Hemoglobinopathies and workup with anemia

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Hemoglobin structure

HB is made by 4 subunits of globin (protein), each subunit is bound with a heme (non protein)

Heme is composed of:

Iron and 4 protoporphyrin ring.

* Without Hb the carrying capacity of O2 will be reduced, as in the rest 3% of O2 is soluble in the plasma (by solubility which equal to 0.03% multiplied by PO2 = 100%), whereas 97% of O2 is carried through Hb

Normally, In adults:

HbA = 95% or 98% of total hemoglobin

HbA2 = 1.5-3% (<3.5%) of total hemoglobin

HbF (fetal hemoglobin) = 1% of total hemoglobin

Hemogloboin in normal adults

HbA is composed of four polypeptide chains: 2alpha, 2beta

HbF: is composed of four polypeptide chains: 2alpha, 2 gamma

HbA2: is composed of four polypeptide chains: 2alpha, 2 delta

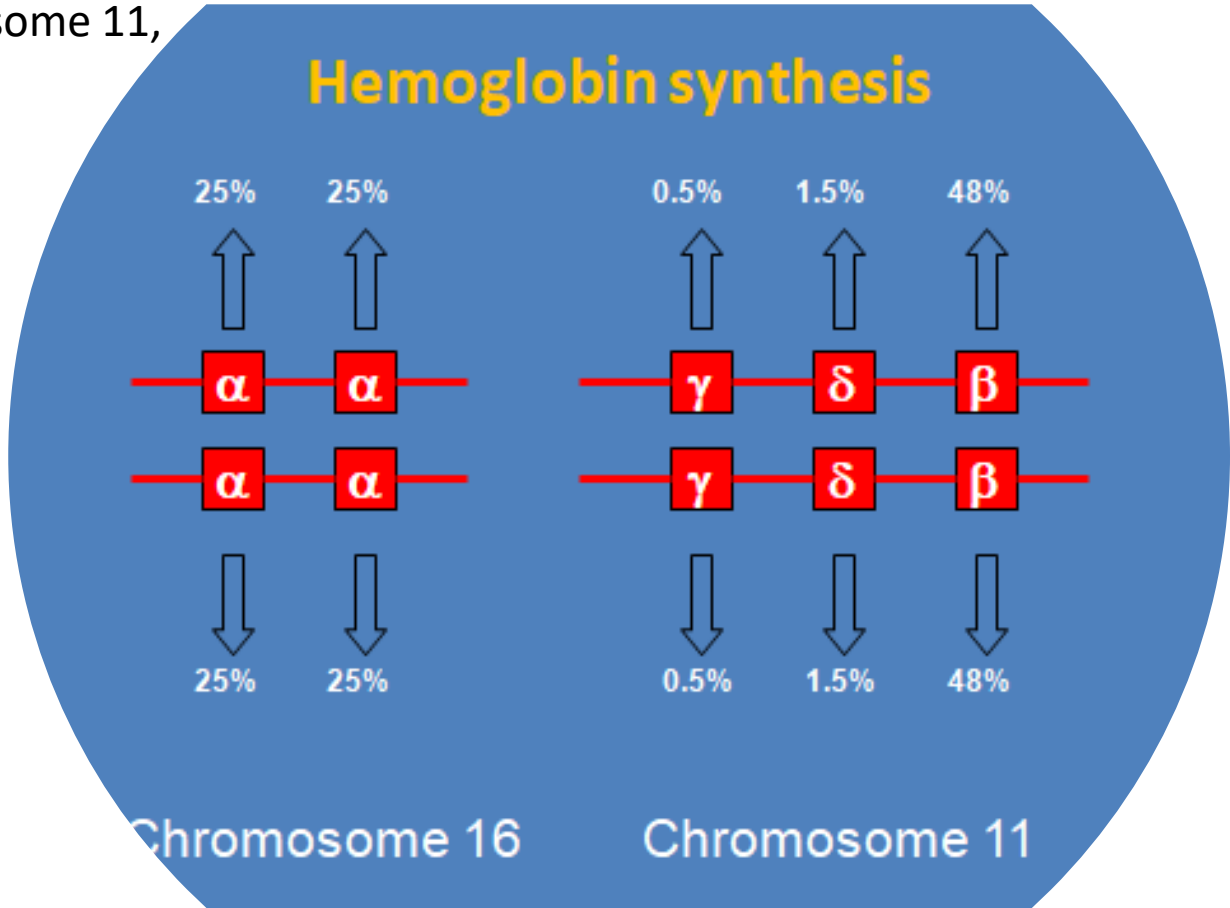
*HbF has a higher affinity for binding with O2 than HbA.

