HEMOLYTIC ANEMIA

- HEMOLYSIS: Premature or accelerated destruction of RBCs
- RBC survival: less than 100 days
- 2 main causes:
  - Intrinsic RBC defects (inherited)
  - Extra-corpuscular causes (acquired)

CLASSIFICATION OF HEMOLYTIC ANEMIAS

- Hereditary Hemolytic Disorders
  - RBC enzymes defects
  - RBC membrane defect
- Hemoglobinopathies
- Thalassemias

CLASSIFICATION OF HEMOLYTIC ANEMIAS

- Acquired Hemolytic Disorders
  - Immune hemolytic anemias
  - Splenomegaly
  - Microangiopathic hemolytic anemia
  - PNH
  - Direct toxic effect (malaria, clostridial infections)
  - Spur cell anemia

ERYTHROCYTE ENZYMOPATHIES

A red cell enzyme defect is a major consideration in the differential diagnosis of inherited, non-spherocytic, Coombs' negative hemolytic anemia
ERYTHROCYTE ENZYMOPATHIES

CLINICAL FEATURES
- Enzymopathies are mostly inherited as **autosomal recessive** disorders
- Glucose-6-phosphate Dehydrogenase (G6PD) and phosphoglycerate Kinase (PGK) deficiencies are **X-linked**

ERYTHROCYTE ENZYMOPATHIES

LABORATORY EVALUATION
- RBCs have increased osmotic fragility
- Increased unconjugated bilirubin, low or absent haptoglobin, increased LDH
- Intravascular hemolysis with hemoglobinemia, hemoglobinuria and hemosiderinuria is rare except in some patients with G-6-PD deficiency

ERYTHROCYTE ENZYMOPATHIES

CLINICAL FEATURES
- Family history is essential to determine the mode of inheritance
- Life-long hemolysis suggests inherited disorder
- Exposure to certain drugs or foods, surgeries or infections can lead to episodic hemolysis in some patients

ERYTHROCYTE ENZYMOPATHIES

LABORATORY EVALUATION
- Diagnosis of enzymopathy is by exclusion
- Peripheral blood smear may be helpful:
  - The RBCs are usually macrocytic and spiculated
  - Basophilic stippling is seen in pyrimidine 5'-nucleotide (P-5'-N) deficiency
  - Presence of spherocytes, elliptocytes, acanthocytes, or schistocytes suggests that the hemolytic process is not caused by an enzymopathy

ERYTHROCYTE ENZYMOPATHIES

DIAGNOSTIC TESTS
- In **acquired hemolysis**: a direct antiglobulin ('Coombs') test should be performed as initial screening
- In **congenital hemolysis**: osmotic fragility, hemoglobin electrophoresis and screening for enzyme deficiencies (G6PD and pyruvate Kinase) represent the initial evaluation
ERYTHROCYTE ENZYMOPATHIES

- During maturation, the RBC loses its ability for protein synthesis and oxidative phosphorylation.
- The mature RBC generates energy through:
  - Anaerobic glycolysis: Embden-Meyerhof (EM) pathway
  - Oxidative glycolysis: Hexose monophosphate (HMP) shunt
  - Nucleotide salvage pathways

ERYTHROCYTE ENZYMOPATHIES

- Structural & biochemical integrity of the RBC depends on:
  - The normal function of more than 20 enzymes involved in these pathways
  - The availability of five RBC substrates: Glucose, Glutathione, NAD, NAD phosphate & Adenosine diphosphate (ADP)

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- Most of RBC adenosine triphosphate (ATP) is synthesized through this pathway.
- 90% of the RBC glucose is converted to lactate via this pathway.
- ATP is necessary for the ATPase-linked Na+/K+ & Ca++ membrane pumps essential for cation homeostasis and RBC deformability.

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- Enzymopathy affecting this pathway leads to ATP-deficient RBCs which are rigid and removed by the spleen.
- Leads to Congenital, non-spherocytic hemolytic anemia.

