Haemolytic Anemia

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**Hemolytic anemias**

Definition: Anemias that result from shortening of RBC life span, RBC destruction could be extravascular or intravascular

**Etiology**

<table>
<thead>
<tr>
<th>congenital</th>
<th>Acquired</th>
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<tbody>
<tr>
<td>1- Membrane abnormalities</td>
<td>1- Immune</td>
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<tr>
<td>• Hereditary spherocytosis</td>
<td>• Autoimmune Warm antibody</td>
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<td>• Hereditary elliptocytosis</td>
<td>• Cold antibody</td>
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<td>• Alloimmune</td>
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<tr>
<td>2-Haemoglobinopathies</td>
<td>2- Non-immune</td>
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<tr>
<td>• Lack of haemoglobin chain synthesis Thalassaemias</td>
<td>• Mechanical</td>
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<td>• Amino acid substitution on the haemoglobin chain Haemoglobin S, C, D</td>
<td>- Artificial cardiac valves</td>
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<td>- Burns</td>
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<td>- Microangiopatetic</td>
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<td>- March haemoglobinuria</td>
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<td>• Infections</td>
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<td>Clostridium perfringens, malaria</td>
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<td>• Drugs, chemicals</td>
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<td>3- Red cell enzyme detects</td>
<td>3- Paroxysmal nocturnal haemoglobinuria (PNH).</td>
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<td>• Glucose-B-phosphate dehydrogenase deficiency</td>
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Approach To Hemolytic anemia

A- Prove The Presence of Hemolysis (Evidence of hemolysis):

- Clinical Features: anemia +jaundice.
- Laboratory Tests

Low Hb

Increased reticulocyte count and percentage.

Indirect hyperbilirubinemia

raised s.LDH,

increased urinary urobilinogen.

Low serum haptoglobin.

Hemosiderinurea, hemoglobinurea in cases of intravascular hemolysis.

B- Find The Cause Of hemolysis

1- Blood Film Morphology: Target Cells, Sickle Cells, Heinz Bodies, Blister Cells, Fragmented RBC, Spherocytes.

2- Hemoglobin Electrophoresis: for Hemoglobinopathies.

3- Osmotic Fragility test for Spherocytosis

4- Enzyme assay for Enzymopathies.

5- Coomb’s test for immune hemolysis.

6- Ham’s test for PNH.

25 years old female accidentally discover she had splenomegally while she had biliary colicy pain, patient has no symptoms apart her new biliary colic no history of transfusion, and she has family history of early cholecystectomy
CBC: showed spherocytosis, increase corrected retic 5%, T.S.B 2mg/dl, coombs was negative

**Hereditary spherocytosis**

This is an *autosomal dominant* disorder in which the principal abnormality appears in red cell membrane protein.

Approximately 25% of patients have no family history.

The erythrocyte envelope is abnormally permeable and the sodium pumps are overworked.

- The exact nature of the red blood cell defect may vary from family to family.

- The severity of the disorder is very variable even within an affected family.

The erythrocytes lose their biconcave shape, become *spherical* and are more susceptible to osmotic lysis.
These spherocytes are destroyed almost exclusively by the spleen. Haemolysis is mainly extravascular.

**Diagnosis & Treatment**

**Clinical Features:**

- The severity of anemia is variable from asymptomatic to transfusion dependent anemia. Jaundice is also variable, as well as splenomegaly.

- Complications include
  1- Crises (hemolytic, megaloblastic, and aplastic).
  2- Pigment Gall stones.

**Lab Tests:**

- Evidence of Hemolysis: Anemia, Reticulocytosis, raised S.LDH...
- Spherocytes on blood film.
- +ve Osmotic Fragility Test.
- -ve Coombs Test.

**Treatment:**

1-Blood Transfusion.

2-Folic acid.

3-Splenectomy for moderate to severe cases.

20 years old male presented with abdominal pain followed by dark color urine. He had history of flu-like illness with history of ingestion of trimethprim.

Family noticed that he become pale within few hours ago.

