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## *PREPARED BY*

**DR. NAITIK D. TRIVEDI,**

**M. PHARM, PH. D**

**LECTURER AT GOVERNMENT AIDED,**

**A. R. COLLEGE OF PHARMACY & G. H. PATEL INSTITUTE OF PHARMACY, VALLABH VIDYANAGAR, ANAND, GUJARAT**

**Mobile:** +91 - 9924567864

**E-mail:** mastermindnaitik@gmail.com

**&**

**DR. UPAMA N. TRIVEDI,**

**M. PHARM, PH. D**

**ASSOCIATE PROFESSOR & HoD (Pharm.D),**

**INDUBHAI PATEL COLLEGE OF PHARMACY AND RESEARCH CENTRE, DHARMAJ, GUJARAT**

**E-mail:** ups.aasthu@gmail.com

## ANEMIA:

Anemia is the condition in which the oxygen carrying capacity of blood is reduced. In the anemia the total number of RBCs decreases so indirectly decreases the oxygen level so decrease the production of ATP and energy.

**GENERAL PATHOPHYSIOLOGY OF ANEMIA:**

Our blood contains RBCs

↓

RBCs contains Hb

↓

Hb contains the iron

↓

Iron transfer the oxygen in body

↓

Oxygen is useful for production of ATP and Heat

↓

ATP provide energy

Anemia is not a single disease entity but a sign of disease. Independent of the cause, anemia is associated with a reduction in circulating Hb because of reduced number of erythrocytes or less Hb per erythrocytes. The number of erythrocytes varies with age, sex and atmospheric pressure.

**CAUSES:**

**The main causes of anemia are:**

1. Blood loss 2. Lack of red blood cell production 3. High rates of red blood cell destruction

**The other causes for the anemia are as under:**

**Blood Loss**

- Blood loss is the most common cause of anemia, especially iron-deficiency anemia. Blood loss can be short term or persist over time.
- Heavy menstrual periods or bleeding in the digestive or urinary tract can cause blood loss. Surgery, trauma, or cancer also can cause blood loss.
- If a lot of blood is lost, the body may lose enough red blood cells to cause anemia.

**Lack of Red Blood Cell Production**

- Both acquired and inherited conditions and factors can prevent your body from making enough red blood cells. "Acquired" means you aren't born with the condition, but you develop it. "Inherited" means your parents passed the gene for the condition on to you.
- Acquired conditions and factors that can lead to anemia include poor diet, abnormal hormone levels, some chronic (ongoing) diseases, and pregnancy.
- Aplastic anemia also can prevent your body from making enough red blood cells. This condition can be acquired or inherited.

**Diet**

- A diet that lacks iron, folic acid (folate), or vitamin B12 can prevent your body from making enough red blood cells. Your body also needs small amounts of vitamin C, riboflavin, and copper to make red blood cells.
- Conditions that make it hard for your body to absorb nutrients also can prevent your body from making enough red blood cells.

**Hormones**

- Our body needs the hormone erythropoietin (eh-rith-ro-POY-eh-tin) to make red blood cells. This hormone stimulates the bone marrow to make these cells. A low level of this hormone can lead to anemia.

**Diseases and disease treatments**

- Chronic diseases, like kidney disease and cancer, can make it hard for your body to make enough red blood cells.
- Some cancer treatments may damage the bone marrow or damage the red blood cells' ability to carry oxygen. If the bone marrow is damaged, it can't make red blood cells fast enough to replace the ones that die or are destroyed.
- People who have HIV/AIDS may develop anemia due to infections or medicines used to treat their diseases.

**Pregnancy**

- Anemia can occur during pregnancy due to low levels of iron and folic acid and changes in the blood.
- During the first 6 months of pregnancy, the fluid portion of a woman's blood (the plasma) increases faster than the number of red blood cells. This dilutes the blood and can lead to anemia.

**Aplastic anemia**

- Some infants are born without the ability to make enough red blood cells. This condition is called aplastic anemia. Infants and children who have aplastic anemia often need blood transfusions to increase the number of red blood cells in their blood.
- Acquired conditions or factors, such as certain medicines, toxins, and infectious diseases, also can cause aplastic anemia.

**High rates of red blood cell destruction**

- Both acquired and inherited conditions and factors can cause your body to destroy too many red blood cells. One example of an acquired condition is an enlarged or diseased spleen.
- The spleen is an organ that removes worn out red blood cells from the body. If the spleen is enlarged or diseased, it may remove more red blood cells than normal, causing anemia.
- Examples of inherited conditions that can cause your body to destroy too many red blood cells include sickle cell anemia, thalassemias, and lack of certain enzymes. These conditions create defects in the red blood cells that cause them to die faster than healthy red blood cells.
- Hemolytic anemia is another example of a condition in which your body destroys too many red blood cells. Inherited or acquired conditions or factors can cause hemolytic anemia. Examples include immune disorders, infections, certain medicines, or reactions to blood transfusions.

**SIGNS & SYMPTOMS**:

**Common symptoms of anemia:**

- fatigue
- decreased energy
- weakness
- shortness of breath
- light headedness
- palpitations (feeling of the heart racing or beating irregularly) and
- looking pale

**Symptoms of severe anemia may include:**

- chest pain, angina, or heart attack
- dizziness
- fainting or passing out; and
- rapid heart rate.

## 3. ANEMIA

**Some of the signs that may indicate anemia in an individual may include:**

- Change in stool color, including black and tarry stools (sticky and foul smelling), maroon-colored, or visibly bloody stools if the anemia is due to blood loss through the gastrointestinal tract;
- rapid heart rate;
- low blood pressure;
- rapid breathing;
- pale or cold skin;
- yellow skin called jaundice if anemia is due to red blood cell breakdown;
- heart murmur; and
- enlargement of the spleen with certain causes of anemia.

**NORMAL HEMOGLOBINE VALUE AS PER AGE AND SEX:**

| Age & Sex | Mean Hb ( g/dl ) |
|---|---|
| 1-3 days | 16.0 – 19.0 |
| 6 Months – 2 Yrs | 12.0 – 14.5 |
| 12 – 18 Yrs<br>Male<br>Female | <br>14.5 – 17.0<br>12.5 – 14.0 |
| Adult<br>Male<br>Female | <br>15.5 – 17.0<br>13.0 – 15.0 |

**Note: Atmospheric pressure**:

- People at high altitudes have more erythrocytes than at low altitudes.