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **Hexokinase (HK) deficiency**
  - First enzyme in the glycolytic pathway
  - Least active of the glycolytic enzymes
  - Activity decreases as RBC matures
  - Decreased HK activity can be quantitative or qualitative
  - Activity increases with reticulocytosis

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **Hexokinase (HK) deficiency**
  - Acquired HK deficiency occurs in Wilson's disease
  - Elevated copper levels inhibit HK intermittently leading to brisk intravascular hemolysis

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **Phosphoglycerate Kinase (PGK) Deficiency**
  - The only X-linked enzymopathy in the EM pathway
  - PGK deficient males develop normally until early childhood
  - Neuromuscular manifestations including seizures, spasticity & mental retardation
  - Cerebellar tumors within the first 4 to 5 years of age

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **Phosphofructokinase (PFK) Deficiency**
  - PFK is a tetrameric enzyme
  - Composed of 3 basic subunits: M (muscle), L (granulocyte), F (fibroblast, platelet)
  - RBC PFK consists of the L4 tetramer

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **PFK Deficiency**
  - First described as a muscle glycogen storage disease
  - Hemolysis is usually mild and well compensated
  - Fresh blood sample for PFK assay
Pyruvate Kinase (PK) Deficiency
- Most common enzymopathy in the EM pathway
- World-wide, multi-racial distribution (more common in northern European)
- Clinical expression ranges from severe hemolytic anemia in neonates to a fully compensated anemia
- Anemia or jaundice or both are recognized in infancy or early childhood

Pyruvate Kinase Deficiency
- Folic acid supplementation
- Splenectomy for pts with poor quality of life, chronic transfusions, need for cholecystectomy and severe anemia
- Hgb level improves in most pts
- Postoperative thromboembolic events

Pyruvate Kinase (PK) deficiency
- RBC osmotic fragility is normal
- Small dense crenated cells (echinocytes) on blood smear
- Quantitative measurement of the enzyme can be performed by reference laboratories

GPI Deficiency
- Peripheral smear shows poikilocytosis, anisocytosis, marked polychromatophilia and reticulocytosis.
- In rare situations myopathy, ataxia and mental retardation have been encountered in conjunction with hemolytic anemia.

Glucosephosphate Isomerase (GPI) Deficiency
- Third most common enzymopathy after G6PD and PK deficiencies
- Encoded by a single gene in all body cells. However, hemolytic anemia may be the only clinical manifestation since only mature RBCs are unable to synthesize the enzyme in an accelerated fashion

Triosephosphate Isomerase (TPI) Deficiency
- TPI present in all tissues.
- TPI deficiency: autosomal recessive inheritance.
- Progressive multi-system syndrome:
  - Hemolytic anemia
  - Hyperbilirubinemia
  - Neurologic abnormalities: spasticity, paraesthesia, weakness, mental retardation
  - Cardiac arrhythmia
  - Systemic infections
**HEXOSE MONOPHOSPHATE SHUNT**

- Major source of reduced nicotinamide-adenine dinucleotide (NADH) in RBCs
- Reduced NADH protects Hgb and other intracellular & membrane proteins from oxidant injury
- 10% of RBC glucose is metabolized via this pathway

**HEXOSE MONOPHOSPHATE SHUNT**

- Normal RBCs can increase the amount of glucose metabolized through this pathway upon exposure to certain oxidants. This leads to production of reduced glutathione to protect the RBCs.

**HEXOSE MONOPHOSPHATE SHUNT**

- Enzymopathy of the shunt renders the RBCs incapable of generating reduced glutathione causing precipitation of hemoglobin and Heinz bodies formation.

**G6PD DEFICIENCY**

- Many G6PD variants have been detected (>300 have been described but few are clinically significant)
- G6PD-B: is the normal enzyme (99% of whites and 70% of African-Americans)
- G6PD-A(+) is the normal African-American variant seen in 20% of this population. Has normal activity but increased electrophoretic mobility.
G6PD DEFICIENCY

- **G6PD-A(-)**: in 10% of African-Americans. Has same electrophoretic mobility as G6PD-A(+) but only 5-15% activity
- **G6PD Mediterranean variant**: in 5% of Arabs, Greeks, Sardinians and Sephardic Jews.