CBP: Hb 7 gm/l, retic 15%, with presence of Heinz body, and blister cell, and spherocytes
Glucose 6 Phosphate Dehydrogenase Deficiency

- G6PD Enzyme is the first one in the hexosmonophosphate shunt, the function of this shunt is to service the enzymes glutathione reductase and glutathione peroxidase, which protect the red cells against damage due to oxidation.

G6PD deficiency

- The deficiency is inherited as an X-linked disorder
- Oxidant damage of RBC followed by intavascular hemolysis is induced by:
  1. Infections.
  2. Ingestion of Fava Beans.
  3. Oxidant drugs like: sulfa, dapsone, antimalarial, chloramphenicol….,

*Clinical manifestations:*

1. Most cases present with fever, rapid anemia, jaundice and deep colored urine.
2. Rarely the hemolysis is chronic.

G6PD deficiency

*Lab Tests:*

1. Anemia, reticulocytosis, indirect hyperbilirubinemia…
2. Blister RBC on blood film, and Heinz bodies.
3- Hemoglobinurea and later hemosideriurea.

4- Enzyme assay is useful after recovery.

Treatment:

1- Avoid fava beans and oxidant drugs.

2- For the hemolytic episode: stop the offending factor, blood transfusion, folic acid.

HAEMOGLOBINOPATHIES

☐ The haemoglobinopathies can be classified into two subgroups:

1- Where there is an alteration in the amino acid structure of the polypeptide chains of the globin fraction of haemoglobin, commonly called the abnormal haemoglobins: the best-known example is haemoglobin S found in sickle-cell anaemia.

2- Where the polypeptide chain production is impaired or absent for a variety of reasons: these are the Thalassemias.

Hemoglobin Structure and Production

☐ Fetal hemoglobin, HbF (α2γ2) switches to adult forms HbA (α2β2) and HbA2 (α2δ2) at 3-6 months of life.

☐ HbA constitutes 97% of adult hemoglobin.

☐ HbA2 constitutes 3% of adult hemoglobin.

☐ 4 α genes are located on chromosome 16.
2 β genes are located on chromosome 11.

There is the possibility of mixed defects
e.g. β-thalassemia minor and sickle cell (HbS) trait.

15 years old child presented with ascitis and leg oedemea. He referred by surgeon planning for splenectomy due to huge spleen. Patient transfusion dependent since childhood and his brother dead on his second decade.
On examination: anemic, with slate grey skin and puffy face, leg oedema, Ascitis, hepatosplenomegally

CBC: Hb: 7.8, retic 7%, NRBC10/50 cell, MCV 56, hypochromic microcytic
WBC: 3.5x10^9
Platelet 40x10^9

Pregnant lady with 24wks gestation referred by her gynecologist as history of increasing anemia and needs of transfusion. Patient denied any previous history of transfusion and her hemoglobin level since childhood around 10gm/l with family history of anemia
ex. patient pale, not jaundice, no organomegaly

CBC: Hb 8gm/l, retic 5%, MCV 60
Hypochromic microcytic
S. Ferritin was normal
THALASSEMIAS

**HETEROZYGOUS β THALASSEMIA** : β-Thalassemia Minor

- Common condition in Mediterranean Basin, Africa, Asia

Clinical Presentation

1. Mild or no anemia.
2. Spleen sometimes is palpable.
3. May be masked by Fe deficiency and sometimes confused with iron deficiency anemia.

Diagnosis

1- Hb 9-12 g/dL, MCV < 70
2- Microcytosis +/- hypochromia, target cells present, basophilic stippling usually present.
3- Hb electrophoresis: Hb A2 increased to 3.5-5% (normal 1.5 - 3.5%), 50% of individuals have slight increase in HbF.

