and/or middle cerebral arteries.
- Multi-vessel involvement is common even in patients will unilateral findings.

Transcranial Doppler (TCD) in SCD

- A measure of cerebral blood flood velocity
- Abnormal TCD predicts increased risk of stroke in SCD.
- Velocity in excess of 200cm/sec is associated with increased risk of stroke.
Prevention of Stroke in SCD

• STOP I (Stroke Prevention) Study: A randomized clinical trial of patients with abnormal TCD, compared chronic transfusion to reduce Hb S level ≤ 30% to a non-transfusion arm.
  • There were 92% fewer strokes in the transfusion arm compared to the non-transfused arm.

Prevention of Stroke in SCD

STOP II

• Patients with abnormal TCD who received transfusion therapy for 36 months randomized to continue or discontinue Tx after 36 months.
  • Study was halted early because of increase stroke in discontinued Tx arm.
Acute Chest Syndrome

• Pulmonary disease (ACS) is the second most common reason for admission to the hospital in patients with SCD.
• It accounts for 25-40% of premature deaths in SCD.

Patho-physiology of ACS:

Pathogenesis:
- Increased Permeability
- Pulmonary Edema
- Vasospasm
- Pulmonary Hypertension
- Platelet/WBC activation
- Endothelial Injury
- Cytokines
Transfusion for SCD

- **Goals**
  - Improve anemia and oxygen carrying capacity
  - Reduce or prevent the occurrence of the complications
  - Reduce Hb S levels

- **Methods**
  - Episodic
  - Chronic
  - Exchange

Indications for Episodic Transfusion

- ACS
- Surgery
- Erythroid aplasia
- Splenic/ Hepatic sequestration
- Intractable Pain
- Priapism
Indications: Chronic Transfusion

- Primary Stroke Prevention
- Prevention of recurrent stroke
- Intractable pain
- Symptomatic anemia
- Pulmonary Hypertension/Sickle Cell Chronic Lung
- Recurrent Splenic Sequestration

Exchange Transfusion

- **Advantages**
  Fast: allows emergent intervention
  Eliminates risks of increase in viscosity and volume
  Decreased iron loading compared to simple transfusion
- **Major role**
  Treatment/prevention of life threatening events
Complications of Transfusion

- Transfusion reactions
- Iron Overload
- Alloimmunization
- Hypersplenism
- Hyperviscosity
- Transfusion transmitted infections

Pharmacological Agents that Stimulate Fetal Hemoglobin Production

- 5-Azacytidine
- Hydroxyurea
- Butyrate
- Erythropoietin
- Combinations
Why is Fetal Hemoglobin Beneficial in Sickle Cell Disease

- Hb F interferes with polymerization of Hb S in vitro.
- Infants with SCD do not have complications during the first 6 months of life.
- SCD patients from Saudi Arabia and India with high Hb F levels have milder clinical disease.
- CSSCD showed an inverse correlation between Hb F levels and clinical severity.

Hydroxyurea

- Hydroxyurea (HU) is widely used in the treatment of sickle cell disease (SCD) in the US and abroad.
- The Multi-center Hydroxyurea Study (MSH), a randomized clinical trials demonstrated a reduction in frequency and severity of ACS and vaso-occlusive crisis by 30% and 50% respectively.
- Clinical benefit in the HU treated patients correlated with cyto-reductive effects.
MSH follow-up Study

299 of the 500 patients enrolled in the original MSH study
9 year follow-up period

- 40% decrease in mortality in patients treated with HU for at least 3 yrs
- 28% of all deaths were pulmonary

PHT : Risk Factor of death in SCD

Gladwin et al NEJM 350:9 2004
- Prevalent in 32% adult pts with SCD

- Development of PHT in SCD associated with anemia, chronic hemolysis and either existing cardiac or renal disease
- Hb F levels or HU therapy did not appear to have protective effect.
Patho-physiology Thalassemia

- Disease results from and imbalance in the synthesis of alpha and beta globin chains
Molecular Genetics of Thalassemia

- There are over 200+ different thalassemia mutations.
- Beta thalassemia from a single base substitution

Beta Globin Gene Expression

- Genes arranged in the order they are expressed during development
- Developmental switching of human globin
Clinical Manifestation

- When Hb F levels fall which are normal offset by increased Hb A levels

Findings:
- Extra-medullary hematopoeisis
- Hemolysis
- Hyperbilirubinemia
- Congestive heart failure.