G6PD DEFICIENCY

- Classified into 5 classes:
  - Class I: chronic hemolysis without precipitating cause
  - Class 2 + 3: Acute hemolysis associated with exposure to oxidant drugs, stress or certain foods (fava bean)
  - Class 4 + 5: harmless

G6PD DEFICIENCY

- **Mediterranean variant**: the most prevalent class 2 deficiency
  - almost all RBCs are oxidant sensitive and hemolysis may be fatal
- **G6PD A(-)variant**:
  - enzyme's half-life is 50% normal
  - after a hemolytic episode, a young RBCs have near normal levels of G6PD
  - African-Americans with this variant stop hemolyzing even if oxidant exposure continues

G6PD DEFICIENCY

- Neonatal jaundice and acute hemolytic anemia
- Peak incidence: days 2 and 3
- Jaundice is more pronounced compared to the anemia
- Eccentrocytes or “bite/blister cells” are present
- Diagnostic assays should be performed during a steady state in the G6PD A- deficiency. In the Mediterranean variant the G6PD level can be checked at any time
HEXOSE MONOPHOSPHATE SHUNT

- Phosphogluconate dehydrogenase & Glutathione reductase deficiencies:
  - Rare
  - Should be considered in cases of oxidant-induced hemolysis & normal G6PD level

DEFECTS OF NUCLEOTIDE METABOLISM

- Pyrimidine 5'-nucleotide (P5N) deficiency
  - Basophilic stippling on Wright-stained blood smear.
  - Lead poisoning inhibits this enzyme.

DEFECTS OF NUCLEOTIDE METABOLISM

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Red Cell Enzyme Deficiencies

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inheritance</th>
<th>Frequency</th>
<th>Anemia</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild to severe</td>
<td>None</td>
</tr>
<tr>
<td>Glucose phosphate isomerase</td>
<td>Autosomal recessive</td>
<td>Unusual</td>
<td>Moderate to severe</td>
<td>None</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild</td>
<td>Myopathy</td>
</tr>
<tr>
<td>Aldolase</td>
<td>Autosomal recessive</td>
<td>Very rare</td>
<td>Moderate to severe</td>
<td>Mental retardation</td>
</tr>
<tr>
<td>Triosephosphate isomerase</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Moderate to severe</td>
<td>Cardiomyopathy and neuropathies</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>X-linked</td>
<td>Rare</td>
<td>Mild to severe</td>
<td>None</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild to severe</td>
<td>None</td>
</tr>
</tbody>
</table>

Red Cell Enzyme Deficiencies (Continued)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inheritance</th>
<th>Frequency</th>
<th>Anemia</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>X-linked</td>
<td>Common in certain ethnic groups</td>
<td>Moderate to severe</td>
<td>None</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>N/A</td>
<td>Very rare</td>
<td>Mild</td>
<td>None</td>
</tr>
<tr>
<td>Glutathione synthetase</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild</td>
<td>Mental retardation, Sorenoponitrate</td>
</tr>
<tr>
<td>Pyrimidine 5'-nucleotide</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild</td>
<td>Thalassemia</td>
</tr>
<tr>
<td>Adenosine deaminase (excess)</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild</td>
<td>None</td>
</tr>
</tbody>
</table>

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ERYTHROCYTE MEMBRANE DEFECTS

- RBC membrane consists of a phospholipid-cholesterol lipid bilayer intercalated by membrane proteins, band 3 (anion transport channel) & glycophorins
- This layer is stabilized by attachment to a membrane skeleton
- Skeleton proteins include: spectrin (75%), actin, ankyrin, protein 4.1 & adducin

RBC MEMBRANE DEFECTS

- Defects in cytoskeletal proteins lead to abnormal shape and flexibility of RBCs causing hemolysis

Spherocytes and Stomatocytes

- Reduced surface-to-volume ratio
- Tolerate less osmotic swelling than normal cells before they lyse

Target cells and dehydrated RBCs:

- Increased surface-to-volume ratio
- Osmotically resistant

HEREDITARY SPHEROCYTOSIS

- Inherited hemolytic anemia
- More common in Northern Europeans (1 in 2000)
- 75% of the cases are Autosomal Dominant
- 25% are Autosomal Recessive or secondary to acquired mutations
HEREDITARY SPHEROCYTOSIS
Molecular Defects
- In dominant HS, ankyrin deficiency or defect is most common
- Both ankyrin & spectrin deficiency in 30-45% of cases
- Spectrin deficiency alone in 30%
- Band 3 mutations alone in 20%

HEREDITARY SPHEROCYTOSIS
Molecular Defects
- Mutations of the B spectrin gene are infrequent in autosomal dominant HS
- Alpha spectrin gene abnormalities identified only in autosomal recessive HS
- Mutations of protein 4.2 gene in Japanese patients with autosomal recessive HS