**NORMAL HEMATOLOGIC & BIOCHEMICAL PARAMETERS**:

| **Component** | **Conventional** |
|---|---|
| **Hematologic** | |
| Hematocrit (Ratio of RBC-Blood Vol.)<br>Male<br>Female | <br>45 – 52 %<br>37 – 48 % |
| Hemoglobin<br>Male<br>Female | <br>14 – 18 g /dL<br>12 – 15 g/Dl |
| Erythrocyte count | 4.2 -5.9 x $10^6$/mm |
| Reticulocyte count | 0.5 – 1.5 % |
| MCV | 80 – 90 fmol |
| MCH | 27 – 30 pg |
| MCHC | 32 – 36 g/dL |
| RDW | 11.5 – 14.5 % |

| **Biochemical** | |
|---|---|
| Iron<br>Male<br>Female | <br>80 – 200 mcg/dL<br>60 – 190 mcg/dL |
| Transferrin | 170 – 370 mg/dL |
| TIBC | 250 – 410 g/ml |
| Transferrin saturation | 20 – 55 % |
| Transferrin receptors | 2.8 – 8.5 mg/L |
| Ferritin | 1.5 – 30 mcg/dL |
| Zinc protoporphyrin | <70 mcg/dL red cell |
| Folate<br>Normal<br>Borderline | <br>2 – 10 ng/ml<br>1 - 1.9 ng/ml |
| Vitamin B12 | 200 – 1000pg/ml |
| Methylmalonic acid | 53 – 376 nmol/ L |
| Homocysteine | 4.1 – 21.3 mcmol / L |

**DIAGNOSIS:**

A detailed medical & medication history along with hematologic & biochemical tests is obtained.

**I. Hematologic tests:**

It includes blood Hb concentration, cell counts, mean corpuscular volume (MCV), Hct, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), etc.

1. **MCV → cell size**

   Microcytic anemia - < 80fL

   Macrocytic anemia - >100fL

2. **MCH & MCHC → cell colour**

   Hypochromic anemia - low MCHC

   Hyperchromic anemia - high MCHC

3. **Red blood cell distribution width (RDW ) → variation in erythrocyte size in blood sample**

   Iron deficiency anemia → increased RDW

4. **Reticulocyte counts → bone marrow activity**
   Iron, B12 or folic acid therapy for respective deficiency states → reticulocytosis

5. **Others :** differential white cell count, platelet count, microscopic examination of peripheral blood smears & bone marrow aspirates.

**II. Biochemical tests**:

It includes measurement of serum vitamin conc, transport proteins, saturation of protein binding sites & storage amounts.

**TYPES OF ANEMIA**

**CLASSIFIED ACCORDING TO THE SIZE OF THE RED BLOOD CELLS:**

a) **Microcytic anemia**:

- If the red blood cells are smaller than normal, this is called **microcytic anemia**. The major causes of this type are iron deficiency (low level iron) anemia and thalassemia (inherited disorders of hemoglobin).

b) **Normocytic anemia**

- If the red blood cells size are normal in size (but low in number), this is called **normocytic anemia**, such as anemia that accompanies chronic disease or anemia related to kidney disease.

c) **Macrocytic anemia**

- If red blood cells are larger than normal, then it is called **macrocytic anemia**. Major causes of this type are pernicious anemia and anemia related to alcoholism.

**ACCORDING TO THE CAUSE, ANEMIA ARE CLASSIFIED AS UNDER:**

**A) IRON DEFICIENCY ANEMIA:**

- It is cause by excessive loss of iron or inadequate absorption of iron.
- It is most often in female than male.
- Iron absorption is regulated by iron needs & body stores.
- When iron stores are low, higher proportion of available iron is absorbed & vice versa. Except, in primary hemochromatosis, thalassemia & sideroblastic anemia iron absorption remains normal & even elevated despite increased iron stores.
- It is primarily absorbed in upper duodenum.

## PATHOPHYSIOLOGY OF IRON DEFICIENCY ANEMIA:

- Iron is an essential element for erythropoiesis, tissue respiration & several enzyme catalyzed reactions. The average adult body contains 3 to 5 g elemental iron.
- Iron is distributed in body in two forms: functional & storage.

### I . Functional iron

It exists as Hb , little as myoglobin , transferring & tissue enzymes.

- Hb is the oxygen binding protein that transports oxygen from lungs to tissues.
- Myoglobin, a hemo protein in muscle accepts oxygen from hemoglobin in the peripheries & acts as an oxygen store in muscle. If oxygen supply is limited, it releases oxygen to cytochrome oxidase leading to oxidative phosphorylation.
- Transferrin is a specific iron binding protein that transports iron through plasma & extravascular spaces. Each molecule of transferrin binds 2 molecules of iron in ferric state. The TIBC is high in iron deficiency & low in iron overload.

### II. Storage iron:

- It is in the form of ferritin & hemosiderin, which is located in parenchymal cells of liver & reticuloendothelial cells of the bone marrow, spleen & liver.
- Low iron stores are an early sign of iron deficiency & may help differentiate between iron deficiency anemia & other causes of anemia.

## DAILY IRON NEEDS:

Recommended daily allowances of iron:

| category | Age ( Yr ) | Iron ( mg ) |
|---|---|---|
| Infants | 0 – 0.5 | 6 |
| | 0.5 - 1 | 10 |
| Children | 1 - 10 | 10 |
| Boys & men | 11 - 18 | 12 |
| | > 19 | 10 |
| Girls & women | 11 - 50 | 15 |
| | > 51 | 10 |
| Pregnant women | | 30 |
| Lactating women | | 15 |

## FACTORS ASSOCIATED WITH IRON ABSORPTION:

| Factor | Associations |
|---|---|
| **Promoting absorption** | |
| Inorganic iron | Ferrous form is better absorbed than ferric iron & organically bound iron. |
| Ascorbic acid | Convert ferric iron to ferrous iron |
| Acids | Gastric HCl promotes the release & conversion of dietary iron to the ferrous form |
| Chelates | Iron chelated to low mol wt sub ( sugars, amino acids, succinates ) facilitates iron binding to the intestinal mucosa |
| Clinical states | Iron deficiency, increased erythropoiesis, pregnancy, anoxia & pyridoxine deficiency |
| **Reducing absorption** | |
| Alkaline | Antacids, alkaline pancreatic secretions containing phosphate convert iron to insoluble ferric hydroxide |
| Dietary | Dietary phosphates & phytates in cereals & tannins in tea probably complex iron |
| Clinical states | Chronic diarrhea, steatorrhea, adequate iron stores,decreased erythropoiesis, acute or chronic inflammation |

## FACTORS ASSOCIATED WITH IRON DEFICIENCY:

| Factor | Association |
|---|---|
| Dietary | Starvation, poverty, vegetarianism, religious practice, food pads |
| Blood loss :<br>Women & Girls<br><br>General | <br>Menstruation, postmenopausal bleeding, pregnancy<br>Esophageal varices, peptic ulcer, drug induced gastritis, carcinomas of colon & stomach, ulcerative colitis, hemorrhoids, renal or bladder lesions, hookworm infestations, frequent blood donation, athletic training, widespread bleeding disorders |
| Malabsorption | Celiac disease, partial & total gastrectomy, chronic inflammation |
| Increased requirements | Rapid growth, pregnancy |

## SIGNS & SYMPTOMS:

- Developmental delays
- Behavioral disturbances
- Altered central nervous system development
- Impaired work capacity
- Preterm delivery
- Delivery of low birth weight baby

- Others like brittle or spoon shaped nails, angular stomatitis, atrophic tongue & pharyngeal & esophageal webs causing dysphagia & atrophic gastric mucosa.