*HbF was in high amount during fetal state, then it continue to reduce in adults to reach 1% of total Hb

Hemoglobin synthesis

Alpha globin gene is on chromosome 16, there are four alpha globin gene in normal adult's chromosome (as each Chromosome 16 carry two alpha gene on two locus)

Beta, gamma and delta globin gene are carried on the chromosome 11, There are two Beta, gamma delta globin in normal adult's chromosome (as each chromosome 11 carry 1 beta,delta and Gamma gene)



• Sickle cell anemia (SCD)

Sickle cell anemia (SCD) is the first and oldest hemoglobinopathy

SCD is an autosomal recessive (must inherit two infected copies of the mutated gene (one from each parent)) which is caused by a point mutation in HBB gene on the chromosome 11 which effect the B-globulin chain of hemoglobin (like HbA), causing a hydrophilic amino acid (glutamic acid)(that binds with water) to be replaced with the hydrophobic amino acid (valine) at the sixth position of amino acid chain forming an abnormal hemoglobin called HbS

HbA will sickle (HbA is effected as it is composed of B-globulin), and thus it forms HbS (an abnormal form of Hb has abnormal b-globulin), HbS will aggregate in deoxygenated state forming a polymer.

Valine will bind with alanine/phenylalanine in another Hb causing a polymerization and aggregation of HbS to make RBC appear as a long sickle shaped fiber, aggregation of HbS will result in thrombus and occlusions which leads to vaso-occlusive crisis.

SCD occurs only in deoxygenated state(WHY?) because in oxygenated state, alanine and phenylalanine will be disappeared, and thus valine can’t bind with them, while in deoxygenated state those Amino acids are present.

SCD occurs in Veins not arteries, as veins lack O2.

Sickle cell anemia (continue…)

HbF will not sickle (HbF is resistant as it is not composed of B-globulin).

New born won't have symptoms until it reaches 6 month after birth (why?) because HbF amount will be reduced (to be 1%) and HbA will be increased

Malaria cause SCD especially among African population by plasmodium, it will change the shape of RBC to sickle (crescent) shape to decrease the amount of O2 in the infected RBC (WHY?) => because it is thought that decreasing O2 of the infected RBC will increase it's opportunity to be more fragile and lysed, so infected sickle RBC has shorter life span 10-20 days.

As infected RBC will decrease its O2 carrying capacity it make Right shift dissociation curve, and thus acidosis occur and the patient's case will be deteriorated due to defect in acid-base balance.

the infected cell make a sickle shape (WHY?) because it increase Ca influx and the outflux of K and H20 out from the cell which cause dehydration

Deoxygenation of SS (HbS) erythrocyte leads to intracellular hemoglobin polymerization, loss of deformobility and changes in cell morphology.

SCD (continue…)

- Diagnose: if HbS > 60% = the paitient suffer from SCD
- Treatment: There is no treatment, however Hydroxyurea is used to increase HbF (which has a higher binding affinity than HbA).
- SCD is considered as Extravascular hemolytic anemia*

*in previous lectures, it was written that SCD, hereditary spherocytosis are intravascular hemolytic anemia, but that is wrong, however, those disease are caused by intrinsic defects but cause extravascular hemolysis.

Thalassemia alpha and beta

is an Autosomal recessive disease it effects globin as it is caused by:

1- a mutation on chromosome 16 cause a deletion of alpha globulin in 1 or 2 locus of the chromosome:

A- deletion of 1 alpha globulin in 1 locus (there will be only 3 alpha globulin), the patient remain asymptomatic (carrier)

B- deletion of 1 alpha globulin in 2 locus (there will be only 2 alpha globulin), the patient remain asymptomatic (carrier) but it may cause minor microcytic anemia (and thus, it can be misdiagnosed with iron deficiency)