Management

1. Add folic acid.
2. Patient and family should receive genetic counseling.

**HOMOZYGOUS β THALASSEMIA (β-Thalassemia Major)**

*Pathophysiology*

Ineffective beta chain synthesis due to point mutation in the beta gene promoter or enhancer on chromosome 11, excess alpha chains relative to
beta chain leading to ineffective erythropoiesis and hemolysis of RBC, compensatory increase in HbF

Clinical Presentation

- Start presenting at 3-6 months because of replacement of HbF by HbA
- Severe anemia developing in the first year of life
- Jaundice
- Stunted growth and development (hypogonadal dwarf)
- Gross hepatosplenomegaly (extramedullary hematopoiesis)
- Skeletal changes (expanded marrow cavity)
  - Skull x-ray has “hair-on-end” appearance
  - Pathological fractures common
- Evidence of increased Hb catabolism (e.g. gallstones)
- Death from
  - Untreated anemia.
  - Infection (early).
  - Iron overload (late, secondary to transfusions), usually 20-30 years old.

PATHOPHYSIOLOGY OF B-TALASSEMIA MAJOR
ß-Thalassemia Major

**Diagnosis**

1. Hemoglobin 4-6 g/dL.

2. Peripheral blood: hypochromic microcytic, increased reticulocytes, basophilic stippling, target cells.
- Postsplenectomy blood film shows Howell Jolly bodies, Nuleated RBC, and thrombocytosis.

3- Hb electrophoresis
   - Hb A: 0-10% (normal > 95%)
   - Hb F: 90-100%

4-DNA analysis.

**Management** (β-Thalassemia Major)

1- Blood transfusions to ensure growth and decrease skeletal deformities, try to keep Hb > 10 gm/dL, add folic acid.

2- Fe chelators to prevent iron overload like desferrioxamine, deferiprone, defrasirox.

3- Ensure good nutrition and try to minimize the frequency of infectious episodes. Infections should be treated adequately.

4- Allogeneic Bone marrow transplant (if suitable donor).

5- Splenectomy for mechanical problems and hypersplenism.

6- Genetic counseling for prevention
ALPHA THALASSEMIAS

Pathophysiology

- Autosomal recessive
- Deficit of alpha chains
- 4 grades of severity depending on the number of defective alpha genes
  1 - silent: αα/α-
  2 - trait: αα/-- or α-/α-
  3 - Hb H Disease (presents in adults): α-/--
  4 - Hb Bart’s (hydrops fetalis): --/--
- Hb Bart’s made of 4 gamma chains; not compatible with life
- Hb H excess of beta chains, is unstable, and leads to inclusion bodies

Diagnosis

1- Peripheral blood film: microcytes, hypochromia, occasional target cells, screen for Hb H inclusion bodies.
2- Hb electrophoresis not diagnostic.
3- DNA analysis using alpha gene probe.

Management: same as beta thalassemia.

PATHOPHYSIOLOGY OF ALPHA THALASSEMIAS
A. Hydrops Fetalis with Hb Bart's in Fetus

- α Genes absent
- α mRNA absent
- α Globin absent
- No Hb F
  - (Hb Bart's)
  - γ₄

- Extremely high O₂ affinity
- Profound tissue hypoxia
- Heart failure
- Liver failure (↓ Albumin)
- Massive edema (hydrops)
- Hypoxic death in utero

B. Hb H Disease in Adult

- 1 of 4 α Gene loci present
- ↓ α mRNA
- ↓ α Globin
- ↓ Hb A

- Excess β Globin

- Moderate hypochromic hemolytic anemia
- with splenomegaly, bizarre morphology

- β₄ (Hb H)

- Moderately unstable

N.B.: During fetal life, similar syndrome but with accumulation of Hb Bart's
HEMOGLOBIN H INCLUSION BODIES

Sickle Cell anemia

- Autosomal recessive
- Amino acid substitution of valine for glutamate in position 6 of beta globin chain.
It has a wide geographical distribution.

Sickle Cell anemia

**Mechanisms of Sickling**

- At low PO2, deoxy Hb S polymerizes, leading to rigid crystal-like rods that distort membrane = SICKLES
- The PO2 level at which sickling occurs is related to the % of Hb S present
  - If the patient is heterozygous (Hb AS), the sickling phenomenon occurs at a PO2 of 40 mmHg
  - If the patient is homozygous (Hb SS), sickling occurs at 80 mmHg
- Sickling is also aggravated by
  1. Acidosis.
  2. Increased CO2.
  3. Increased 2,3-DPG.
  4. Increased temperature and osmolality.
Clinical Consequences Of SCA

Heterozygous SCA: Hb S Trait

**Clinical presentation:**

- the patient will appear normal except at times of extreme hypoxia and infection, elderly patients may suffer from loss of renal concentration ability.

