



HEMATOLOGY

& LYMPH SYSTEM

Pathology

sheet

Number

5

Done BY

رناد زكريا

Correction

Dr. ahmad

Doctor

Dr. ahmad

Before we start....

- This sheet was written according to section 2's record and reviewed according to section 1's record by Ruba Hussien with all thanks and I referred to last year sheets.
 - In this sheet, the topic about anemia will end and we'll start talking about *polycythemia*.
 - For you who want to go through the book this sheet will cover the pages 416-417 & 425 & 447-448.
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In the previous lecture, we were talking about hemolytic anemia of intrinsic factor and we discussed the heredity types:

- 1-Membranopathies→spherocytosis.
- 2-Hemoglobinopathies→thalassemia and sickle cell disease.
- 3- Enzymopathies→G6PD deficiency (it's our topic in this sheet).

And the Acquired type, which is *Paroxysmal nocturnal hemoglobinuria* (it's our topic in this sheet as well).

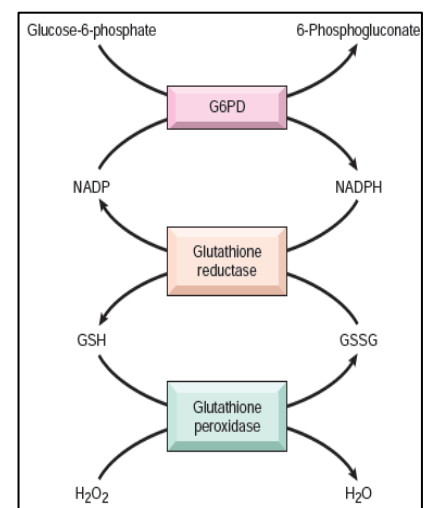
Enzymopathies→

G6PD deficiency

G6PD is an enzyme which produces NADPH; NADPH will in turn reduce glutathione which will find reactive oxygen species and get rid of them.

- So if we have G6PD deficiency, this cycle (shown in the figure) will break, making RBCs more prone to oxidative stress.

- Cells that are more prone to oxidative stress are senescent RBCs; in other words, young RBCs are not affected as severely as old RBCs.
- Remember that the half-life of an RBC is 120 days and with daily production and turnover of cells, old and new RBCs are found in the blood.



- G6PD deficiency is an X-linked disorder → more common in males.

- Many G6PD variants exist (over 400); those associated with disease are mainly two:
1- G6PD A- (common in African Americans).

2- G6PD Mediterranean (common in Middle East).
- the Mediterranean is more severe than G6PD A-.

*Hemolysis in G6PD deficiency is acute and episodic compared to hemolysis in spherocytosis where it is chronic. For example, if a patient who doesn't have chronic hemolysis and got exposed to an acute attack, he will suffer from hemolytic anemia; on the other hand, spherocytosis patients always have hemolysis even if it was pre-symptomatic or subclavian.

**The acute attacks that cause acute episodic hemolysis in G6PD deficiency are:

1-Infections: most common cause.

2-Drugs: antimalarials, nitrofurantoin (old antibiotic for UTIs, especially in females).

3-Certain foods: fava beans; one of the least common causes.

- Once the oxidative stress occurs, the old RBCs will undergo hemolysis. The acute episodic hemolysis will stop even when the patient takes the drug that causes the hemolysis or still has an infection. Why?

Because the new RBCs have the ability to fight this oxidative stress more than the old ones, so once the old RBCs undergo hemolysis, the patient will come back to being normal.

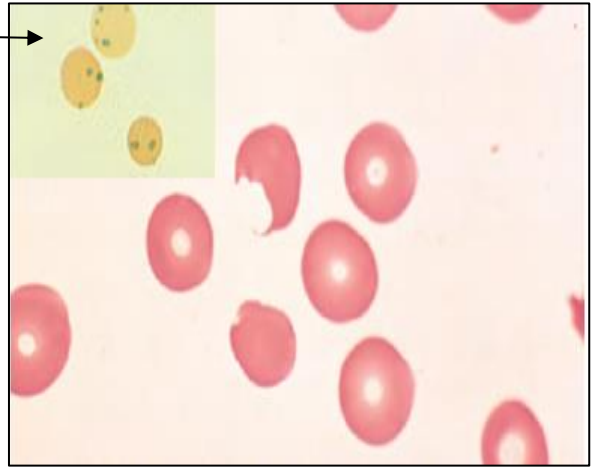
- After oxidation, the oxidized hemoglobin denatures and precipitates, forming intracellular inclusions called **Heinz bodies**.