**DIAGNOSIS**:

- Medical history, full blood count & peripheral smears.
- Blood Hb concentrations & erythrocyte numbers are normal in mild cases.
- As deficiency worsens MCV & erythrocyte count & Hb decreases & RDW increases.
- Hypochromia or poikilocytosis shown when Hb conc are 7.0 g/dl or less for women & 9.0 g/dl or less for men.
- Absence of stainable iron in bone marrow aspirates is ultimate proof of deficiency but is painful & expensive so not used routinely.
- After hemorrhage or iron therapy reticulocytes increase.
- Serum ferritin is the first parameter to change in iron deficiency. It fall ( < 15 mcg/ml) in deficiency but increase abnormally in iron storage conditions.
- ZPP (zinc protoporphyrin )→ is an early indicator of iron deficient erythropoiesis than anemia. It represents the amount of protoporphyrin not incorporated into heme, it increase when insufficient iron is available for Hb synthesis.
- TfR (transferring receptor )→ provides information on later stages of iron deficiency, increasing only after iron stores are depleted.
- Since ZPP & TfR are not affected by inflammatory processes are useful in differentiating it from iron deficiency anemia.
- Serum iron is low & TIBC is high.

**PREVENTION:**

It can be done by identifying the underlying cause of iron deficiency & correcting it through diet or supplementation.

- **Dietary manipulation :**

- Food fortification is best recommended.
- When dietary iron supplementation is not possible or adequate, oral supplementation should be initiated.
- In **infants** CDC recommends following guidelines :
    1. breastfeeding for 4 – 6 months after birth.
    2. use of 1 mg/kg/day of iron from supplemental foods or iron drops when breastfeeding is stopped.

3. use of only iron fortified infant formula as a substitute for breast milk.
4. use of 2 – 4 mg/kg/day of iron drops ( max 15mg/day ) for preterm or low birth weight infants starting at 1 mth & continuing until 12 mths after birth.
5. introduction of iron fortified infant cereal at age 4 to 6 mths.

- The CDC recommends universal treatment with 30 mg iron/day during **pregnancy** to prevent iron deficiency.but since iron can cause side effects & potentially affect absorption of other nutrients, it is recommended only for women at risk of iron deficiency anemia.

**Screening of iron deficiency**:

- The CDC recommends screening of infants who are at risk ( preterm, low birth weight, low iron diet ) at 9 to 12 mths & at 15 to 18 mths of age.
- Women with risk factors (poor diet, excessive menstrual bleeding, chronic blood loss) should be screened annually.

**TREATMENT:**

1. **Oral iron therapy** :

- Generally, 30 to 40 mg elemental iron is used to treat iron deficiency states.
- This can be calculated from maximum rate of Hb regeneration. 0.25 g Hb/100 ml blood/ day x 5000 ml blood x 3.4 mg Fe/1g Hb = 40 mg Fe/day
- Since only 10 to 20 % of iron is absorbed, 200 to 400 mg iron would result in absorption of 40 mg elemental iron.
- Maximum absorption occurs if iron is taken before meals or between meals.

**Side effects**:

- It includes epigastric distress, abdominal cramping, nausea, diarrhoea & constipation caused by gastric irritation.
- This can be minimized by reducing daily dosage, taking the iron with food or changing to once a week dosing.
- Use of enteric coated products to minimize the side effects is not preferred since it prevents the dissolution & so decrease the absorption.
- Iron therapy can also cause the stools to appear black about which patients should be educated to differentiate from that of GI bleeding.

**Drawbacks:**

- Iron absorption may be reduced in patients with reduced gastric acid production or prior GI surgeries.
- When an ability to absorb iron is suspected, oral bolus dose of 325 mg ferrous sulphate should be administered. The serum iron levels after 2 & 4 hours should be 21 to 23 mcmol/ L. otherwise it indicates decreased iron absorption.
- Antacids, H 2 blockers & proton pump inhibitors may also decrease iron absorption
- Fails in cases of malabsorption, non-compliance with oral iron therapy, severe uncontrolled intolerance to iron therapy, excessive iron loss or erythropoiesis as seen in patients on renal dialysis receiving erythropoietin.

***common oral iron preparations includes salts of iron such as ferrous sulphate, ferrous fumarate, ferrous gluconate, etc

**2. parenteral iron therapy :**

To overcome the drawbacks of iron therapy, parenteral iron therapy is preferred.

- The amount of parenteral iron needed to replenish iron stores & restore Hb levels in patients with iron deficiency anemia can be obtained by formula :
  - Dosage (mg) = 0.3 x body weight (lb) x [ 100- {Hb (g/dl) x 100/14.8} ]

    Or
  - Dosage (mg) = 0.66 x body weight (kg) x [100-(Hb{g/dl} x 100/14.8}]
- The iron dose calculated is divided by 50 mg iron / ml to provide the volume of iron dextran injection needed.
- For children weighing < 15 kg, a normal mean of Hb of 12g/L is used in place of 14.8 g/dL.
- To determine iron replacement dosage in patients with active blood loss, one assumes that 1 ml of normochromic, normocytic erythrocytes contains 1 mg elemental iron :
  - Dosage (mg) = 1 mg iron / ml blood x blood loss ( ml ) x Hct
- It is administered by deep IM inj into the upper quadrant of the buttock or IV, eitheras a bolus or a total dose infusion (TDI).

**Contraindications to iron therapy:**

- In hemochromatosis & hemosiderosis ( iron load )
- In thalassemia & anemic conditions with chronic inflammatory disease such as rheumatoid arthritis ( have normal to high iron stores due to impaired use of iron )
- In alcoholics ( elevated iron stores )
- In enteritis, diverticulitis, colitis & ulcerative colitis ( local effects )
- In patients receiving repeated blood transfusions.

**Iron toxicity**:

- It can be acute ( overdose or accidental poisoning ) or chronic ( hemochromatosis, hemosiderosis, thalassemia )
- An iron overloaded person usually have more than 4 g body iron.
- Computed tomography & magnetic resonance imaging have been used to determine hepatic iron content.

  **Causes**

  - Alcohol consumption
  - Fortified food may be good for women but may lead to excessive iron intake by men.
  - Iron overload secondary to anemia :
    1. hypoplastic bone marrow → blood transfusion ( eg. Aplastic anemia )
    2. hyperplastic bone marrow → increased iron absorption secondary to ineffective erythropoiesis (eg. Thalassemia major, sideroblastic anemia, some hemolytic anemia )
- Treatment of tranfusional iron overload generally consist of chelation therapy such as deferoxamine.

