***“Hemolytic anemia –part 3”***

This part will discuss sickle cell anemia and thalassemia

***First: sickle cell anemia:***

Sickle cell anemia is the most common hemoglobinopathy (a group of hereditary disorders caused by inherited mutations that lead to structural abnormalities in hemoglobin).also sickle cell anemia is the most common familial hemolytic anemia in the world.

1. **Cause:**

Mutation in the Beta globin gene that creates sickle hemoglobin (HbS). HbS is produced by the substitution of valine for glutamic acid at the sixth amino acid residue of beta globin.

1. **Mechanism of the disease:**

On deoxygenation--🡪 HbS molecules form long polymers---🡪these polymers distort the red cell membrane ,which assumes an elongated crescentic or sickle shape ----🡪the sickling of red cells is initially reversible upon reoxygenation ----🡪but continues distortion of the membrane that is produced by each sickling episode leads to an influx of calcium ,which causes loss of potassium and water and also damages the membrane skeleton ----🡪over time ,continues damage creates irreversibly sickled cells ,which are rapidly hemolyzed.

1. **Sickling of red cell is influenced by many variables:**
2. The presence of hemoglobins other than HbS :

* In heterozygots approximately 40% of Hb is HbS and the remainder is HbA which interacts weakly with deoxygenated HbS .so the presence of HbA greatly retards the polymerization of HbS , and thus the red cells have little tendency to sickle .

Such persons are said to have sickle cell trait.

* HbC, another mutant beta globin has a lysine residue instead of the normal glutamic acid residue at position 6 (common among African Americans). Persons with those 2 hemoglobins (HbS +HbC) are compound heterozygots. HbC has a greater tendency to aggregate with HbS than does HbA .such persons with those 2 hemoglobin have a symptomatic sickling disorder called HbSC disease.
* HbF (fetal hemoglobin) interacts weakly with HbS ,so newborns with sickle cell anemia do not manifest the disease until HbF falls to adult levels within 5 -6 months after birth.

1. The intracellular concentration of HbS :

* The polymerization of deoxygenated HbS is concentration dependant. Thus, red cell dehydration which increases the HbS concentration facilitates sickling.
* In contrast, the coexistence of alpha thalassemia, which decreases the Hb concentration, reduces sickling.
* The relatively low concentration of HbS in heterozygots (sickle cell trait) also contributes to the absence of sickling.

1. The transit time for red cells through the microvasculature:

* The normal transit time of red cells through capillaries is normally too short for significant polymerization of deoxygenated HbS to occur. Hence sickling is confined to areas with sluggish blood flow such as the bone marrow and the spleen (most affected organs)
* Sickle red cells have a greater tendency to adhere to endothelial cells: because repeated episodes of sickling causes membrane damage that make them sticky.
* Inflammation: it slows down the flow of blood by increasing the adhesion of leukocytes and red cells to endothelium and by inducing the exudation of fluids through leaky vessels.

1. **Sickling of red cell consequences (complications):**
2. Homozygous sickle cell disease usually is asymptomatic until 6 months when the shift from HbF to HbS is complete. While heterozygous patients rarely have symptoms under severe hypoxic conditions such as high altitudes.
3. red cell membrane damage and dehydration caused by repeated episodes of sickling produce a chronic hemolytic anemia (the life span of RBCs is 20 days)
4. Red cell sickling produces microvascular obstructions which result in ischemic tissue damage and pain crises. This vaso occlusion increases with variables that increase red cell sickling (mentioned in point 3).
5. Vascular congestion, thrombosis, and infarction can affect any organ (bones, liver, kidney, retina…). The bone marrow is prone to ischemia because of its sluggish blood flow and high metabolic rate.
6. Priapism can lead to penile fibrosis and erectile dysfunction.
7. Acute chest syndrome: triggered by pulmonary infections or fat emboli from infarcted marrow .the blood flow in the inflamed ischemic lung becomes sluggish and “spleen like --🡪 check section d in point 5” , leading to sickling within hypoxemic pulmonary dysfunction, creating a vicious circle of :

* Pulmonary and systemic hypoxia
* Sickling
* Vaso-occlusion.

1. Stroke: which sometimes happen in the setting of acute chest syndrome.

Note: acute chest syndrome & stroke are the leading cause of death of ischemia -related death.