- Or the less severely damaged cells lose their deformability and suffer further injury when splenic phagocytes attempt to "pluck out" the Heinz bodies, creating so called bite cells.

Remember: Heinz bodies are denatured hemoglobin molecules; when there is oxidative stress on the hemoglobin, the sulfhydryl groups within the amino acids will combine with each other causing precipitation (Heinz bodies).



This is the denatured globin



Why are cells in the picture above cells (containing denatured globin) yellow compared to the normal color of RBCs?

Because Heinz bodies are not seen by Wright Giemsa stain which we normally use (our routine stain that we use in every blood test; i.e. in staining peripheral blood and bone marrow); that's why we use a special stain known collectively as supravital stains, specifically crystal violet.

** Hemolysis in G6PD can be either intra- or extravascular depending on how much the oxidizing is or how strong the attack is.

** Since it's episodic and acute (rather than chronic) hemolysis, features related to chronic hemolysis (splenomegaly and gallbladder stones) are typically absent.

Anemia's where splenomegaly and gallbladder stones are absent are:

a. Aplastic anemia b. Sickle cell anemia c. G6PD deficiency

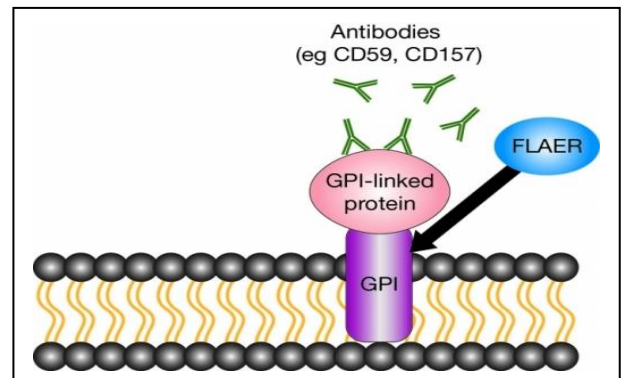
Acquired: Paroxysmal nocturnal hemoglobinuria (PNH):

- PNH is rare, is due to an acquired mutation (not an inherited disease), and is caused by a mutation on the protein found on RBCs that protect them from the complement system.

- Not all cells are affected; one clone in the body has this mutation. It's kind of similar to leukemia or any cancer; one clone of the disease is affected not the whole cells in the body, but here this condition is not neoplastic.

****Mutations don't mean they are inherited.**

-To be inherited, the mutations must be germ cell mutations.



- PNH results from acquired mutations in the phosphatidylinositol glycan complementation group A gene (PIGA), an enzyme that is essential for the synthesis of certain membrane-associated complement regulatory proteins

-PIGA gene is present on the X chromosome.

- A mutation in PIGA gene will result in decreased production of PIGA protein on the cellular membrane → less CD55 and CD59 to counter complement proteins → the cell will be more prone to complement fixation and hemolysis.

- It is the only hemolytic anemia resulting from an acquired genetic mutation.

Clinical manifestations

1- Low level chronic hemolytic anemia.

2- It's NOCTURNAL, why? Because the fixation of complement is enhanced by the slight decrease in blood pH that accompanies sleep, rendering cells more prone to hemolysis.

3- It has some association with aplastic anemia and thrombosis that's why the most encountered clinical scenario is when you suspect PNH in a young patient with anemia and thrombosis.

4- High incidence of bone marrow disorders like myelodysplastic syndrome and leukemia.

**** The treatment of those patients will increase the risk of Neisseria infection, why?**

Because the treatment will fight the complement proteins, and complements specifically the last 5 proteins, which are called membrane attack complex (MAC) are really important in fighting Neisseria. If you inhibit these proteins, you will risk that the

patient might be affected by Neisseria.

** That was the last topic in anemia lectures.

We will start with the new topic which is:

Polycythemia

Denotes an abnormally high red cell count, usually with a corresponding increase in the hemoglobin level.

**In polycythemia, these two criteria usually go side by side, so whenever you hear polycythemia, it means high RBC count as well as elevated hemoglobin level.