Hereditary Spherocytosis
Pathophysiology
- Aberrant interaction between the lipid bilayer and the skeleton
- Spectrin loss is caused by a defect in one of the membrane proteins involved in the attachment of spectrin to the membrane rather than a primary defect in the spectrin molecule

Hereditary Spherocytosis
Pathophysiology
- Lipid bilayer is destabilized, with associated loss of membrane lipid and surface area leading to a spherocyte
- The reduced deformability of spherocytes leads to their retention and premature destruction in the spleen

CLINICAL FEATURES OF HS
- Anemia, Jaundice, Splenomegaly
- May present at any age
- Severe in Neonates
- Partially compensated hemolysis after neonatal period

CLINICAL FEATURES OF HS
- Severe hemolysis and anemia can be precipitated by illnesses that cause splenic hypertrophy (infectious mononucleosis) and by long term intensive physical activity (increased splenic blood flow)
DIAGNOSIS OF HS

- Spherocytosis and Reticulocytosis
- Hyperbilirubinemia (increased indirect bilirubin) in 50%-60% of cases
- Negative direct antiglobulin test
- HS red cells have high MCHC because of cellular dehydration (MCHC ≥ 36 in 50% of HS patients)

DIAGNOSIS of HS

- EMA (eosin-5-maleimide) binding
  - EMA is an eosin-based fluorescent dye that binds to RBC membrane proteins, especially band 3 and RH-related proteins. EMA fluorescence in HS is 2/3 of controls
  - High sensitivity and specificity (93-99%)
  - Rapid turnaround time (2 hours)
  - False negative in mild HS
  - Positive in hereditary pyropoikilocytosis and Southeast Asian Ovalocytosis

Osmotic fragility of RBC in hereditary spherocytosis (HS). The results from two patients are compared to those from a normal individual.

COMPLICATIONS OF HS

- Hyperhemolytic crises:
  - Most common
  - Associated with infections
  - Accelerated hemolysis & splenic enlargement
- Aplastic crises:
  - Rare, severe, caused by parvovirus B19. Lasting immunity after 1st infection
- Cholelithiasis:
  - Increased incidence (55%-75%) after 5th decade.
  - 50% are radiopaque. Ultrasonography is most reliable
COMPLICATIONS OF HS
- Gout, indolent ankle ulcers, chronic erythematous dermatitis on the legs, extramedullary tumors in the thorax
- Distal renal tubular acidosis in HS patients with band 3 mutation (anion channel protein)
- Spinocerebellar degeneration and familial cardiomyopathy have been described

TREATMENT OF HS
- Splenectomy
  - Corrects anemia but not the RBC defect
  - Relapses can occur due to hypertrophy of accessory spleens
  - Benefits of splenectomy to be weighed against the risks of post splenectomy infections

HEREDITARY ELLIPTOCYTOSIS
- Three subtypes:
  - Common HE
  - Spherocytic HE
  - Southeast Asia Ovalocytosis

- Heterogeneous disease
- Autosomal dominant
- Only 10%-15% have significant hemolysis
- No anemia, splenomegaly or reticulocytosis
- Osmotic fragility is normal or slightly resistant
HEREDITARY ELLIPTOCYTOSIS

- Splenectomy corrects the hemolysis but not the red cell abnormality. It should not be performed before the 3rd year of life.

SPHEROCYTIC ELLIPTOCYTOSIS

- Rare, autosomal dominant disorder
- Features of HS and HE
- All patients reported are from Europe
- Mild to moderate hemolytic anemia
- Round elliptocytes, and occasional spherocytes

SPHEROCYTIC ELLIPTOCYTOSIS

- Poikilocytes and red cell fragments are uncommon
- Positive osmotic fragility test
- Splenectomy is curative

SOUTHEAST ASIAN OVALOCYTOSIS

- Autosomal dominant disorder
- Prevalent in Melanesia, Indonesia, Malaysia and the Philippines
- Associated with band 3 mutation
- Heterozygotes have rounded elliptocytes, some with a transverse bar that divides the central clear space (spoon-shaped)
- Elliptocytes are rigid and resist invasion by malarial parasites
**SOUTHEAST ASIAN OVALOCYTOSIS**

- Rigidity appears to be caused by micro-aggregation of the mutant protein within the lipid bilayer
- Red cells circulate freely
- Mild hemolysis and no anemia
- Homozygous state is lethal