**Diagnosis:**

1- Hb level is normal

2- Peripheral blood: normal except for a few target cells

3- Hb electrophoresis (confirmatory test): Hb A fraction of 65% ; Hb S fraction of 35%

**Treatment:**

1- Avoid hypoxia during flying and surgery.
2- Folic acid for pregnant.
3- Genetic counseling.

HEMOGLOBIN ELECTROPHORESIS IN SCA

Homozygous SCA: Hb SS Disease

Clinical presentation

1- Chronic hemolytic anemia with jaundice in the first year of life.
2- Retarded growth and development +/– skeletal changes.
3- Susceptibility to infections by encapsulated organisms due to hyposplenism.
4- Spleen enlarged in children and atrophic in adults.
5- Crises:

Vaso - occlusive crises (infarction) leading to pain, fever, leukocytosis, acidosis & dehydration. Any organ or tissue can be involved.

Hyperhemolytic crises associated malaria.

Sequestration crises presenting with anemia and rapidly enlarging spleen or liver.
Aplastic crisis due to parvovirus infection or folate deficiency, leading to rapid anemia and reticulocytopenia.

Acute Chest Syndrome presenting as fever, chest pain, cough and hypoxia.

6- Iron overload due to repeated blood transfusion (less likely compared to Thalassemia).

7-Gall stones, leg ulcers.

Homozygous SCA: Hb SS Disease

**Diagnosis**

1- Peripheral blood: sickled cells, target cells, reticulocytosis.

2- Indirect hyperbilirubinemia, raised s.LDH

3- Screening test: sickle cell preparation searching for sickling phenomenon.

4- Hb electrophoresis (confirmatory test): Hb S fraction > 80%, the rest is Hb F.

**Management** (Homozygous SCA: Hb SS Disease)

1- Prevention Of Sickling Attacks:

   - Avoid conditions that favor sickling (hypoxia, acidosis, dehydration, fever).
   - Vaccination in childhood e.g. pneumococcus, meningococcus.
   - Good hygiene and nutrition.

2- Genetic counseling.

3- Blood transfusion to keep Hb>8 g/dl + Iron chelation for frequent transfusions.

4- Folic acid to avoid folate deficiency.

5- Hydroxyurea to enhance production of Hb F, presence of Hb F in the SS cells decreases polymerization and precipitation of Hb S.
**Note:** Hydroxyurea is cytotoxic and may cause bone marrow suppression it is indicated in severe cases.

6- Experimental anti-sickling agents.

7- Allogeneic Bone marrow transplant for selected patients.

Homozygous SCA: Hb SS Disease

**Treatment of Vaso-Occlusive Crisis**

1- Oxygen.

2- Good Hydration (reduces viscosity).

3- Antimicrobials.

4- Correct acidosis if severe.

5- Analgesics/narcotics (give enough to relieve pain).

6- Exchange transfusion for CNS crisis.

25 years old female presented with one week history feature of anemia. Examination revealed tinge of jaundice and palpable spleen. All the following are true except

Hb:4g/l, WBC 15X109, 80% neutrophil, retic corrected 16%, DAT positive for IgG, TSB 4mg/dl peripheral blood film showed spherocytosis.

a- idiopathic cause is the main cause of hemolysis

b- connective tissue disorder need to exclude

c- destruction mainly by phagocytosis in the liver

d- antibody or complement may coated the RBC

e- blood transfusion may be a cause of spherocytosis
AUTOIMMUNE HEMOLYTIC ANEMIA

Types:

1- Warm Antibody type: usually IgG.
2- Cold Antibody type: usually IgM.

AUTOIMMUNE HEMOLYTIC ANEMIA (Warm Antibody type)

Pathophysiology: RBC are coated with IgG or complement (C3d) or both leading to extravascular hemolysis in RES (mainly spleen).

