RBC Enzymes, Membranes and Metabolism

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DISCLOSURES

Off-Label Usage
  • None

Interests
  • None
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.
Enzymopathies are mostly inherited as \textit{autosomal recessive} disorders

Glucose-6-phosphate Dehydrogenase (G6PD) and phosphoglycerate Kinase (PGK) deficiencies are \textbf{X-linked}
ERYPHTHROCYTE 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

CLINICAL FEATURES

- Icteric sclerae and splenomegaly are present in chronic hemolysis and absent in episodic hemolysis.
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

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

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)
ERYTHROCYTE ENZYMOPATHIES

- The functions of the products of glucose catabolism include:
  - Maintenance of protein integrity, cellular deformability & RBC shape
  - Preservation of hemoglobin iron in the ferrous form
  - Modulation of the oxygen affinity of Hgb
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.
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**
  - Activity should be measured taking into consideration the extent of reticulocytosis
  - Hemolytic anemia can vary from mild to severe
  - RBC morphology is normal with the presence of reticulocytosis, polychromatophilia and few spherocytes
EMBDEN-MEYERHOF (GLYCOLOYTIC) 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
EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **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
EMBDEM-MEYERHOF (GLYCOLYTIC) PATHWAY

- **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*
EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **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
EMBDEN-MEYERHOFF (GLYCOLYTIC) PATHWAY

- **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.
EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

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

- **Glucose-6-phosphate-Dehydrogenase (G6PD) deficiency**
  - Most prevalent inborn error of RBC metabolism.
  - Affects more than 200 million people worldwide.
  - Gene located on long arm of the X-chromosome (band X q28).
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

- **Hyperactivity of Adenosine Deaminase (AD)**
  - AD catalyzes deamination of adenosine to inosine
  - The excessive deaminase activity prevents normal salvage of adenosine and leads to ATP depletion and hemolysis
  - Autosomal dominant inheritance
# 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>
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<tr>
<td>Triosephosphate isomerase</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Moderate to severe</td>
<td>Cardiomyopathy and neuropathies</td>
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<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>
<tr>
<td>Enzyme</td>
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</tr>
<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, 5-oxoprolinuria</td>
</tr>
<tr>
<td>Pyrimidine 5’-nucleotidase</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild</td>
<td>?Mental retardation</td>
</tr>
<tr>
<td>Adenosine deaminase (excess)</td>
<td>Autosomal recessive</td>
<td>Rare</td>
<td>Mild</td>
<td>None</td>
</tr>
</tbody>
</table>
ERYTHROCYTE MEMBRANE DEFECTS
RBC 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
Defects in cytoskeletal proteins lead to abnormal shape and flexibility of RBCs causing hemolysis
RBC MEMBRANE DEFECTS

- 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
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
- Incubated osmotic fragility test at 37 degrees C:
  - almost always abnormal and most reliable
  - The osmotic fragility test measures the ability of RBC to swell in a graded series of hypotonic solutions.
- HS red cells have high MCHC because of cellular dehydration (MCHC ≥ 36 in 50% of HS patients)
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 radioopaque. 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 myocardiopathy 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
TREATMENT OF HS

- All splenectomized patients should receive vaccination with pneumococcal, meningococcal and H-influenza B vaccines, several weeks prior to splenectomy.
- Prophylactic Pen VK or equivalent post splenectomy in pediatric patients.
- Folic acid therapy should be instituted at diagnosis.
HEREDITARY ELLIPTOCYTOSIS

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

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

- Bioconcave elliptocytes and in severe cases rod-shaped cells, poikilocytes and fragments
- Caused by defects in the interactions of red cell cytoskeletal proteins
- Spectrin abnormalities are the most common
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
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.
HEREDITARY 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 (over hydrated), the MCHC is decreased and the MCV is increased
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.
Stomatocytes are more commonly seen as an acquired condition without permeability defect or hemolysis in patients with liver disease or excessive alcohol abuse.
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°C, secondary to mutant spectrin
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
Rh Deficiency Syndrome

- Absent ($\text{Rh}_{\text{null}}$) or markedly reduced ($\text{Rh}_{\text{mod}}$) 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
Rh Deficiency Syndrome

- The RBCs are dehydrated leading to stomatocytes or rarely spherocytes
- Increased osmotic fragility
- Splenectomy improves hemolysis