## B) MEGALOBLASTIC ANEMIA/PERNICIOUS ANEMIA:

- It is cause by insufficient of hemopoiesis.
- In this condition stomach decreases the production of intrinsic factors because they decrease the absorption of vitamin $B_{12}$.
- It is a sub class of the macrocytic anemias.
- It is characterized by a lowered blood Hb mass due to reduced erythropoiesis secondary to defective DNA synthesis in the developing erythroid cells of the bone marrow.

## CAUSES:

- It can be mainly due to deficiencies of **vitamin B12 and folate.**
- It can be by drug induced interferences, either direct or indirect, i.e. with DNA synthesis or nutritional status.

## 1. VITAMIN B12 DEFICIENCY MEGALOBLASTIC ANEMIA:

**Stages of vitamin B12 deficiency anemia:**

| Stage | B12 concentration | MCV | Hb | Signs & symptoms |
|---|---|---|---|---|
| **Normal** | Normal | Normal | Normal | None |
| **Negative balance** | Normal | Normal | Normal | None |
| **Depletion of stores** | Slight decrease | Normal | Normal | Possible |
| **B12 deficient erythropoiesis** | Mod decrease | Increased | Normal | Possible |
| **B12 deficiency anemia** | Severe decrease | Increased | Decreased | Probable |

**Vitamin B12 Needs:**

1. Daily requirement for humans is 0.5 to 1 mcg.
2. The total body stores amount to 2-5 mg mainly into liver.

## PATHOGENESIS OF MEGALOBLASTIC ANAEMIA DUE TO VIT B12:

- Vit B 12 is well absorbed from GIT in a sequence by three different binding proteins i.e. R proteins, IF, & transcobalamin II (TCII).
- Extravascular R proteins also known as cobalophilins, are the first binding proteins for B12.

- The cobalamin remains bound to R protein in the upper small intestine until pancreatic proteases viz, trypsin partially degrade the complex releasing B12.
- Then B12 binds to IF, a specific B12 binding glycoprotein.
- The IF – B12 complex is highly resistant to proteolysis, passes down the small intestine to the distal ileum where it attaches to specific receptors.
- This attachment with receptor is not energy dependent but requires extracellular calcium & pH higher than 5.4.
- The majority of B12 in the circulation binds to intravascular R protein i.e. transcobalamin I ( haptocorrin ).
- But transcobalamin II (TCII) is a functional binding protein responsible for releasing B12 to the tissues.
- Patients with TC II deficiency may have normal serum B12 concentration as binding to TC I compensates for it.
- Features of severe B12 deficiency occur as TCI – B12 complex does not deliver the vitamin to the tissues.
- Another mechanism of absorption is diffusion, which is a potential method of providing oral B12 therapy to people with low IF levels (pernicious anemia).
- Lack of B12 allows folic acid to be trapped as non-functional methyl tetrahydrofolate(folate trap) So deficiency of functional FH4 causes impairment of formation of deoxy thymidine monophosphate(dTMP) which is needed for DNA synthesis As a result large proerythroblast fails to divide rapidly to make mature RBC rather immature precursors of erythocyte(blast cell) appear to cause megaloblastic anaemia.

**ETIOLOGY:**

**I Dietary** → inadequate intake.

**II Impaired transport** → transcobalamin II deficiency

**III Malabsorption:**

- **Pernicious anemia**: It is a B12 malabsorption caused by the loss of gastric IF secretion. Its an auto immune reaction against gastric parietal cells.
- **Gastric disorders:** gastrectomy (absolute deficiency of IF), atrophic gastritis, achlorhydria, vagotomy, partial gastrectomy & the use of H 2 receptor antagonists (prevent release of vitamin from food).

- **Intestinal problems**: Zollinger – Ellison syndrome, surgical resection or bypass of the ileum, Crohn's disease, celiac disease, lymphomas, Whipple's disease, bacterial overgrowth.
- **Drug induced**: colchicine, PAS, neomycin, H2 blockers, proton pump inhibitors, ethanol, Cholestyramine, etc decrease vitamin B12 absorption.

**SIGNS & SYMPTOMS:**

- Peripheral neuropathy
- Strange feeling in extremities
- Loss of hand coordination
- Deterioration in hand writing
- Tingling of extremities
- Loss of propioception
- Depression
- Psychosis
- Spinal cord degeneration
- Sore tongue or mouth, glossitis, beefy red tongue
- Lateral column disruption results in weakness & spasticity, exemplified by myoclonus, hyperreflexia, & a positive Babanski's sign. If it remains untreated, instability of gait & virtual paralysis results.

**DIAGNOSIS:**

- Measurement of holo - TC to differentiate between B12 deficiency or TC II deficiency. But clinically not used because of limited assay availability.
- **TC II saturation**→ it decreases in early B12 deficiency.
- Assessment of metabolite production – **MMA & Hcy** where MMA is more specific for B12 deficiency. But MMA also increases in renal disease so renal function should be assessed.
- **MCV** > 100 fL indicating macrocytosis indicates its deficiency.
- **Schilling test** (with or without IF)

- Stage I → An oral dose of Co labeled B12 is given, followed by an IM dose of unlabelled B12. The large IM dose saturates B12 binding proteins in the blood. So there are few finding sites for labeled B12 & a substantial portion is excreted in urine.

Urine is collected over 24 hours & the amt of labeled B12 is measured. If B12 conc is less than 10 % absorption is impaired & if its <5% than it indicates pernicious anemia.

- Stage II → Same process is repeated but now IF is given with labeled B12.If IF deficiency is there than stage I will be abnormal & stage II will be normal.
- Stage III → Patients are given antibiotics (tetracycline). So if there is bacterial overload stage I & II will be abnormal & stage III will be normal.
- If all the stages show abnormal absorption, it indicates ileal disorder.
- Results of test :

| Condition | Stage I | Stage II | Stage III |
|---|---|---|---|
| Normal | Normal | | |
| Inadequate diet | Normal | | |
| Pernicious anemia | Low | Normal | |
| Bacterial overgrowth | Low | Low | Normal |
| Ileal defect | Low | Low | Low |

- **Evaluating smear for megaloblastic changes** viz, neutrophil hypersegmentation & oval shaped erythrocytes, generally differentiates a B12 or folate deficiency from other causes.
- If iron deficiency occurs with vit B12, the MCV may appear normal but the blood smear should show both megaloblastic & microcytic cells.
- **Others** : thymidine uptake ( deoxyuridine suppression test [DUST] ) by bone marrow cells, food cobalamin absorption, erythropoietin measurement, folate concentrations & gastrin & pepsinogen analysis.