**Disorders of Erythrocyte Hydration**

- Heterogeneous disorders with abnormalities of RBC solute content
- Classification:
  - Primary due to intrinsic defects of RBC hydration
  - Secondary due to other intrinsic defects of RBC that also influence RBC hydration such as dehydrated RBC in sickle cell disease
- Primary hydration defects include:
  - Hereditary xerocytosis syndromes (dehydrated phenotype)
  - Hereditary hydrocytosis syndromes (overhydrated phenotype)
  - And intermediate syndromes

---

**Hereditary Xerocytosis**

- Most common erythrocyte hydration disorders
- Autosomal dominant
- Passive loss of intracellular K+ in excess of accumulation of intracellular Na+ leading to obligate water loss to maintain osmotic balance
- HX erythrocyte is rigid with decreased deformability leading to a shortened life span
- Missense mutations in PIEZO1 in most HX patients
- Mild to moderate, well compensated hemolytic anemia
- Splenomegaly uncommon
- Pseudohyperkalemia
- Iron overload, unrelated to chronic transfusion, requiring chelation

**Hereditary Hydrocytosis**

- Increased reticulocytosis than expected for degree of anemia
- Few target cells and stomatocytes
- Elevated MCHC & MCV
- Osmotic fragility is decreased (increased osmotic resistance) in contrast to hereditary spherocytosis
- No specific treatment is needed or available
- Splenectomy of little or no benefit associated with life-threatening thrombotic episodes

**Hereditary Hydrocytosis (Stomatocytosis)**

- Autosomal dominant disorder
- The red cell defect consists of a sodium leak, leading to increased intracellular sodium and water content and mild decrease in intracellular potassium
- The RBC is swollen (overhydrated), the MCHC is decreased and the MCV is increased

**Hereditary Hydrocytosis (Stomatocytosis)**

- Most patients have hemolytic anemia and splenomegaly
- Splenectomy decreases the hemolytic process but does not correct it completely
- Splenectomy is associated with increased incidence of thrombotic events

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HEREDITARY STOMATCYTOSIS

- Stomatocytes are more commonly seen as an acquired condition without permeability defect or hemolysis in patients with liver disease or excessive alcohol abuse.

Rh Deficiency Syndrome

- Absent (Rh\(sub:n\)) or markedly reduced (Rh\(sub:m\)) expression of Rh antigen
- Associated with mild to moderate hemolytic anemia
- Autosomal recessive inheritance of either a suppressor gene unrelated to the Rh locus or a silent allele at the locus itself

HEREDITARY PYROPOIKILOCYTOSIS

- Rare autosomal recessive disorder
- Severe hemolysis, bizarre poikilocytosis and red cell fragmentation
- Markedly low MCV (50-60 fl)
- Marked defect in Spectrin self-association
- Heat-sensitive RBCs that fragment when heated at 45\(^\circ\)C, secondary to mutant spectrin

Rh Deficiency Syndrome

- Stomatocytes or rarely spherocytes
- Increased osmotic fragility
- Splenectomy improves hemolysis, but is associated with thrombotic events

HEREDITARY PYROPOIKILOCYTOSIS

- More severe variants feature Spectrin deficiency (20%-40%) and spherocytosis
- Splenectomy partially corrects the hemolysis. RBC morphology and heat sensitivity are not affected
- Splenectomy should not be performed before the 3rd year of life
ACANTHOCYTOSIS

- Acanthocytes or spur cells are RBCs with multiple irregular projections (vary in length, width and surface distribution)
- Seen in severe liver disease (end-stage alcoholic cirrhosis-Zieve syndrome), cardiac cirrhosis, Wilson's disease, fulminant hepatitis & metastatic liver disease

ACANTHOCYTOSIS

- **Abetalipoproteinemia**
  - Congenital absence of apolipoprotein-b in plasma
  - Steatorrhea in the first month of life
  - Marked increase in membrane sphingomyelin
  - Autosomal recessive disorder
  - Retinitis pigmentosa, ataxia, intention tremors & death by 2nd or 3rd decade

ACANTHOCYTOSIS

- Seen also in patients with the McLeod phenotype
- In this condition, red cells have reduced surface Kell antigen because of absence of Kx protein needed for its expression
- Kx protein encoded by the X chromosome
- Males have mild compensated hemolysis & females are identified by flow cytometric analysis of Kell antigen expression