Classification:

1- idiopathic

2- secondary to

  • Lymphoproliferative disorders (CLL, Hodgkin’s disease, Non - Hodgkin’s lymphoma)
  • Autoimmune (SLE)

3- Drug induced (penicillin, quinine/quinidine, alpha methyl dopa)

Clinical Features:

Usually insidious: anemia, jaundice and splenomegaly.
Mechanism of extravascular hemolysis in autoimmune hemolytic anemia. (A) Macrophage encounters an IgG-coated erythrocyte and binds to it via its Fc receptors. Thus entrapped, the RBC loses bits of its membrane as a result of digestion by the macrophage. The discoid erythrocyte transforms into a sphere. (B) RBC lightly coated with IgG (and therefore incapable of activating the complement cascade) is preferentially removed in the sluggish circulation of the spleen. (C) RBC with a heavy coat of IgG; thus, C3b (black circles) can be removed both by the spleen and the liver

AUTOIMMUNE HEMOLYTIC ANEMIA (Warm Antibody type)

**Diagnosis**

1- Spherocytes in blood film, reticulocytosis.

2- Indirect hyperbilirubinemia, raised s.LDH.

3- Positive direct antiglobulin test (direct Coombs’) best detected at 37ºC (hence “warm-reacting antibodies”)

4- Exclude delayed transfusion reaction.

**Management**

- Treat underlying cause
- Corticosteroids: prednisolone 1mg/Kg until response then taper over 2-3 months.
- Splenectomy for relapsed and corticosteroid resistant cases.
- Immunosuppressives like azathioprine for relapses after splenectomy
- Blood transfusion is used with caution.
- Add folic acid.
Autoimmune Hemolytic Anemia with Cold-Reacting Antibodies

Pathophysiology

- Either monoclonal or polyclonal IgM Antibodies attach to RBC surface antigens in peripheral circulation where $T < 37^\circ$C.
- Antibodies will detach from the surface antigen if $T >$ thermal amplitude
- Thermal amplitude is the temperature at which IgM is attached to RBC surface
- Associated with intravascular hemolysis

Classification

1- Idiopathic
2- Secondary to
   - Lymphoproliferative disorders (CLL, Hodgkin’s disease, non-Hodgkin’s lymphoma)
   - Infections (Mycoplasma pneumoniae, EBV).

Clinical Features:

Anemia, acrocyanosis, joint pain, vasculitic rash, Raynaud phenomena, and rarely splenomegaly.

COLD AGGLUTININ
Autoimmune Hemolytic Anemia with Cold-Reacting Antibodies (IgM)

**Diagnosis**

1- Anemia, mild reticulocytosis, RBC agglutination in blood film.
2- Positive cold agglutinin test best at 4ºC.
3- Positive direct Coombs’ for complement at any temperature.

**Management**

1- Treat underlying cause
2- Warm the patient above the thermal amplitude of the antibody
3- Plasmapheresis.
4- Immunosuppressives like chlorambucil.

Non Immune Hemolysis

1- Infections: Bacterial, Malaria, Babesia.

2- Mechanical:

1- Microangiopathic Hemolysis (MAHA): TTP, HUS, DIC.
2- March Hemoglobinurea.
3- Mechanical Cardiac Valves.

3- Snake bite.
4- Burns.

**Drug Induced Hemolysis**

1- Hapten Mechanism: high dose Penicillin
2- Complement Fixation : quinidine , phenacetin.
3- Autoantibody production: L-dopa, methyldopa.
4- Nonspecific: cephalothin.
5- Metabolic: sulfa drugs.

**Hypersplenism**

It is a state of sequestration of one or more of blood elements in an enlarged spleen.

**Causes**

1- Portal hypertension
2- Myeloproliferative disorders.
3- Thalassemia major.
4- Others.

**Diagnosis**

1- Reduction of one or more of blood elements.
2- Normal cellularity of bone marrow.

**Treatment**
Treat the underlying condition, splenectomy may be indicated for increased transfusion requirements.

in Hypersplenism ,the following are:

a- Portal hypertension may be a cause
b- there may be a reduction only in platelet
c- hypocellularity of bone marrow.
d- splenectomy may be indicated for sever cytopenia
E- Howell-jolly body may be seen in peripheral blood