Types of Beta Thalassemias

<table>
<thead>
<tr>
<th>Type</th>
<th>Hb levels</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta^0\beta^0$ Thal Major</td>
<td>Hb F 100%</td>
<td>Tx dependent</td>
</tr>
<tr>
<td>$\beta^+\beta^0$ Thal intermedia</td>
<td>Hb F 40-80%</td>
<td>+/- Tx</td>
</tr>
<tr>
<td>$\beta^+\beta^+$ Thal intermedia</td>
<td>Hb F 30-95%</td>
<td>+/- Tx</td>
</tr>
</tbody>
</table>
Complications of Disease

- Bone expansion
- Short stature
- Osteoprosis/Arthopathy
- Delayed puberty
- Endocrinopathy/Hypopituitary
  - IDDM, Hypothyrodism, Hypo
  - Testicular and ovarian failure
- Cardiomyopathy
- Pulmonary hypertension

Therapy

Transfusion:

Mainstay of the therapy to correct the anemia.

Suppress the ineffective erythropoiesis

Prevent excessive marrow expansion
When to transfuse?

- Thal intermedia Hb levels 6-9 gm/dl
- Thal Major Hb levels 3-4 gm/dl

Hb Level that are insufficient and that lead to cardiovascular compromise, impaired linear growth and excessive marrow expansion.

Transfusion therapy

- 10-20 cc/kg every 4-6 weeks to maintain Hb in 9.5-11 gm/dl range.
- Suppression of the erythropoietic drive
- Decrease GI iron absorption, less bone demineralization and
- Splenectomy can reduce transfusion requirements.
Iron Balance in Thalassemia

Other Therapies for Thalassemia

- Erythropoetin
- Fetal Hb Augmentation
- Antioxidant

Curative: BMT/HPSC
Support Therapies

• Chelation Therapy
  Subcutaneous Desferoxamine
  Deferipone, L1
  Deferasirox (oral chelator)

• Osteoclast repelacement therapy
  bisphosphonates and Vitamin D

• Hormonal repalcement
<table>
<thead>
<tr>
<th>α Gene Map</th>
<th>α Genotype</th>
<th>α Clinical Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Heterozygous</td>
<td>Silent Carrier of α Thalasemia</td>
</tr>
<tr>
<td></td>
<td>Heterozygous</td>
<td>α - Thalasemia Trait</td>
</tr>
<tr>
<td></td>
<td>Homozygous</td>
<td>α - Thalasemia Trait</td>
</tr>
<tr>
<td></td>
<td>Compound Heterozygous</td>
<td>Hb - H Disease</td>
</tr>
<tr>
<td></td>
<td>Homozygous</td>
<td>Hydrops Fetalis</td>
</tr>
</tbody>
</table>
The interaction of Hb E and α thalassemia results in a variety of genotypes (see table below). Presence of α thalassemia reduces the amount of Hb E usually found in Hb E heterozygotes.

Some interactions of hemoglobin E with α thalassemia

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Clinical findings</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>αα/αα β⁺β⁻</td>
<td>Normal</td>
<td>A + E</td>
</tr>
<tr>
<td></td>
<td>Red cells slightly hypochromic</td>
<td>Hb E 25-30%</td>
</tr>
<tr>
<td>−α/−α β⁺β⁻</td>
<td>Normal</td>
<td>A + E</td>
</tr>
<tr>
<td></td>
<td>Hypochromic red cells</td>
<td>Hb E 20-25%</td>
</tr>
<tr>
<td>−α/−α β⁺β⁻</td>
<td>Normal</td>
<td>A + E</td>
</tr>
<tr>
<td></td>
<td>Hypochromic red cells</td>
<td>Hb E 17-20%</td>
</tr>
<tr>
<td>−/−α β⁺β⁻</td>
<td>Hb E/Hb H disease (see below)</td>
<td>A + E + Bart's</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hb E about 14%</td>
</tr>
<tr>
<td>−α/−α β⁺β⁻</td>
<td>As for heterozygous Hb E (mild anemia)</td>
<td>E + trace Bart's</td>
</tr>
<tr>
<td>−/−α β⁺β⁻</td>
<td>Severe thalassemia intermedia</td>
<td>E + F + Bart's</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hb E 80%, HbF 13%</td>
</tr>
</tbody>
</table>

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