** While in anemia, the decrease in hemoglobin level does not necessarily reflect low RBC Count. For example, in beta-thalassemia minor, there's a normal or increased RBC count (Erythrocytosis) associated with low hemoglobin level.

** Polycythemia may be absolute or relative.

Relative Polycythemia

- Occurs when there's a decrease in plasma volume with no change in the total RBC mass.

* It results from dehydration, which occurs with diarrhea, vomiting or diuretic therapy.

* Remember the case of the pregnant woman that has low blood count as a result of fluid retention that leads to an increase in RBC concentration, but she doesn't have anemia. The same concept is applied here.

Absolute Polycythemia

- In absolute polycythemia, the increase of RBC count is due to overproduction of RBCs in the bone marrow.

* It's classified into primary and secondary absolute polycythemia.

* It's described as primary when increased red blood cell mass results from autonomous proliferation of erythroid progenitors, independent of erythropoietin. While in secondary, this overproduction is induced by erythropoietin (no problem in the RBC).

In other words, →

-Primary polycythemia is associated with low erythropoietin levels

-Secondary polycythemia is associated with elevated erythropoietin levels

1- Secondary absolute polycythemia:

As we just mentioned, secondary absolute polycythemia results from excess stimulation of erythroid progenitors by erythropoietin.

****But how does this occur and what is the relation between erythropoietin and oxygen?**

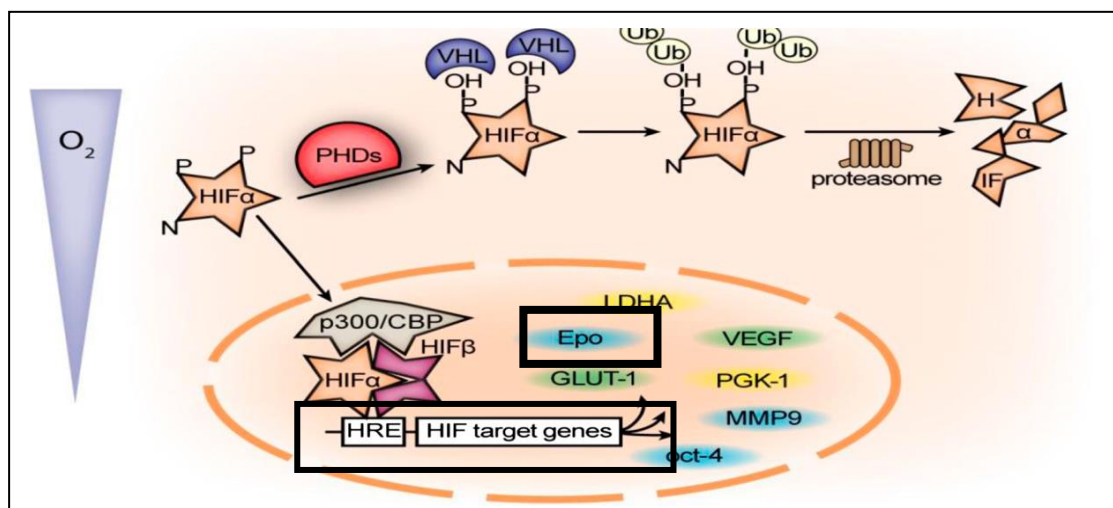
1- Hypoxia is sensed by kidney cells.

2- Kidneys cells also have a transcription factor called HIF (hypoxia induced factor).

3- If hypoxia occurs, kidney cells will sense it and HIF factor will be stabilized; therefore, it will act as a transcriptional factor for many genes. One of them is the gene that codes for erythropoietin.

4- Finally, increased erythropoietin secretion by kidney cells would increase RBC production.

**** Keep in mind, in high oxygen tension, HIF will be destroyed and thus inactivated.**



****Two types of hypoxia occur → generalized hypoxia and localized hypoxia:**

a. Generalized hypoxia, like in smoking, High altitude and High affinity hemoglobins.

Hemoglobin in these cases have high affinity to oxygen.

b. Localized hypoxia, like in renal artery stenosis and polycystic kidney disease

** Moreover, some tumors can secrete erythropoietin. Examples are Wilms tumor, Renal cell carcinoma, Cerebellar hemangioma, Hepatocellular carcinoma.