**TREATMENT:**

- Identify B12 deficiency early ( before anemia or neurologic symptoms develop )
- Correct the cause of deficiency if possible
- Replenish the depleted stores
- If necessary administer maintenance B12 therapy
- Dietary changes & supplemental B12 given orally, intranasally or parenterally.

**PHARMACOTHERAPY:**

1. **Oral vitamin B12 therapy :**

- Usual oral dose of vitamin B12 supplementation is 1 to 10 mcg/day.
- In patients with malabsorption, B12 dosages should be 1000 mcg or higher to produce favorable long term results.
- Concerns with oral therapy are the potential for erratic absorption, poor compliance & subsequent development of neurologic symptoms.
- Patient evaluation & monitoring for compliance & therapeutic response should whether long term oral therapy is optimal for each patient.
- Preparations containing iron in different forms are used eg. Ferrous fumarate, ferrous gluconate, etc.

2. **Parenteral vitamin B12 therapy :**

- Being safe, dosages of 100 to 1000 mcg can be given.
- It is given **IM** or by deep **Sc** injection .
- Peak serum concentrations after IM are reached in about 1 hr.
- The half-life of the parenteral B12 is about 6 days & its half-life in liver is 400 days.
- Two synthetic forms of B12 are available: **cyanocobalamin & hydroxocobalamin.**
- Hydroxocobalamin is more protein bound & so requires less frequent dosing. Cyanocobalamin has a side effect of optic neuropathy.
- It is well tolerated & has fewer allergic & anaphylactic reactions.
- Iron dextran is commonly used.

3. **Intranasal Vitamin B12 therapy:**

- Intranasal sprays & gels are available for patients who refuse or cannot tolerate parenteral therapy & don't respond to oral treatment.

**2. FOLATE DEFICIENCY MEGALOBLASTIC ANEMIA:**

- Folate deficiency occurs in stages, with depletion of stores leading to deficiency that can result in megaloblastic anemia & other hematologic abnormalities.
- Role of folate deficiency is evaluated in pregnancy, heart disease, stroke & peripheral arterial disease.

## PATHOPHYSIOLOGY OF FOLATE RELATED MEGALOBLASTIC ANEMIA:

- Dietary folate is usually in polyglutamate form, which must be converted to monoglutamate form for absorption.
- Active absorption of dietary folate occurs mainly in the proximal part of the small intestine.
- Synthetic folate is already in monoglutamate form & has greater stability & better absorption.
- Folic acid from formulations is completely absorbed in the upper duodenum, even in the presence of malabsorption.
- The principle circulating form is extensively protein bound & undergoes enterohepatic cycling but not reabsorbed from the bile.
- Reduced form of folate ( tetrahydrofolate ) are cofactors for transformylation reactions in the biosynthesis of purines & thymidylates of nucleic acids.
- Folate deficiency, so leads to defective DNA synthesis resulting in megaloblast formation & bone marrow suppression.
- It is needed in Hcy metabolism or any other methylation reactions.

## FOLATE NEEDS:

- The minimum daily requirement of folate is 50 to 100 mcg/day in general & in pregnancy an additional 400 mcg/ day is recommended.
- The average amount stored in the body is 5 to 10 mg, one half of which is found in liver.

## EPIDEMIOLOGY:

- Malnutrition
- In alcoholics due to poor diet & altered absorption.
- In pregnancy folate needs increase thrice the normal requirement due to large increase in nucleic acid synthesis associated with growth of the fetus, placenta & uterus & with increased maternal erythrocyte mass.
- Folate needs also increase during malignancy, increased erythropoiesis, conditions causing rapid cell turnover, chronic hemolytic anemia, exfoliative dermatitis, generalized psoriasis or extensive burns.
- Drugs can also cause folate deficiency by either reducing absorption or by altering metabolism.

- Reduced absorption → ethanol, metformin, oral contraceptives, Sulfasalazine, sulfamethoxazole
- Altered metabolism → methotrexate, trimethoprim, triamterene, alcohol

**SIGNS & SYMPTOMS:**

- They are similar to those of other anemias.
- In addition may cause megaloblastosis, glossitis, diarrhoea & weight loss.

**DIAGNOSIS:**

- Serum or erythrocyte **folate concentrations** are assessed where erythrocyte folate concentration reflects tissue status & is a better indicator of depletion.
- **Hcy concentration** is increased in deficiency. (also increased in B12 deficiency & decreased renal function)
- A blood smear & hematologic evaluation show macrocytosis with megaloblastosis.( also shown by B12 deficiency ).

**TREATMENT:**

- Primary prevention is by dietary manipulation or oral supplementation.
- Women planning to become pregnant should take atleast 400 mcg/ day, to prevent fetal neural tube defects.
- Folate deficiency is usually treated with oral folic acid 1 mg daily.
- Parenteral administration is indicated when oral administration is unacceptable or not possible.
- Long term therapy is needed in chronic hemolytic states, myelofibrosis, refractory malabsorption, postgastrectomy states, prolonged stress or infection, chronic fever & persistent diarrhoea.

## C. ANEMIA OF RENAL FAILURE:

- The severity of the anemia correlates with the extent of uremia.
- Most of the patients with serum creatinine concentration higher than 310mcmol/L & 97% of those on maintenance dialysis are affected.
- The cells are normochromic & normocytic but often are irregular in shape.

## PATHOPHYSIOLOGY:

- It is a multifactorial process but primarily due to reduced secretion of erythropoietin by the diseased kidneys.
- **Accumulation of the inhibitors of erythropoiesis, reduced RBC life span & chronic blood loss.**
- Parathyroid hormone & the polyamine spermine have been implicated in reducing marrow responsiveness to erythropoietin.
- Erythrocyte survival also decreases to an average of one-half of normal uremia due to mild, chronic hemolysis.
- Chronic blood loss both from a GI source & during hemodialysis may also contribute to it.
- Others: folic acid deficiency caused by losses to the dialysate, the accumulation of fat sol vit A, aluminum toxicity caused by long term hemodialysis & the use of aluminum containing phosphate binders & osteitis fibrosa, a complication of hyperparathyroidism in which myelofibrosis reduces viable erythroid cellular mass.

## TREATMENT:

- Iron & folate supplementation should be provided as necessary, & blood loss & use of aluminum containing antacids should be minimized whenever possible.
- For treatment of acute symptoms of hypoxia transfusions of RBCs is done.
- Risks of transfusions : Hs reactions, transmission of viral hepatitis, bone marrow suppression, iron overload, etc.
- Drugs : androgens, recombinant human erythropoietin, etc.

## D. APLASTIC ANEMIA

- Aplastic anemia is distinguished by hypocellularity of the bone marrow & subsequent pancytopenia that is unrelated to malignancy or myeloproliferative disease.
- The characteristic anemia , neutropenia & thrombocytopenia result from failure of the pluripotent stem cells due to congenital or acquired process.