1. Aplastic crises: caused by sudden decrease in red cell production ( as in hereditary spherocytosis it is usually triggered by parvovirus B19 infection of RBCs) ,it is self limited.
2. Susceptibility to infections:

* because both children and adults are functionally asplenic (please refer to section d in point 5):
* in adults hyposplenism is caused by autoinfarction
* in children (in the phase of splenic enlargement ) congestion caused by trapped sickled red cells interferes with bacterial sequestration and killing

so patients become prone to encapsulated bacteria (such as pneumococcai).

* Patients become prone to salmonella osteomyelitis which may be due to defects in complement system

1. **Sickle cell anemia anatomic & histologic changes (diagnosis):**
2. In peripheral smears of homozygous patients, elongated, spindled, or boat shaped irreversibly sickled red cells are evident. While in patients with sickle cell trait sickling can be induced in vitro (outside the body) by exposing cells to high degree of hypoxia.
3. Hyperplasia of erythroid progenitors in the marrow which leads to bone resorption and secondary new bone formation , resulting in:

* Prominent cheekbones
* Changes in the skull resembling a “crewcut” in radiographs.

1. Extramedullary hematopoiesis may appear in the liver and spleen
2. Splenomegaly in children due to red pulp congestion caused by entrapment of sickled red cells. However, the chronic splenic congestion produces hypoxic damage and infarcts, which reduces the spleen to a useless fibrous tissue (autosplenectomy) .this process is completed by adulthood.
3. Both the anemia and the vascular stasis lead to hypoxia induced fatty changes in the heart, liver, and renal tubules.
4. Hemosiderosis, gallstones, hyperbilirubinemia and reticulocytosis.
5. Diagnosis is confirmed by electrophoresis.
6. Prenatal diagnosis can be performed by analyzing fetal DNA obtained by amniocentesis or biopsy of chorionic villi.
7. **Treatment:**
8. Supportive care is important
9. Prophylactic treatment with penicillin to prevent pneumococcal infections
10. A very important therapy is Hydroxyurea: a gentle inhibitor of DNA synthesis.

Hydroxyurea reduces pain crises and anemia by:

* Increasing red cell levels of HbF.
* Anti-inflammatory effect due to inhibition of WBCs production
* Increases red cell size which lowers the mean cell Hb concentration (refer to point 3 section b)
* Its metabolism to NO , a potent vasodilator and inhibitor of platelet aggregation

1. Bone marrow transplantation may provide some encouraging results.
2. **Notes:**

HbS has a significant protective effect against plasmodium malaria and that’s why this HbS is common in Africa where malaria is endemic.

***Second: thalassemia:***

Thalassemia: inherited disorders caused by mutations that decrease the synthesis of alpha or beta globin chains. As a result there is a deficiency of Hb and additional red cell changes due to the relative excess of the unaffected globin chain.

1. **Mode of inheritance:**

Autosomal codominant.

1. **Mutations:**

Note: each hemoglobin A contains: 2 alpha chains (encoded by 2 alpha globin genes on chromosome 16) and 2 beta chains (encoded by 1 gene on chromosome 11)

1. Beta –thalassemia mutations :
2. fall into 2 types according to the amount of beta chain produced:

* Β0 : no beta globin chains are produced
* Β+: reduced beta globin synthesis

1. Type of mutations : single base changes( unlike alpha thalassemia where gene deletions is common), such as:

* Mutations in RNA splicing (most common) may create B+ OR B0
* Mutations in the promoter produces B+
* Mutations in the coding region have severe consequences like producing B0

1. Classified according to the number of abnormal alleles:

* Beta thalassemia minor (beta thalassemia trait) : one abnormal allele (B0/B ,B+/B)
* Beta thalassemia intermedia : (B0/B+ ,B+/B+ ,B0/B ,B+/B)
* Beta thalassemia major : 2 abnormal alleles (B0/B0 , B0/B+ ,B+/B+)

NOTE: I don’t know what the difference between major is and intermedia with regard to genotype, I just wrote what is in the book …. It is confusing!!!

1. Alpha thalassemia mutations:
2. Type of mutations: gene deletions involving one or more of the alpha globin genes
3. Classified according to the number of abnormal alleles (remember 2 genes express alpha chain):

* Silent carrier: loss of 1 alpha (\_/a , a/a)
* Alpha thalassemia trait: loss of 2 alpha( \_/\_ , a/a 🡪in Asians // \_/a ,\_/a 🡪 in Africans)
* HbH or Hb Bart: loss of three alpha genes results in a relative excess of beta globin and γ globin (early in life) .excess β globin and γ globin form relatively stable β4 and γ4 tetramers known as HbH and Hb Bart respectively. (\_/\_ ,\_/a)
* Hydrops fetalis: loss of 4 alpha genes, lethal in utero because the red cells have no oxygen delivering capacity. (\_/\_ ,\_/\_)