C- deletion of 1 alpha globulin 3 loci (there will be only 1 alpha globulin), so the blood try to compensate by producing 2 beta globulin in ch.16 forming B4 tetramers (2 abnormal b-globulin from ch.16 and 2 normal B-globulin from ch.11) => called **HbH hemolytic anemia**. The same will occur in fetus but causing gamma tetramers (as there is no beta-globin in HbF) => **called Hb Barts**

D-deletion of alpha globulin in 4 loci (all of alpha- globin are deleted) will decrease the amount of O2 (not effective oxygenation) causing hydrops fetalis (death of fetus)

Thalassemia (continue…)

2- a mutation on ch.11 causing a deletion on B-globulin:

A- deletion of 1 beta globulin (B minor) is asymptomatic, however it may cause microcytic anemia (not dangerous)

B- deletion of 2 beta globulin (B major), the patient can't live without blood transfusion, the patient can live max to 15 -25yrs*

* As time pass, blood transfusion may cause an accumulation in iron, and thus forming fenton reaction and free radicals which leads to death.

Thalassemia (ملخص)

In health, equal quantities of a- and b- globin chains are produced. Abnormalities in the Transcription of either a or Beta globin genes lead to the excessive production of the Other chain, and these chains may precipitate, causing hemolysis and anemia.

The gene for the alpha globin chain is duplicated on each chromosome 16, so in health, Four alpha globin genes exist. Alpha thalassemia results from the deletion of between one all four genes, with an associated variation in clinical severity. The deletion of all four genes Is incompatible with life.

B-thalassemia is usually due to a single gene mutation and results in the reduced production Of Beta globin chains. It normally becomes clinically apparent at between 3 and 6 months of age when
Fetal hemoglobin begins to be replaced by HbA. The excess a-globin chains combine with the available B, delta or gamma chains, formin abnormal amounts of HbA2 (delta chains) and HbF (gamma chains)

Art of Anemia work up

If we want to diagnose (approach) Anemia, first we use new school Medicine method (kinetic approach – to identify basic mechanisms of anemia) by measuring the response of BM (to make sure it has good response and produce enough reticulocyte or not) so we check on reticulocyte count (RPI – reticulocyte production index), if reticulocyte was in high amount (RPI >2) then there is an increase in RBC destruction (either by hemolysis or bleeding).

If RPI is less than 2, it indicates that there is a problem in RBC production, then we use Old school medicine method (diagnose anemia based on MCV) called morphologic approach.

Morphologic approach

It is done by looking at MCV, it is benefitial in small clinics, if it was:

Macrocytic (>100): caused by folate, B12 deficiency, Reticulocytosis (Big reticulocyte) or a liver disease (like alcoholic Liver disease). We use Liver panel (Liver function test – to test the overall health of the liver), however if the patient macrocytic anemia we firstly suspect he has folate deficiency (because folic acid is stored in little amount and any deficiency in its amount will cause anemia, unlike Vit.b12 which is stored in high amount and for a long period so the body can uitilize it for 3-5years without causing anemia.

Normocytic: we suspect there is a blood loss or a hemolysis (so we test LDH, bilirubin, haptoglobin amount) or a renal disease (so we do test renal function), we measure reticulocyte count. If everything was normal, then we suspect a problem in Bone marrow (leukemia, tumor, granuloma)

***In normocytic, there may be a kind of autoimmune hemolysis, can be discriminated and differentiated through Coomb test like:**
A- warm hemolytic anemia: IGG binds with RBC (in increased temperature) to destruct RBC (extravascular hemolysis)

B-Cold hemolytic anemia: IGM binds with RBC (in low temperature) to destruct RBC (intravascular hemolysis)

Morphologic approach (continue…)

Microcytic anemia:

A- If Fe and ferritin are decreased, high TIBC => IDA

B-If Fe and TIBC are decreased, high ferritin => ICA

C- if Fe and ferritin are increased => sideroblastosis (It is caused by a deficiency of protoporhyrin ring, so iron level will be increased in serum and it will be aligned as a blue ring around RBC's membrane) (we use blood smear test and Liver panel test to confirm the disease and rule out any liver disease)

D-Normal Fe => unknown cause (we use reticulocyte count test, HgB electrophoresis to know the disease (suspect thalassemia))

Morphologic approach

- Diagnosis by observation of cell changes.
- Start by looking at the MCV.