**ETIOLOGY**:

- It is mostly idiopathic.
- Myelosuppression is a component of several congenital disease but is more commonly acquired after exposure to :

- drugs ( chloramphenicol, anticonvulsants, acetazolamide, etc)
- chemicals ( arsenic, benzene, ethanol, etc)
- viral ( HIV, influenza, rubella etc )
- others ( ionizing radiation, pregnancy, mycobacterial infection, etc.)

**PATHOPHYSIOLOGY :**

- It develops when hematopoiesis is interrupted because of deficient or defective stem cells.
- Others : immune mediated suppression of stem cell function, disturbances in the bone marrow microenvironment, & alterations in the cellular or humoral interactions that normally sustain hematopoiesis.

**CLINICAL PRESENTATION:**

- Pallor & fatigue initially mild but more pronounced when accompanied by bleeding due to thrombocytopenia.
- Ecchymoses & petechiae
- Fever due to infection caused by underlying neutropenia.

**DIAGNOSIS:**

- Peripheral blood - ↓ number of morphologically normal cells.
- Erythrocytes – normochromic, normocytic or slightly macrocytic
- Reticulocyte count, absolute granulocyte count - ↓
- Bone marrow biopsy – extensive areas of hypocellularity interspersed with small patches of hematopoietic cells.

**TREATMENT:**

- Removal of potential causative agents, supportive care & restoration with normal hematopoiesis with pharmacologic therapy or BMT.
- Blood transfusions, preferably with leukocyte depleted products, antiplatelet antibodies, broad spectrum antibiotics, cyclophosphamide, androgens, hematopoietic growth factors.

**PRECAUTIONS FOR TREATMENT:**

- Blood products from family members should not be used in candidates for marrow transplantation.
- Due to risk of hematoma, IM inj should be avoided in patients with thrombocytopenia & so should be avoided aspirin, NSAIDS & other agents with antiplatelet properties.

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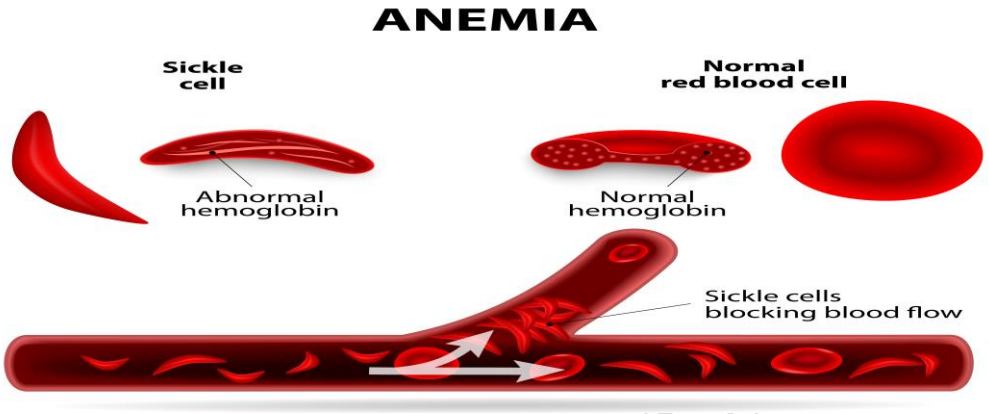
**E. SICKLE CELL ANEMIA:**

- The term sickle cell disease encompasses a variety of hemoglobinopathies, including sickle cell anemia, sickle Hb C (SC) disease, & sickle cell thalassemia.
- Although the clinical presentations of all are often similar, the manifestations of sickle cell are more severe & so mainly considered.

**PATHOPHYSIOLOGY:**

- Hb is distinguished as Hb A1, HbA2, Hb C, Hb F & Hb S of which Hb A1, Hb A2 & Hb F are normal.
- Hb A1 – a tetramer consist 2 pairs of globin chains α & β
- Substitution of valine for glutamic acid in both the β chains.
- Each parent contributes a single β chain gene, the heterozygous genotype AS is also possible & is expressed as the sickle cell trait phenotype.
- Deoxygenation in capillaries induces rapid polymerization of the sickling Hb, Hb S & results in formation of helical strands of parallel fibres.
- The elongated, crescent shaped cells characteristic of sickle cell anemia are so produced.
- The affected erythrocytes are rigid & unable to pass through the microvasculature. Vasooclusion with subsequent painful ischemia & chronic organ damage.

- Sickling is reversible upon reexposure to oxygen, however repeated sickling episodes eventually damage the cell membrane.
- The rate of Hb polymerization depends on its concentration in the erythrocyte.
- The co polymerization of Hb S with Hb F inhibits further polymer growth ; intracellular Hb F concentrations are inversely correlated with severity of disease.




**CLINICAL PRESENTATIONS**

- **Constitutional**
  - Impaired growth & development
  - Increased risk of infection viz meningitis, pneumonia, septicemia
- **Hematologic**
  - Hemolytic anemia
  - Aplastic crises
  - Splenic sequestration crises
- **Vasoocclusive :**

**1. Cardiovascular:**
- Cardiac enlargement
- Systolic murmur

**2. GI**:
- Autosplenecetomy
- Gallstones / cholecystitis
- Hepatic insufficiency
- Intrahepatic cholelithiais

**3. Genitourinary:**
- Hematuria
- Impotence
- Priapism
- Renal insufficiency

**4. Neurologic :**
- Cerebral thrombosis
- Intracerebral hemorrhage
- Seizures
- Subarachnoid haemorrhage

**5. Ocular:**
- Retinopathy
- Secondary glaucoma

**6. Painful crises**

**7. Pulmonary**:
- Acute chest syndrome
- Chronic obstructive disease
- Infarction

**8. Skin & skeletal :**
- Arthropathy
- Aseptic necrosis
- Leg ulcers

**DIAGNOSIS:**

- Hb electrophoresis → types & proportion of Hb present.
- Is rapid & inexpensive screening test

- It establishes the patients genotype.
- If both parents have the AS genotype there is a 1 in 4 chance that their child will have homozygous SS disease.
- Prenatal diagnosis also possible

**TREATMENT:**

**1. Management of major complications**:

**a. Anemia**

- Blood transfusions
- Folate supplementations

**b. Infection**

- Cefuroxime for Pneumonia & erythromycin & azithromycin for Mycoplasma pneumonia treatment. Prophylactic penicillin for pneumococcal septicemias.
- Ampicillin & cephalosporins for salmonella infections.

**c. Painful crisis**

- Vigorous hydration is initiated & oxygen administered if hypoxia is present.
- Ketorolac is given if codeine or oxycodones singly or in combination with acetaminophen are not effective.