1. **How these mutations contribute to anemia??**
2. Beta thalassemia:
3. The reduced synthesis of β globin leads to inadequate HbA formation and result in the production of pale cells with low hemoglobin (hypochromic) and small in size (microcytic)
4. Imbalance in β globin and α globin synthesis: there is an excess of alpha globin that aggregate into insoluble precipitate, which bind and severely damage the membranes of both red cells and erythroid precursors. So a large number of erythroid precursors die in the bone marrow by apoptosis this is known as (ineffective erythropoiesis) ,and the few red cells that are produced have a short life span due to extravascular hemolysis.

Note: Ineffective erythropoiesis has 2 effects:

* Lowers the amount of RBCs in the blood
* Causes an inappropriate increase in iron absorption (low levels of hipcidin) which over time leads to iron overload which results in secondary hemochromatosis.

1. Alpha thalassemia:

The same effects of beta thalassemia but there are few notes:

* The severity of the disease is proportional to the number of alpha genes lost (the more loss of alpha the higher severity)
* HbH & Hb Bart will cause less membrane damage than the α globin aggregates in β thalassemia ;as a result there is less ineffective erythropoiesis and thus less iron overload.

But!!!

HbH and Hb Bart have an abnormal high affinity to oxygen which decreases the amount of oxygen delivery to tissues.

1. **Manifestations and symptoms:**
   1. Β thalassemia minor & α thalassemia trait:

* Asymptomatic
* Mild microcytic hypochromic anemia
* Normal life expectancy
  1. B thalassemia major :
* Manifest postnatally as HbF synthesis is reduced
* The ineffective erthropoietic precursors consume nutrients and produce growth retardation and cachexia (general weakness seen in cancer patients)
* Skeletal deformities (please refer to point 5 section 3 )
* Iron overload and secondary hemochromatosis result in cardiac dysfunction
  1. B thalassemia intermedia & HbH :
* Not as severe as b thalassemia major
* Hematopoiesis is more effective
* Anemia of moderate severity
* Iron overload is rarely seen
  1. Hydrops fetalis :
* Lethal in utero

1. **Morphology:**
2. B thalassemia minor & α thalassemia trait :

* Smears of peripheral blood not the bone marrow show microcytic hypochromic cells but regular in shape (the regularity of shape distinguish this anemia from iron deficiency anemia)
* Target cells are seen (cells with increased surface area to volume ratio that allows the cytoplasm to collect in a central dark red puddle .

1. B thalassemia major:

* Marked microcytic hypochromic poikilocytic (variation in shape) anisocytic (variation in size) cells in the peripheral blood
* Nucleated red cells are seen which reflects the increased hematopoiesis
* The ineffective erythropoiesis and compensatory erythropoiesis -in response to hemolysis -results in : hyperplasia of erythroid progenitors / expanded marrow which will result in skeletal deformities
* Extramedullary hematopoiesis and mononuclear hyperplasia results in splenomegaly ,hepatomegaly ,and lymphadenopathy
* Growth retardation and cachexia
* hemosiderosis

1. B thalassemia intermedia & HbH :

* Peripheral smear findings lie between b thalassemia major and (b thalassemia minor and α thalassemia trait).

Those are less severe than b thalassemia major

* Splenomegaly
* Erythroid hyperplasia
* Growth retardation results from anemia

1. **Diagnosis:**

* B thalassemia: Prenatal diagnosis of b thalassemia can be done by DNA analysis

1. B thalassemia major:

* Clinical grounds (symptoms and manifestations)
* Hb electrophoresis shows reduction of Hb A and ( increase in Hb F or Hb A2 )

Note: Hb A2 can be normal

1. B thalassemia minor :

* Similar to b thalassemia major but less severe
* In Hb electrophoresis Hb A is low and Hb A2 is high
* Alpha thalassemia:

1. HbH : detection of β4 tetramers by electrophoresis.

**Note: morphologic changes in point 5 can be used in diagnosis**

1. Treatment:
2. B thalassemia major:

* Repeated blood transfusion which adds to the already increasing iron overload
* Iron chelators to lower iron overload
* Bone marrow transplantation is the treatment of choice at an early age

1. B thalassemia minor and HbH :

* Don’t require iron overload

1. **Notes:**

Thalassemia is high in regions endemic with malaria because globin mutations may be protective against falciparum malaria

Done by: Fekra….Good luck !!!