2- Primary absolute polycythemia:

- Erythropoietin production will be low.

The most common cause of primary absolute polycythemia is polycythemia vera.

** Polycythemia vera is characterized by increased marrow production of red cells, granulocytes, and platelets (panmyelosis), so when we take a bone marrow biopsy, we will see proliferation of all myeloid cells.

- So, it's an acquired, clonal neoplasm of bone marrow stem cells (Chronic myeloproliferative neoplasm).

** PCV is Strongly associated with JAK2 mutation. Such mutation is present in more than 97% of PV cases:

- It's strongly associated with PCV, but it's not specific as seen in 50% of cases of some other chronic myeloproliferative disorders, such as essential thrombocythemia and primary myelofibrosis.

- The most common JAK2 mutation is a Valine-to-phenylalanine substitution at residue 617.

** At first, the patient will have mild splenomegaly which will then become more severe (as a result of the congestion of the spleen). Also, the bone marrow will become hypercellular.

** Late in the disease course, bone marrow fibrosis and significant organomegaly is present, PCV carries a 2% risk for transforming to acute myeloid leukemia.

- In myeloproliferative neoplasm, the spleen will be very large.

** Myeloproliferative neoplasms (we will talk about them in other lectures). They are four major neoplasms:

- a. polycythemia vera
- b. primary myelofibrosis
- c. essential thrombocythemia
- d. chronic myelogenous leukemia

Clinical Features:

- 1- Pruritis.
- 2- Headache; dizziness.
- 3- Hyperuricemia and gout.
- 4- Increased risk of both major bleeding and thrombotic episodes, why? Because of the high levels of RBCs that cause viscosity in the blood, bleeding would occur because of dysfunctional platelets, so what kills in PCV is the thrombosis not the neoplasm itself.

Examples:

- a. Deep venous thrombosis.
- b. Stroke.
- c. Myocardial infarction.
- d. Bowel infarction.
- e. Budd-Chiari syndrome → formation of a blood clot within the hepatic veins which may progress to cirrhosis.
- f. Epistaxis and bleeding gums.

** Major hemorrhage can occur in ~10% of the patients.

** Spent phase → fibrosis and splenomegaly.

** 2% might transform to acute myeloid leukemia

THROMBOSIS IS THE MAJOR RISK FOR MORTALITY IN THESE PATIENTS.

Treatment:

Treatment is Phlebotomy and administration of JAK2 inhibitors:

- If you have high count of RBCs, you have to donate them, as simple as that.
- And recently, JAK2 inhibitors have been developed.



Criteria for PCV diagnosis according to WHO:

Major criteria:

1. Haemoglobin >18.5 g/dL in men, 16.5 g/dL in women. Or other evidence of increased red cell volume.
2. Presence of JAK2 V617F or other functionally similar mutations such as JAK2 exon 12 mutation.

Minor criteria:

1. Bone marrow biopsy showing hyper-cellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic and megakaryocytic proliferation.
2. Serum erythropoietin level below the reference range for normal.
3. Endogenous erythroid colony formation in vitro. (Not commonly used).

- To diagnose PCV, you have to observe two major and minor criteria, or the first major criterion with two minor criteria.

- The second major criterion is not sufficient, because JAK2 mutation is not specific for PCV.

The Doctor's Questions:

1- Relative polycythemia occurs in the setting of:

- A. Wilms tumor
- B. Dehydration
- C. Renal cell carcinoma
- D. Polycythemia vera.
- E. Hypoxia

2- All of the following are examples of secondary absolute polycythemia, except:

- A. Smokers
- B. Renal artery stenosis
- C. Polycystic kidney
- D. Polycythemia vera
- E. High altitude

3- The risk of acute myeloid leukemia in polycythemia vera is:

- A. 2%
- B. 10%
- C. 30%
- D. 50%
- E. 70%

4- One of the following is a major criterion for PV:

- A. High hemoglobin
- B. Hypercellular bone marrow
- C. Low erythropoietin level
- D. High erythropoietin level
- E. Endogenous erythroid colony formation in vitro

Answers: 1: B, 2: D, 3: A, 4: A



"If you are not willing to risk the usual, you will have to settle for the ordinary."

Sorry for any mistake 😊