**2. Management of the sickle cell disease:**

a. Transfusion therapy

b. Pharmacologic management : clotrimazole. Pentoxiphylline, antineoplastics, hydroxyurea.

c. Bone marrow transplantation

**F. THALASSEMIAS:**

- It is an inherited disease.
- Thalassemia is also the hemolytic anemia in which hemoglobin production is decreased.
- The RBCs are small, pale and short lived.
- It required the blood transfusion for life.
- Hemolytic disease of the newborn Rh+ antibodies of a sensitized Rh– mother cross the placenta and attack and destroy the RBCs of an Rh+ baby.
- Rh– mother becomes sensitized when exposure to Rh+ blood causes her body to synthesize Rh+ antibodies.
- The drug RhoGAM can prevent the Rh– mother from becoming sensitized
- Thalassemias are a group of hereditary disorders of Hb synthesis characterized by impaired production of one or more of the normal polypeptide chains of globin.
- The most prevalent thalassemia syndromes are those that involve diminished or absent synthesis of the α or β globin chains of HbA1.

**PATHOPHYSIOLOGY**:

- The thalassemia syndromes are collectively one of the most common genetic disorders of the human.
- Reduced production of normal α2 β2 tetramer of HbA1 results in the production of smaller erythrocytes with a low Hb content.
- The synthesis & accumulation of excess normal globin chains within the red cells lead to the formation of unstable aggregates, which may precipitate & cause cell membrane damage
- These deformed cells undergo premature destruction either in the bone marrow (Extravascular hemolysis) or the peripheral circulation ( intravascular hemolysis).
- Chronic hemolysis is a primary complication of the clinically significant α & β thalassemia syndromes.(Hb H disease & β thalassemia major ).
- The ineffective erythropoiesis & microcytic, hypochromic anemia described earlier are associated with a compensatory ↑ in the absorption of dietary iron.

**CLINICAL PRESENTATION:**

- This may contribute to the iron overload due to blood transfusion therapy.
- ↑ in erythropoietic activity in the bone marrow& in extramedullary sites.

- In severe form, excessive erythropoiesis causes significant bone marrow hypertrophy, growth retardation, lymphadenopathy & hepatosplenomegaly.
- Bone marrow expansion in untreated patients leads to skeletal deformities & fragility

**i. α – THALASSEMIA :**

- Four genes are involved in the production of α globin chains, with one pair occurring on each DNA strand (αα/αα).
- The most common form of it result from deletion of one or more of these genes.
- Excess production of β & γ chains result in the formation of unstable & nonfunctional γ4 (Hb Bart's) & β4 (Hb H) tetramers.

**Comparison of α-thalassemia syndromes :**

| **Syndrome** | **Genotypes** | **Hb conc (g/L)** | **RBC morphology** | **Clinical manifestation** |
|---|---|---|---|---|
| **Silent carrier** | -α/αα | 150 | Normal | None |
| **α-thalassemia trait** | -α/-α or -/ αα | 120-130 | Microcytic | Mild anemia |
| **Hb H disease** | -/-α | 60-100 | Microcytic, deformed | Chronic hemolysis, splenomegaly |
| **Hydrops fetalis** | -/- | - | Nucleated RBC | Intrauterine or neonatal death |

ii. **β-THALASSEMIA:**

- It results from faulty mRNA transcription of the β gene.
- Excess of α-chain accumulate & cause membrane damage in RBC precursors.
- So premature destruction of the cells in the bone marrow or peripheral blood.
- α- & δ- chain production is usually unaffected & so ↑ levels of Hb A2 (α2δ2).

**Comparison of β-thalassemia syndromes**:

| **Syndrome** | **Hb conc (g/L)** | **Clinical manifestation** | **Conventional treatment** |
|---|---|---|---|
| **Heterozygous** | | | |
| Minima | Normal | None | None |
| Minor (trait) | >100 | Mild anemia | Genetic/medical counseling |
| **Homozygous** | | | |
| Intermedia | 70 - 100 | Mod – sev anemia, impaired growth & splenomegaly | Intermittent blood transfusion & chelation therapy |
| Major | 20 - 70 | Sev anemia, abnormal skeletal growth, splenomegaly, iron load complications | Chronic blood transfusion & chelation therapy |

G. **HEMOLYTIC ANEMIAS:**

- It is caused by an increased rate of RBC destruction.
- It can be because of the production of the defective or damaged RBC's (megaloblastic anemias, thalassemias, sickle cell anemias) or drug induced.

**CLASSIFICATION:**

**1. Inherited:**

**a. Globin synthesis defect**

- Sickle cell anemia
- Thalassemia
- Unstable Hb disease

**b. Erythrocyte membrane defect**

- Hereditary spherocytosis
- Hereditary elliptocytosis
- Hereditary stomatocytosis

**c. Erythrocyte enzyme defect**

- HMP shunt defect
- Glycolytic enzyme defect
- Other enzyme defect ( adenylate kinase )

**2. Acquired**

**A. Immune mediated**

**a. Warm reacting Ab ( IgG )**

- Primary ( idiopathic )
- Secondary ( collagen vascular disease, lymphoproliferative disorders )
- Drug induced

**b. Cold agglutinin disease ( IgM )**

- Acute (mycoplasma pneumonia, infectious mononucleosis )
- Chronic ( lymphoid neoplasms, idiopathic )

**c. Paroxysmal nocturnal hemoglobinuria**

**d. Transfusions reactions**

**e. Hemolytic disease of newborns**

**B. Microangiopathic & traumatic**

- Disseminated intravascular coagulation
- Hemolytic uremic syndrome
- Thrombotic thrombocytopenic purpura
- Prosthetic or diseased heart valves

**C. Infection**

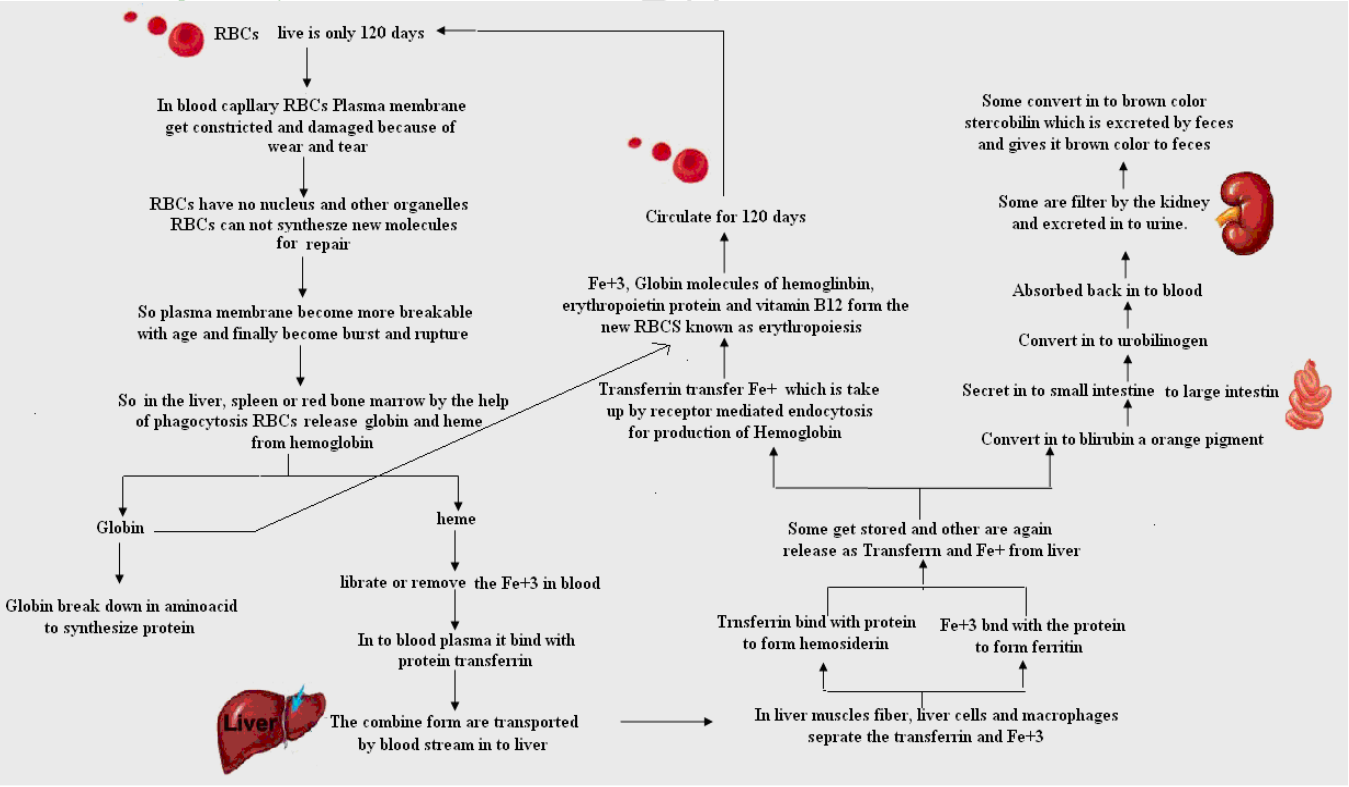
**D. Exogenous substances**

**E. Others**

- Liver disease
- Hypophosphatemia

**PATHOPHYSIOLOGY:**

- It involves hemolysis of RBC within the circulation (intravascular haemolysis) or taken up by the RES & destroyed ( extravascular hemolysis ).
- Intravascular hemolysis may be caused by trauma to the RBC, complement fixation to the RBC (immune mediated), or exposure to exogenous substances.




- During hemolysis, if haptoglobin binding capacity is exceeded, unbound Hb levels ↑, resulting in hemoglobinemia.

- Free Hb is filtered through the glomerulus & usually is reabsorbed by the proximal tubules.
- In severe intravascular hemolysis, the reabsorptive capacity is exceeded, causing hemoglobinuria.
- Some Hb molecules are transferred from hemopexin to albumin forming methemalbumin.

**CLINICAL PRESENTATION & DIAGNOSIS:**

| | **Moderate hemolysis** | **Severe hemolysis** |
|---|---|---|
| **Physical findings** | | |
| Jaundice | + | + |
| Hemoglobinuria | 0 | + |
| **Laboratory indices: urine** | | |
| Hemoglobin | 0 | + |
| Hemosiderin | 0 | + |
| Laboratory indices: plasma/ serum | | |
| Reticulocytosis | + | ++ |
| Plasma Hb | + | ++ |
| RBC Hb | Low | Low |
| Hematocrit | Low | Low |
| Bilirubin (unconjugated ) | + | ++ |
| Haptoglobin | Low | Low / - |
| Hemopexin | Normal / low | Low / - |
| Methemalbumin | 0 | + |
| Lactate dehydrogenase | 0 | + |

**I Inherited hemolytic anemia : G6PD deficiency :**

- It is the most prevalent inherited RBC enzyme defect, a sex linked ( X chromosome ) disorder.
- The G6PD enzyme, with glutathione & NADP acts as a protective antioxidant for RBCs against external oxidative stresses.
- In G6PD deficiency, oxidative stresses on the RBC viz drugs, infection or acidosis can lead to denaturation of the globin chains which ppts intracellularly onto the cell membrane as Heinz bodies & premature hemolysis occurs→ oxidative hemolysis.

*** Several factors affecting patient's susceptibility for deficiency:**

- The type of G6PD genetic variant present (i.e. type A- or mediterranean type).
- Patient age
- Other sources of oxidant stress
- Dosage of an offending drugs (nalidixic acid, cephalosporin, nitrofurantoin, etc.)
- Patient metabolism & excretion of offending drugs.

**TREATMENT**

- Withdrawal or avoidance of any potentially oxidant drugs or other substances.
- In patients with A- variant G6PD deficiency, hemolysis usually is mild & self-limited, so no need of therapy.
- For mediterranean type blood transfusions
- Folic acid supplementation.

**II Acquired hemolytic anemia: Autoimmune hemolysis**

- Autoimmune hemolytic anemia results from the binding of complement or anti-RBC antibodies to the red cell membrane in affected patients.
- These disorders are classified according to the temperature at which the antibodies have the greatest affinity for & interaction with red cells.

**1. Cold agglutinin hemolytic anemia**

- Here, IgM antibodies bind to RBCs at low temperatures (4° C).
- This agglutination process is reversed quickly during warming.
- Most don't appreciably shorten RBC survival.
- It is associated with mycoplasma pneumonia or infectious mononucleosis.
- Chronic disease occurs with lymphoproliferative disorders & results in poor peripheral circulation.
- It involves preventing exposure to cold environments.
- Folic acid supplementation
- Blood transfusions ( if necessary )
- Treating any underlying diseases.
- Occasssionaly patients may respond to plasmapheresis or cytotoxic agents such as cyclophosphamide or chlorambucil

**2. Warm autoimmune hemolytic anemia :**

- IgG or occasionally IgA have greatest affinity for red cells at room temperature (37°C).
- Hemolysis involves the attachment & subsequent destruction of IgG coated erythrocytes to receptors on macrophages in the RES.
- It may be idiopathic, secondary to an underlying disease that affects immune system (chronic lymphocytic leukemia, non Hodgkins lymphoma, or systemic lupus erythematosus), or secondary to certain drugs.
- When hemolysis is clinically significant, corticosteroid therapy is effective & blood transfusions may be needed.
- Splenectomy
- Alternative therapies include immunosuppressive agents, danazol, IVIG & cyclosporine